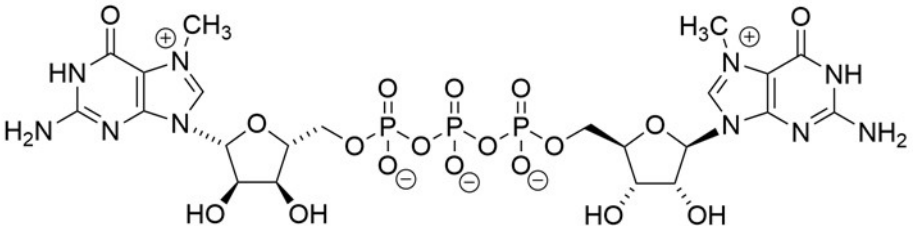
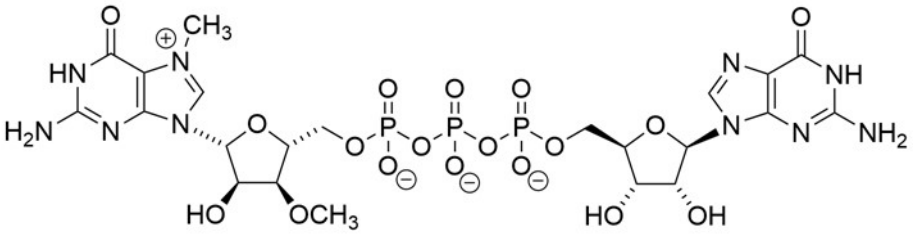
Electronic Supplementary Material (ESI) for Faraday Discussions.

