Isomorphic emissive GTP surrogate facilitates initiation and elongation of in vitro transcription reactions

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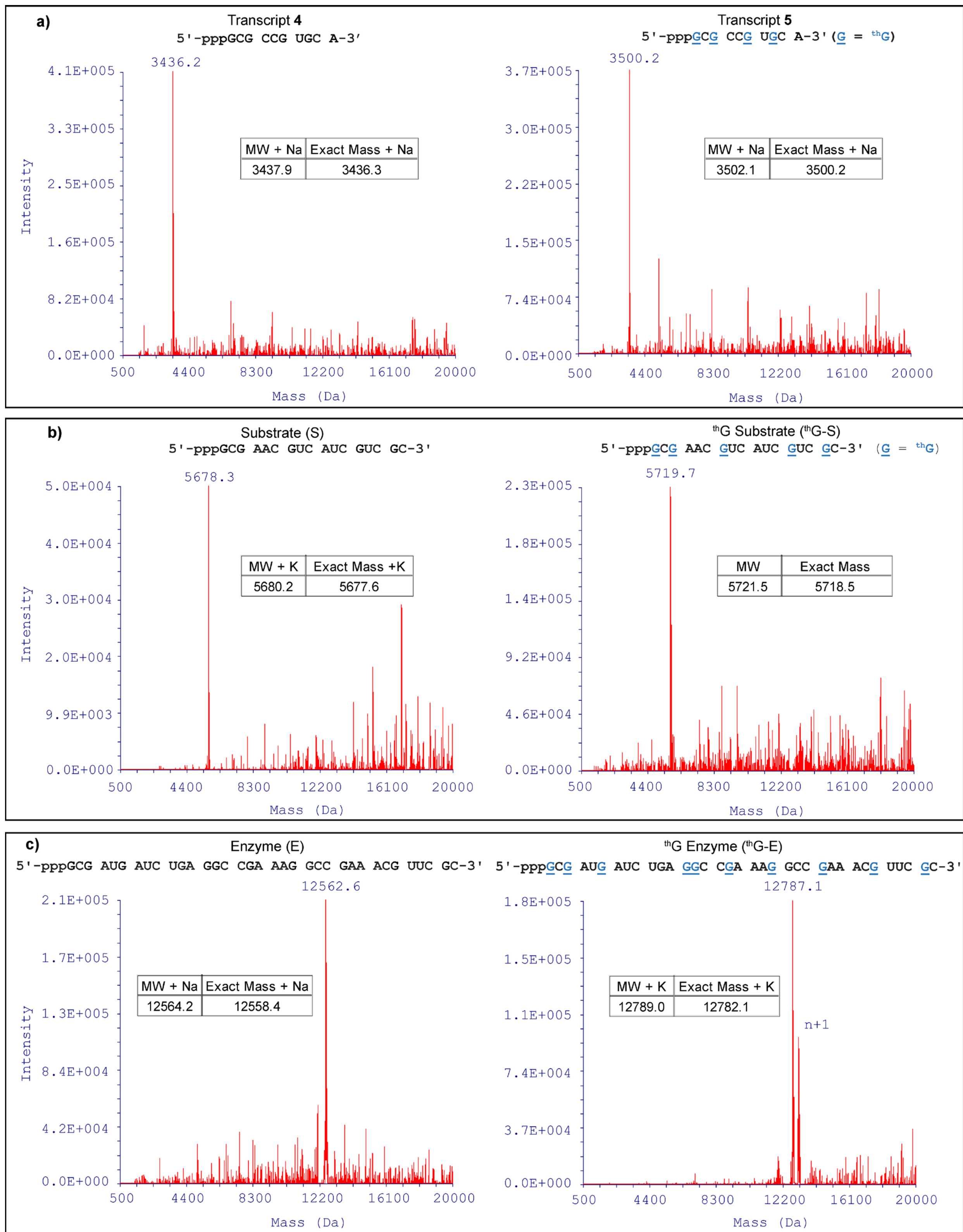
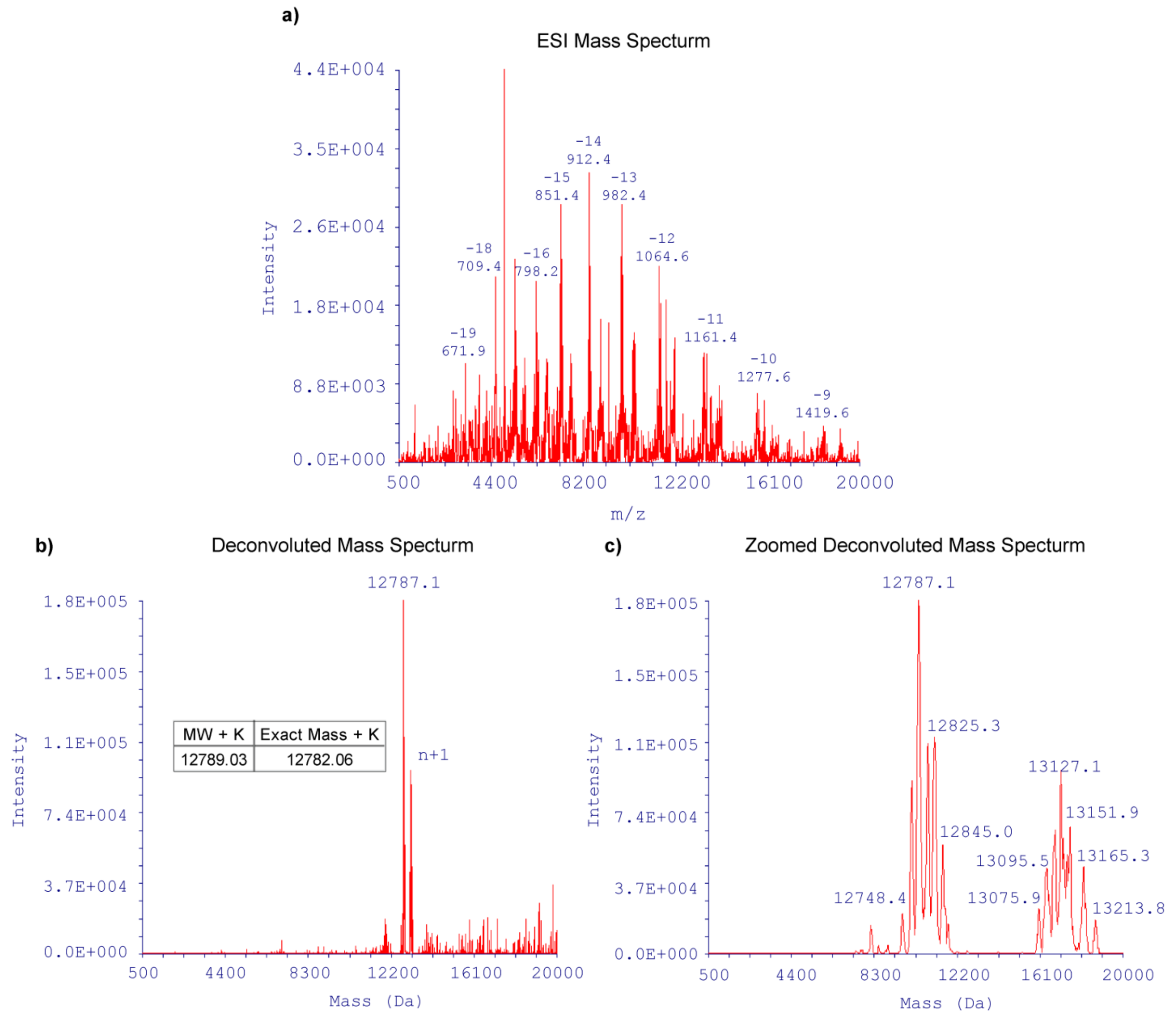


