Supplementary information

Rational design of oligonucleotides for enhanced in vitro transcription of small RNA

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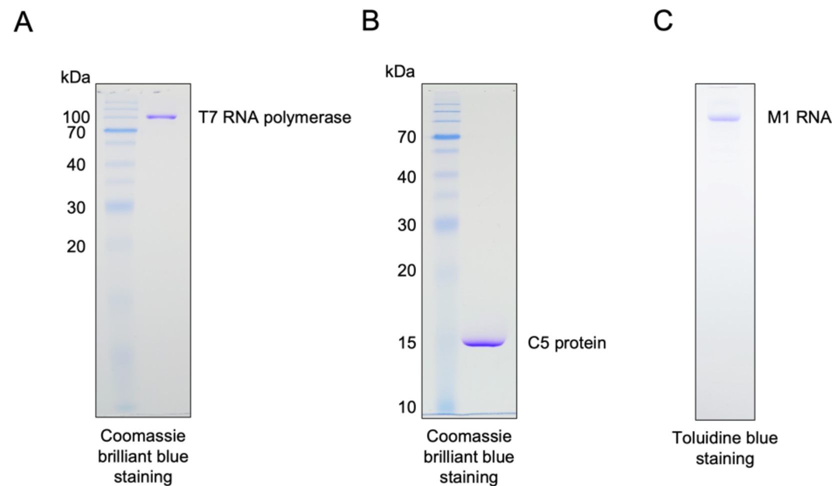

Figure S1. Purified reagents. (A) T7 RNA polymerase used for all in vitro transcription reactions. After purification, 2 µg protein was separated on 10% SDS-PAGE and stained with Coomassie brilliant blue as described in the Materials and Methods. (B-C) RNase P components: (B) Purified *E. coli* C5 protein (2 µg) stained with Coomassie brilliant blue after SDS-PAGE and (C) an in vitro synthesized *E. coli* M1 RNA (0.03 OD) stained with Toluidine blue after 10% denaturing PAGE (7 M urea).

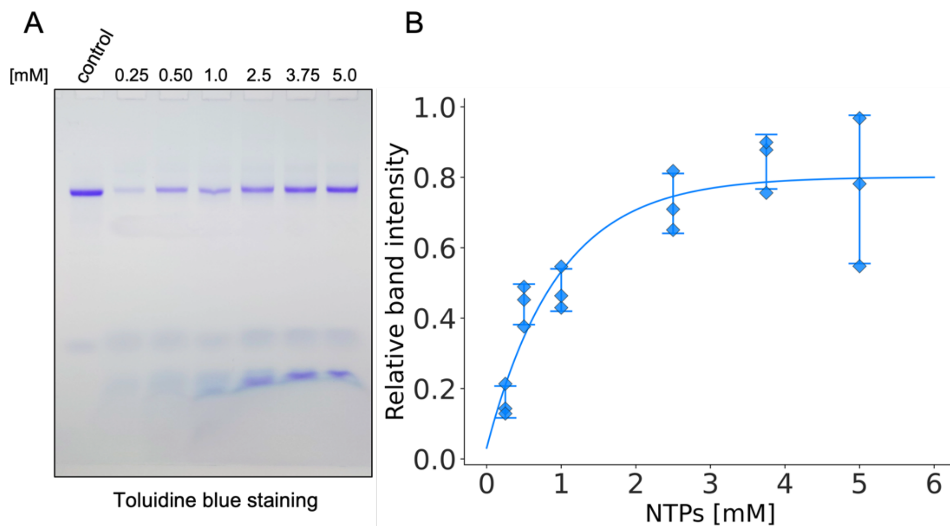

Figure S2. RNA transcription under various concentrations of NTPs. (A) A representative gel image of the transcription assay. The final concentration of 0.25, 0.5, 1.0, 2.5, 3.75, and 5.0 mM NTPs were tested by $tRNA^{His}$ transcription at 37 ºC for 4 hours. The tRNA transcripts were separated by 10% denaturing PAGE (7 M urea) and stained with toluidine blue. (B) The relative transcription yield was calculated from band intensities quantified with ImageJ where we set the band intensity of the control RNA ran on the same gel as 1.0. Standard deviations were calculated from three independent experiments.