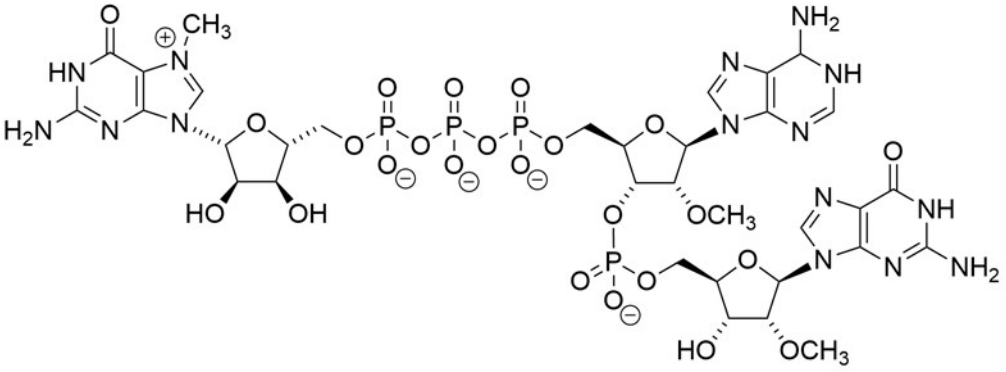
A



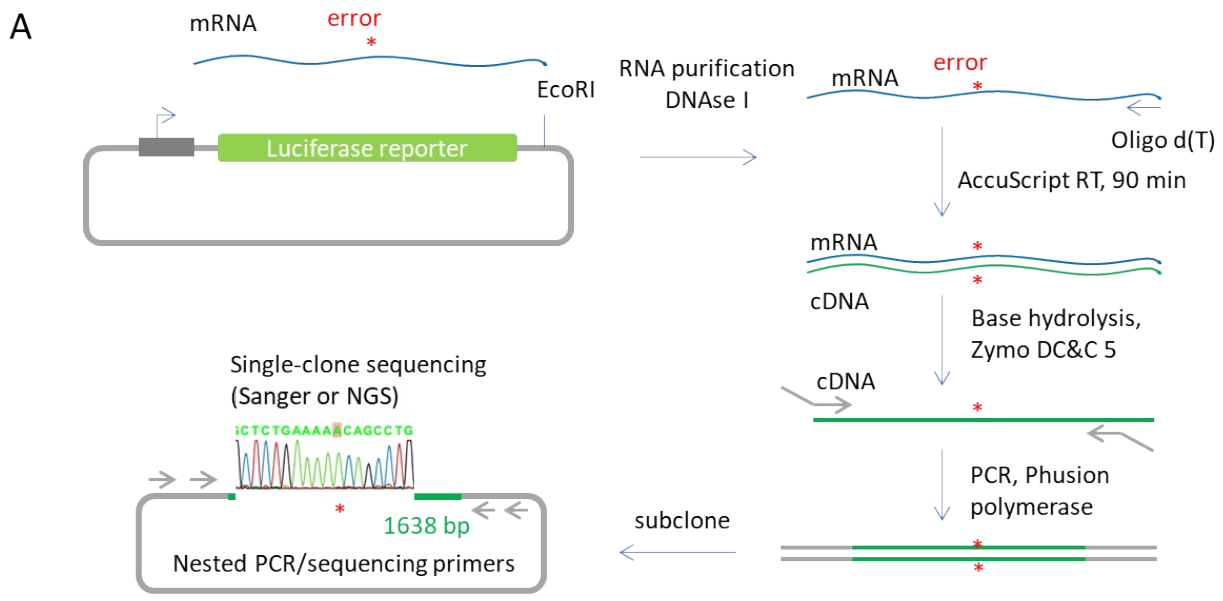
B



C

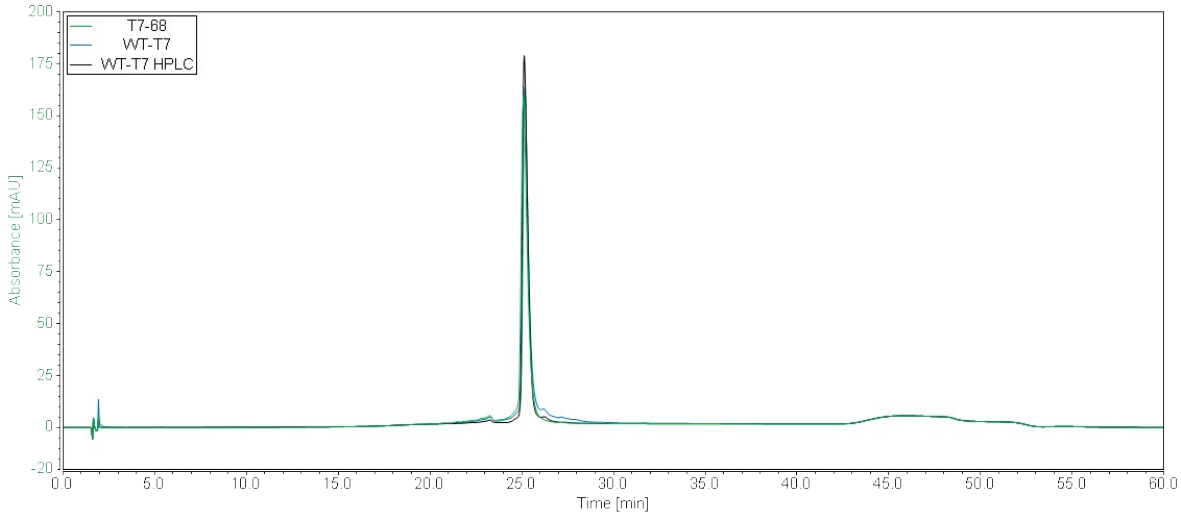


S1 Cap analog structures for (A) sCap, (B) ARCA, and (C) CleanCap™ AG

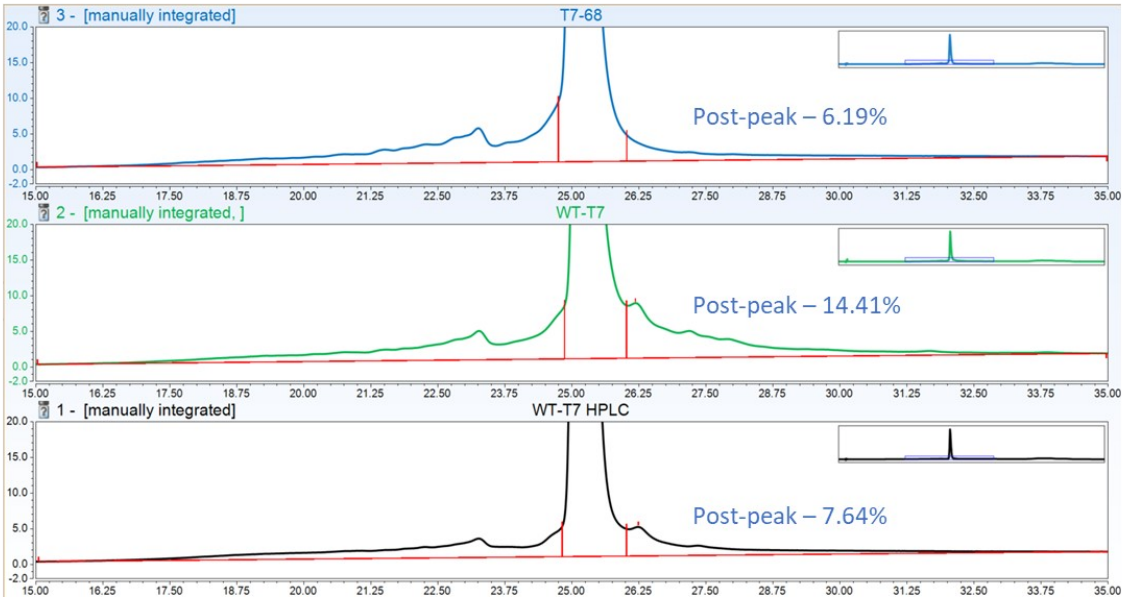


S2 A flow diagram for an RNA polymerase fidelity assay**.** Polymerase fidelity was measured based on directly sequencing a large number of RT-PCR clones derived from mRNA transcribed from variant polymerases. A 1.7 kb firefly luciferase DNA template DNA was used with wild-type and variant T7 RNAPs to transcribe full-length mRNA transcripts. RNA was isolated and residual DNA was removed from the RNA samples by nuclease treatment. Samples were reverse-transcribed with Accuprime Reverse Transcriptase (Agilent) using an oligo-$(dT)_{25}$ primer annealing to the (A) tail on the luciferase template. The RT reaction was then PCR amplified using PHUSION® high-fidelity DNA polymerase. Individual clones were picked and sequenced to >20x coverage depth on the Ion Torrent PGM platform (Thermo Fisher) in multiplex. Reads were mapped against the expected sequenc, and mutations including small insertion, deletions, and single-nucleotide polymorphisms were observed.

A

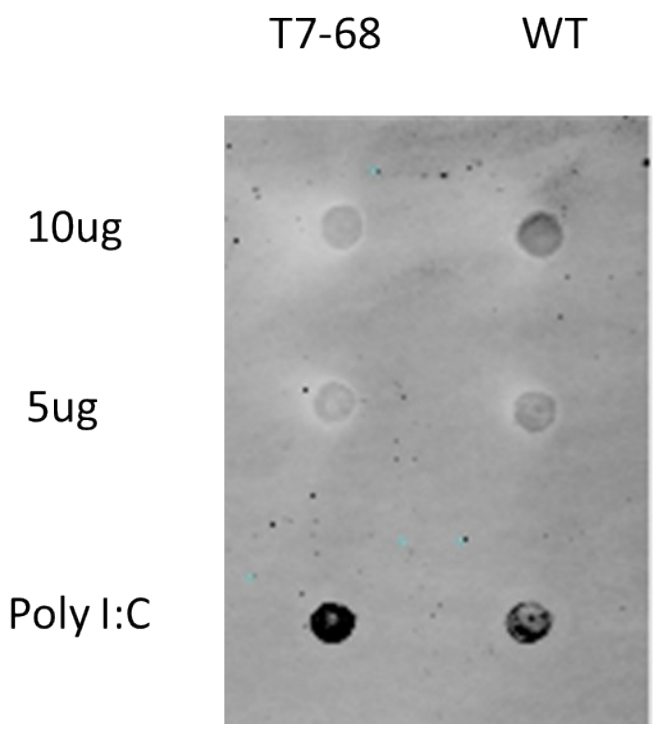


B



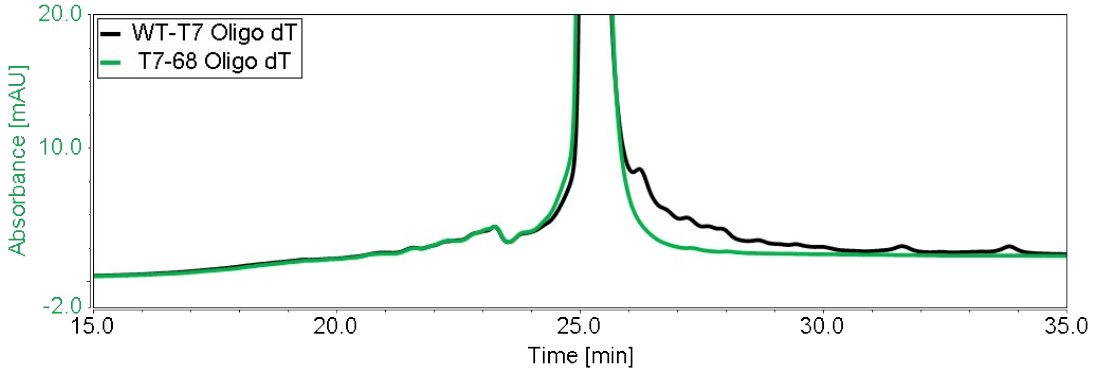
S3 Reverse Phase-HPLC analysis of mRNA 3’ extensions. (A) Full chromatograms for three mRNA samples transcribed using the polymerases indicated (WT, T7-68). The WT sample was analyzed before and after preparative HPLC purification of the mRNA. Equal amounts of each mRNA were injected for each run (B) Integration of apparent higher MW species. Because the baseline does not resolve between the expected mRNA and higher MW species, integrations don’t

directly indicate the fractional amount of 3’ extensions in the sample. However, relative comparisons of these numbers between samples can be used to rank their purity with respect to 3’ extension contaminants.