Figure S1: ESI-MS deconvolution mass results of a) transcripts **4** and **5**, b) S and thG-S and c) E and thG-E.



MW + K	Exact Mass + K
12789.03	12782.06

Figure S2: ESI-MS showing (a) mass over charge (m/z), b) deconvoluted and c) zoomed deconvoluted spectra.

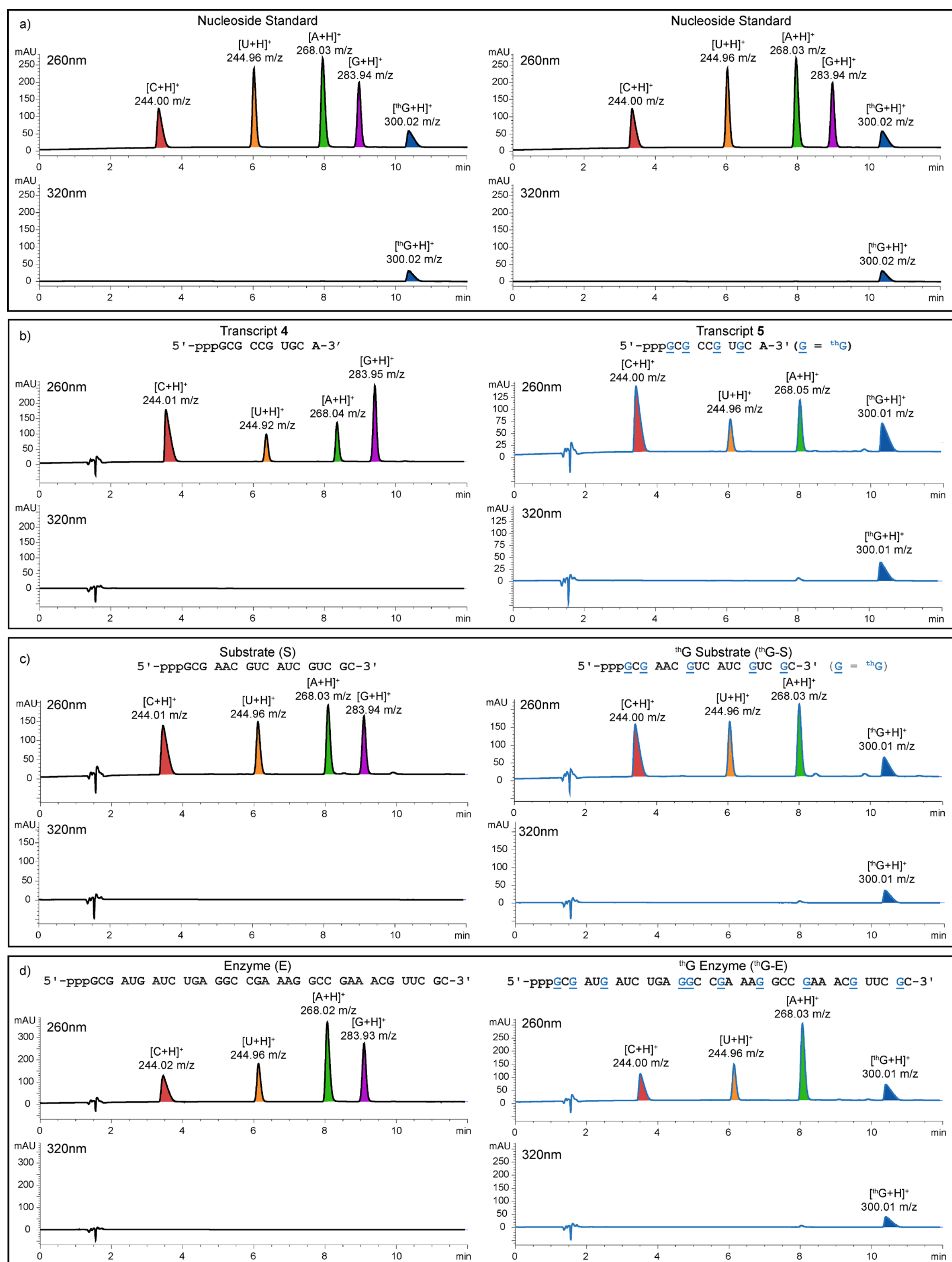


Figure S3: a) The mixture of nucleosides used as a standard. This is shown in both panels to compare to the natural and modified transcripts. Digestion results of b) transcript **4** and **5**, c) S and thG-S, and d) E and thG-E. Digestion of 0.5-2 nmol of transcript was carried out using S1 nuclease for two hours at 37 °C, and was followed by dephosphorylation with alkaline phosphatase for two hours at 37°C. The ribonucleoside mixture obtained was analyzed by reverse-phase