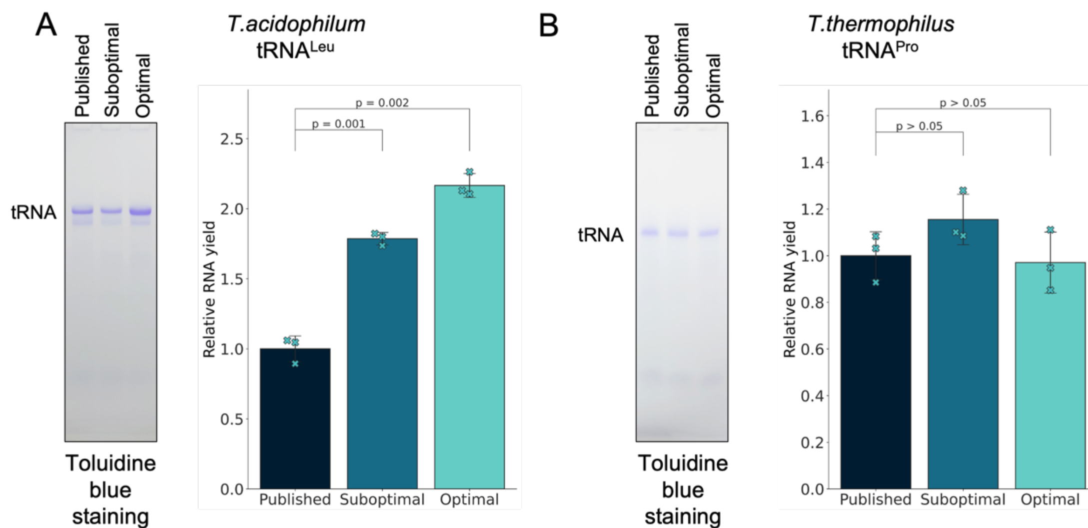

Figure S3. Comparison of transcription yields. Transcription yields for (A) *T. acidophilum* $tRNA^{Leu}$ and (B) *T. thermophilus* $tRNA^{Pro}$. RNA was visualized by Toluidine Blue staining after denaturing PAGE (7M urea) of 5 µL transcription mixture (left panels). Band intensities quantified as described in the Materials and Methods are shown including errors (x), S.D. (n=3) and p-values (right panels).

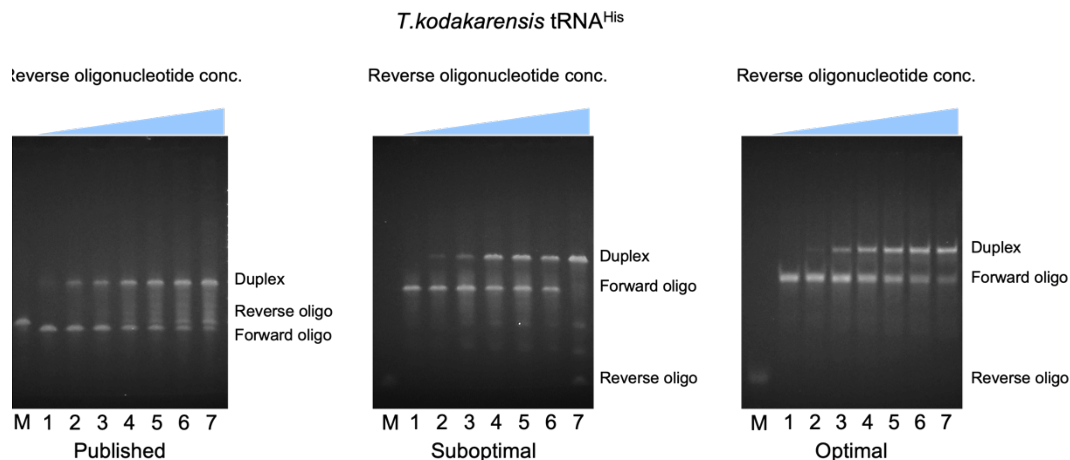

Figure S4. Electrophoretic mobility shift assays. Duplex formation by forward and reverse oligonucleotides was assessed by EMSA as described in the Materials and Methods. Oligonucleotides selected by ROCKET (optimal (right) and suboptimal (middle)) or that have been published (left) were stained with SYBR bold. No reverse oligonucleotide was present in lanes 1; Lane M contains reverse oligonucleotide by itself. Reverse oligonucleotide concentrations were 0.01 µM (lanes 2), 0.02 µM (lanes 3), 0.04 µM (lanes 4), 0.06 µM (lanes 5), 0.08 µM (lanes 6), and 0.10 µM (lanes 7).

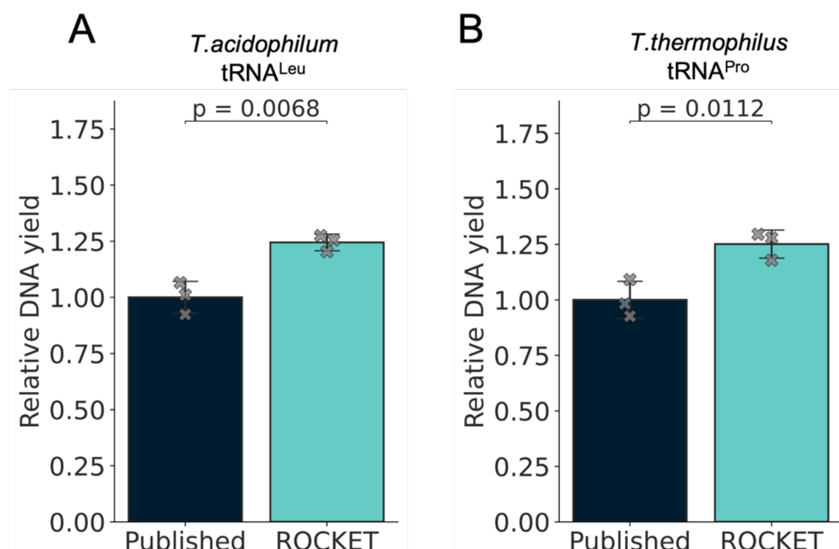

Figure S5. Template DNA yields after 10 cycles of the DNA polymerase extension reaction. (A and B) Amounts of extended DNA obtained from 10 cycles of DNA polymerase extension reactions in the presence of 1 μM oligonucleotides and ~10 nM Dream-Taq DNA polymerase (0.01 units) were quantified for (A) *T. acidophilum* $tRNA^{Leu}$ and (B) *T. thermophilus* $tRNA^{Pro}$. Band intensities quantified as described in the Materials and Methods are shown including errors (o), S.D. (n=3), and p-values.