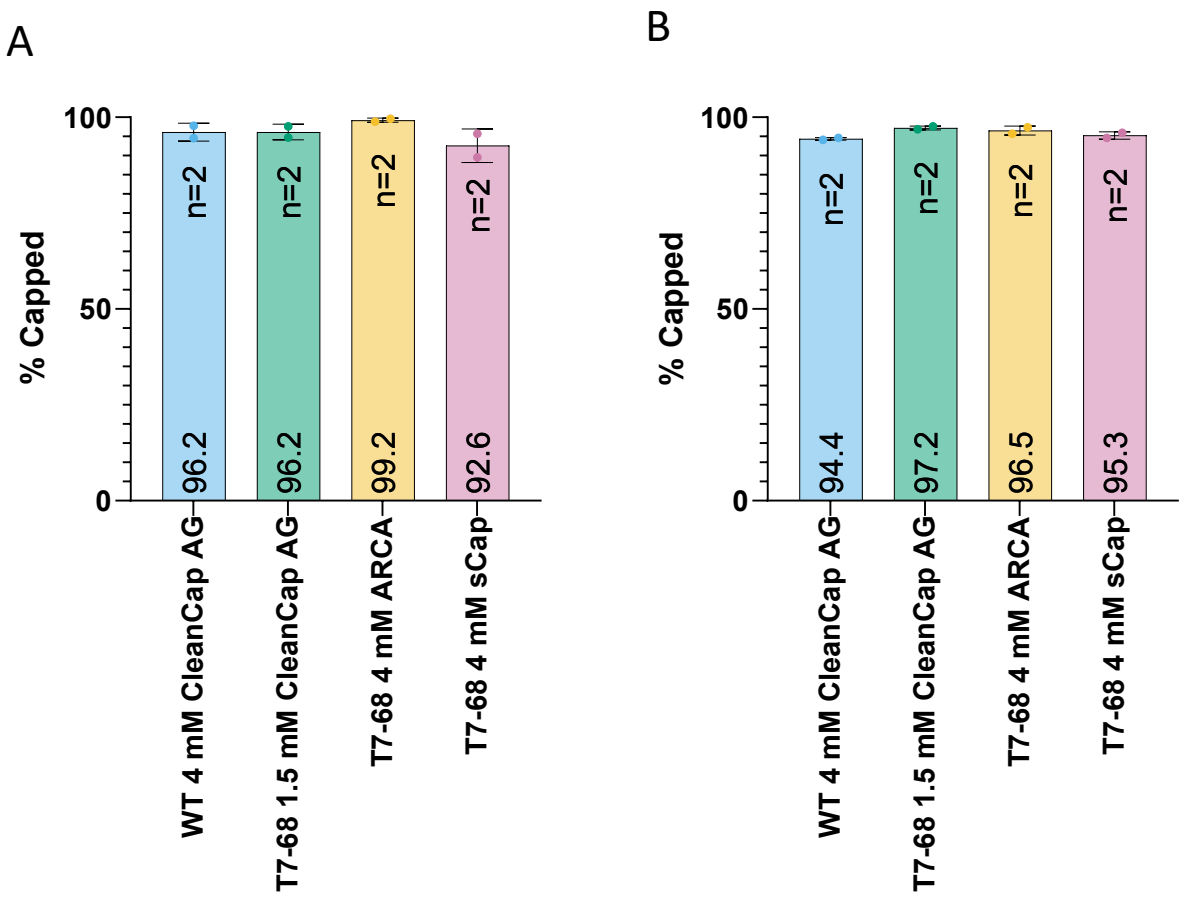


S4 A dsRNA dot blot using the dsRNA-specific J2 antibody. IVT samples transcribed in Buffer B were prepared and spotted onto the membrane in the amounts indicated, and poly I:C was included as a positive control. Higher signal was apparent in crude samples transcribed using the WT RNA polymerase relative to those transcribed using T7-68.

B



S5 HPLC chromatography of 3’ extension products for WT and T7-68 –produced mRNAs following Oligo-dT affinity purification. Oligo (dT) purification does not remove 3’ extension products, but this scalable process can be used to select for full-length mRNAs. The reduced 3’ extension profile from the T7-68 sample is apparent after Oligo-dT affinity purification.



S6 Capping assays were performed under process-relevant reaction conditions for the WT and T7-68 polymerases for each cap analogue shown. **A** Co-transcriptional capping reactions were performed with the recommended buffer for the the WT polymerase (Buffer C) and with Buffer B for T7-68 reactions using 5mM nucleotides (A,C,G,U). Duplicate results for capping efficiency are plotted as points, and the range of is indicated by the error bars. **B** Reactions were performed as in (A), but substituting magnesium acetate for magnesium chloride in the reaction buffer.