analytical HPLC, using a mobile phase of 0–6% acetonitrile (0.1% formic acid) in water (0.1% formic acid) over 12 min; flow rate 1 mL/min. Detection wavelengths are listed.

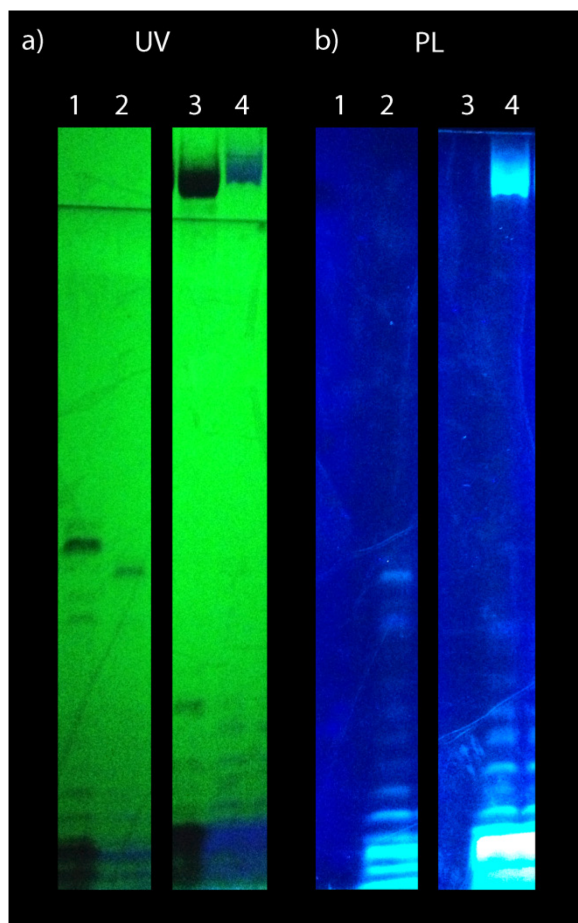


Figure S4: Gel of large scale transcription reactions using templates **6** and **7** with all natural NTPs (lane 1 and 3) or ATP, UTP, CTP and thGTP (lane 2 and 4) with UV light at a) 254 nm (on TLC plate) and b) 302 nm (PL). The reaction was resolved by gel electrophoresis on a denaturing 15% polyacrylamide gel. The isolated yields for thG-S compared to S was 61±3% (or 53±13%), with an average thGTP incorporation of 91±1% (or 88±2%). The isolated yields for thG-E compared to E was 28±2%, with an average of each of the thirteen thGTP incorporations was 91±1%.

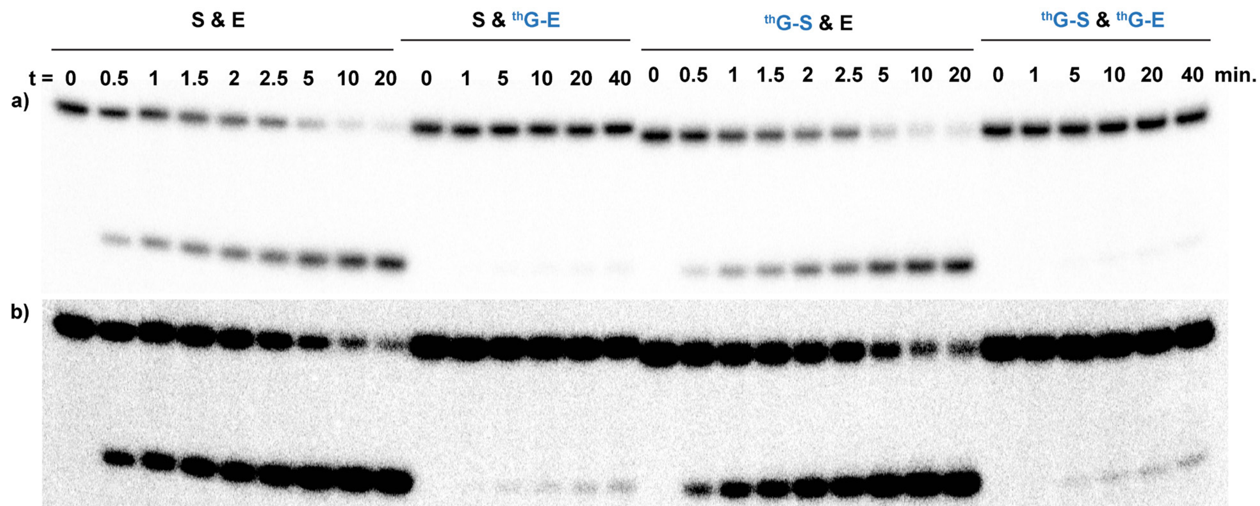


Figure S5: a) Light and b) darker contrast images of HH ribozyme cleavage reactions results were followed by ^{32}P radioactive labeling of substrate strands S and thG-S. S and P_1, and thG-S and thG-P_1 indicate substrate and product strands. All reactions were conducted at 37 °C and contained 0.3 µM substrate (including a trace of 5'-^{32}P labeled material), 3 µM enzyme, 50 mM Tris pH 7.0, 200 mM NaCl, and 10 mM $MgCl_2$. Cleavage for S and thG-S by thG-E was estimated at around 2% at 40 min.

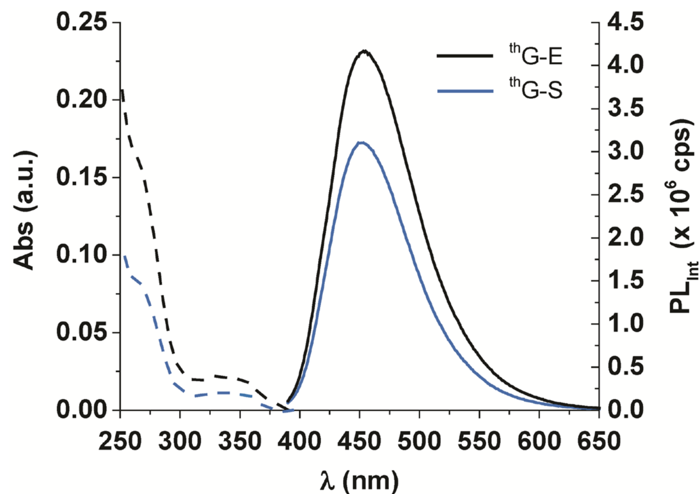


Figure S6: Absorption (dotted) and emission (solid) spectra of 0.6 µM of thG-S (blue) and thG-E () in 50 mM Tris pH 7.0 and 200 mM NaCl. The excitation wavelength was 470 nm and emission was recorded from 390–650 nm. Slit widths for excitation and emission were 8 nm.

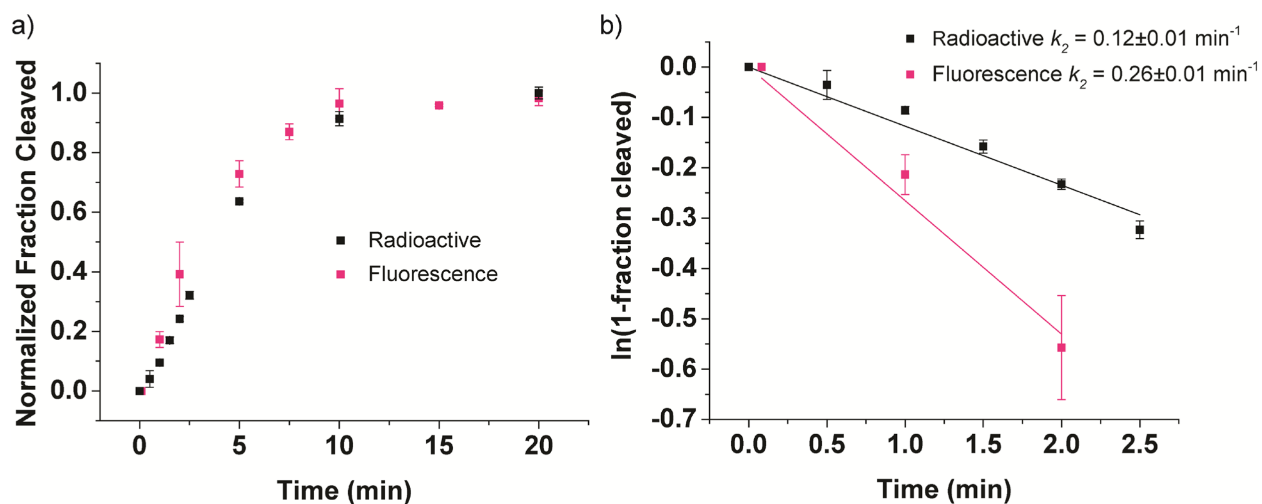


Figure S7: Comparison of fluorescence and radioactive experiment data for thG-S and E, including a) normalized fraction cleavage detected by radioactive experiments or normalized intensity at 450 nm for the fluorescence experiments and b) rates.

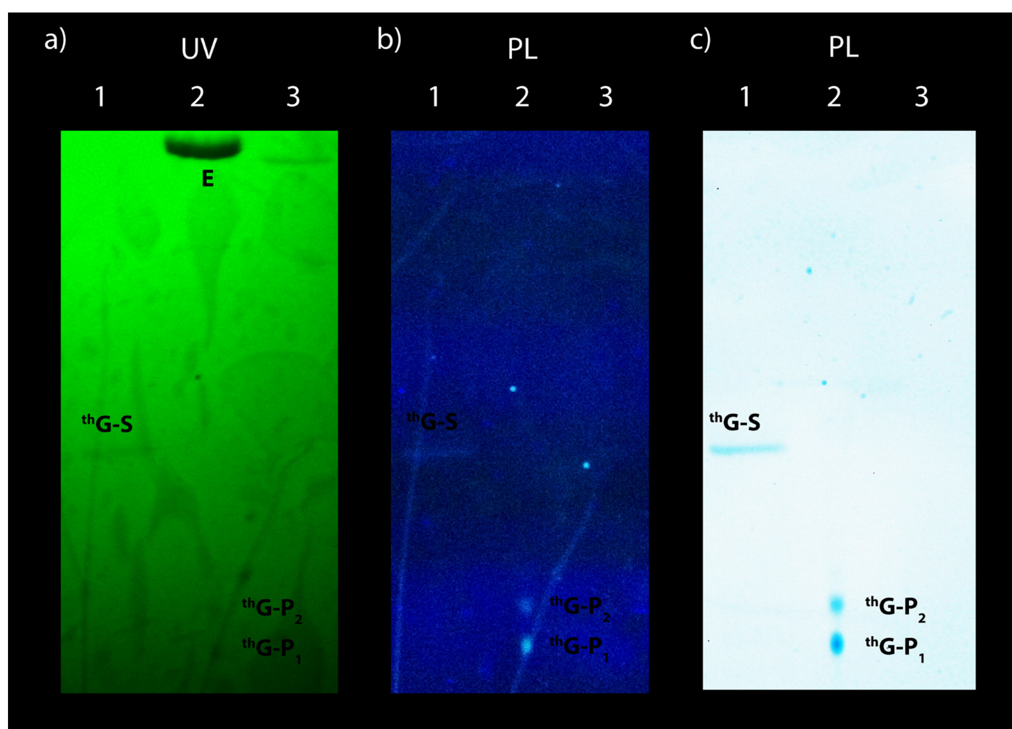
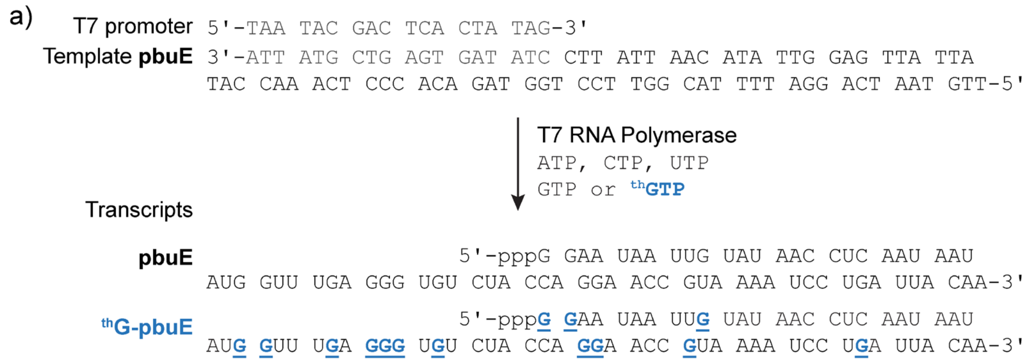


Figure S8: Lane 1 is thG-S, lane 2 is the cleavage reaction of thG-S & E after 20 minutes, and lane 3 is E. The reaction was conducted at 31 °C in a micro fluorescence cell (125 µL) and contained 0.3 µM thG-S, 3 µM E, 50 mM Tris pH 7.0, 200 mM NaCl, and 10 mM $MgCl_2$. The reaction was resolved by gel electrophoresis on a denaturing 20% polyacrylamide gel and imaged with a) UV light at 254 nm (on TLC plate), b) 302 nm (PL), and c) in a BioRad gel imaging system with 302 nm (PL).



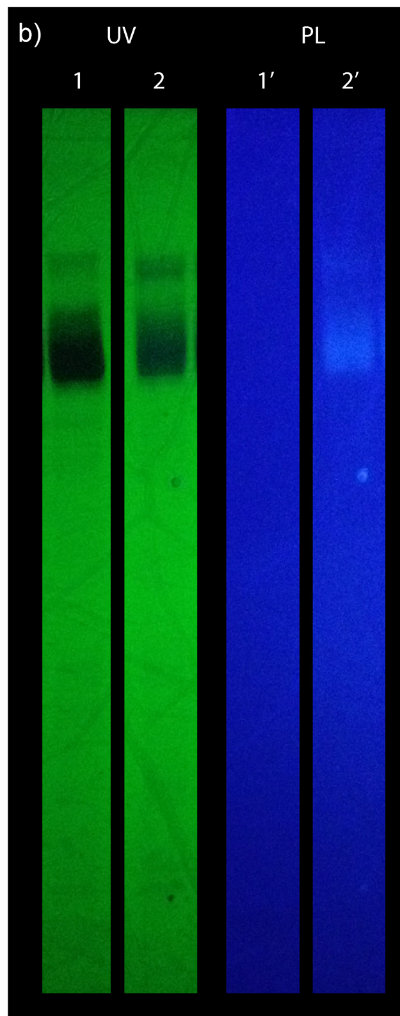


Figure S9: a) T7 promoter and template pbuE depicting the enzymatic incorporation reaction using natural NTPs and GTP or thGTP resulting in transcripts pbuE or thG-pbuE. thG is underlined and bolded blue in transcript thG-pbuE. b) Large scale transcription reaction using template pbuE with all natural NTPs (lane 1 and 1') or ATP, UTP, CTP and thGTP (lanes 2 and 2') with UV light at 254 nm (on TLC plate) and 302 nm (PL). The reaction was resolved by gel electrophoresis on a denaturing 8% polyacrylamide gel. The isolated yields for thG-pbuE compared to pbuE was 54%, with an average of each of the fourteen thGTP incorporations was 95%.

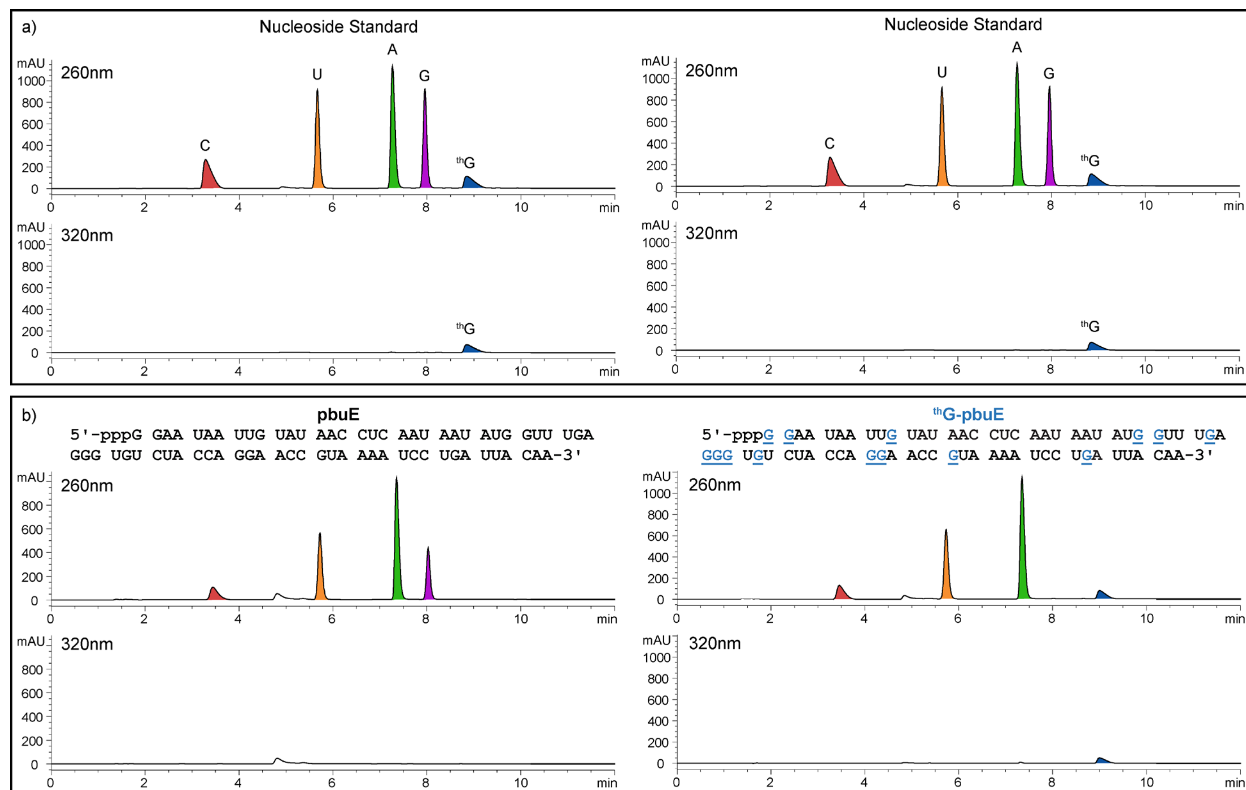


Figure S10: a) The mixture of nucleosides used as a standard. This is shown in both panels to compare to the natural and modified transcripts. b) Digestion results of transcript pbuE and thG-pbuE. Digestion of 0.3 nmol of transcript was carried out using S1 nuclease for two hours at 37 °C, and was followed by dephosphorylation with alkaline phosphatase for two hours at 37°C. The ribonucleoside mixture obtained was analyzed by reverse-phase analytical HPLC, using a mobile phase of 0-5% acetonitrile (0.1% formic acid) in water (0.1% formic acid) over 10 min; flow rate 1 mL/min. Detection wavelengths are listed.