Supporting Information

An *In Vitro* Selection System for TNA

Justin K. Ichida,[1] Keyong Zou,[1] Allen Horhota,[2] Biao Yu,[3] Larry W. McLaughlin,[2] and Jack W. Szostak.[*1]

[1] Howard Hughes Medical Institute, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, [2] Department of Chemistry, Boston College, Chestnut Hill, MA 02467, and [3] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai, China

Figures 1 template sequence:

5'-
TTCTCGCTCTTCACCGCTCACTCTTCACTTGCTTCACTTGCTTTCACTCTCCTCTCCCTATAGTGAGTC
GTACCC -3'

Figure 1 primer sequence:

5'- GGGTACGACTCACTATAGGGAGAGG -3'

Figure 2 hairpin oligonucleotide sequence:

(BsrBI site is underlined)
5'-
TTCTCGCTCTTCACCGCTCACTCTTCACTTGCTTCACTTGCTTTCACTCACTCGCTTTTAGGCGCGTGC
ATAAGCGTGTCACAACCTCGGCCGTCACCACGACACGCTTATGCACGCGCC -3

Figure 2 strand displacement primer sequence:

5'- GTGGTGACGGCCGAGGTTG-3'

Supporting Figure 1. PAGE analysis of fidelity assays. 31 nM primer/template complex was annealed in 2x Thermopol buffer. tNTPs (120 μM tDTP, 31 μM tTTP, 18 μM tCTP, 2 μM tGTP), .5 Units of *Tth* Pyrophosphatse, and .25 Units of Therminator polymerase were added in a total volume of 5 μl. 2,6-diaminopurine(D) was used in the DNA templates to improve the efficiency of polymerization.[5] Reactions were incubated at 75 degrees Celsius. Time course samples were denatured by boiling for 5 minutes in 7M Urea/ 20mM EDTA/ 400mM NaOH and analyzed by 20% denaturing PAGE.

A. tGTP Fidelity

Primer Sequence:

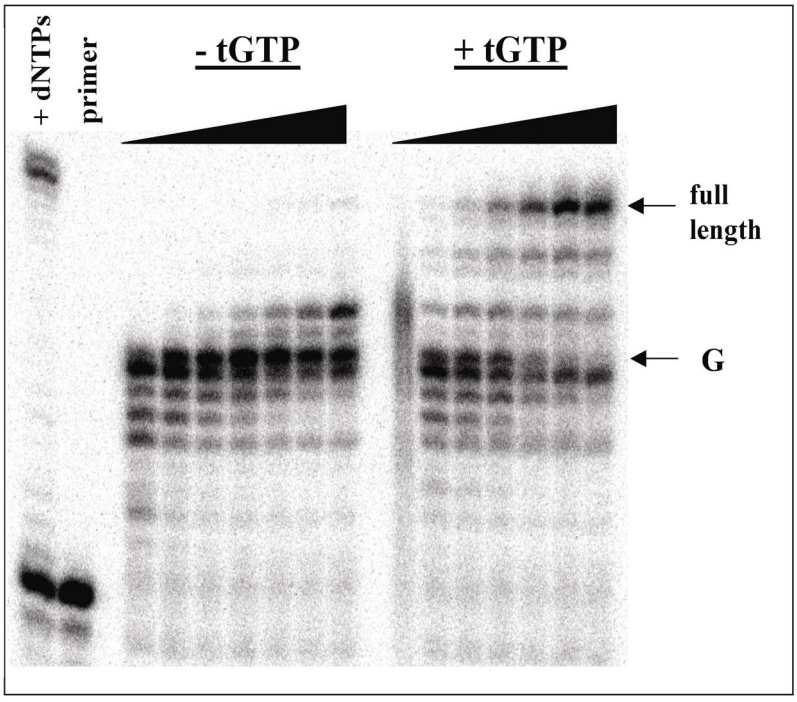
5'- GGGTACGACTCACTATAGGGAGAGG –3'

Template Sequence:

5'- TTTTGTTCTTTGTTTGTTCCTCTCCCTATAGTGAGTCGTACCC –3'

Time points:

10 min, 20 min, 30 min, 45 min, 75 min, 120 min, 180 min



B. tCTP Fidelity

Primer Sequence:

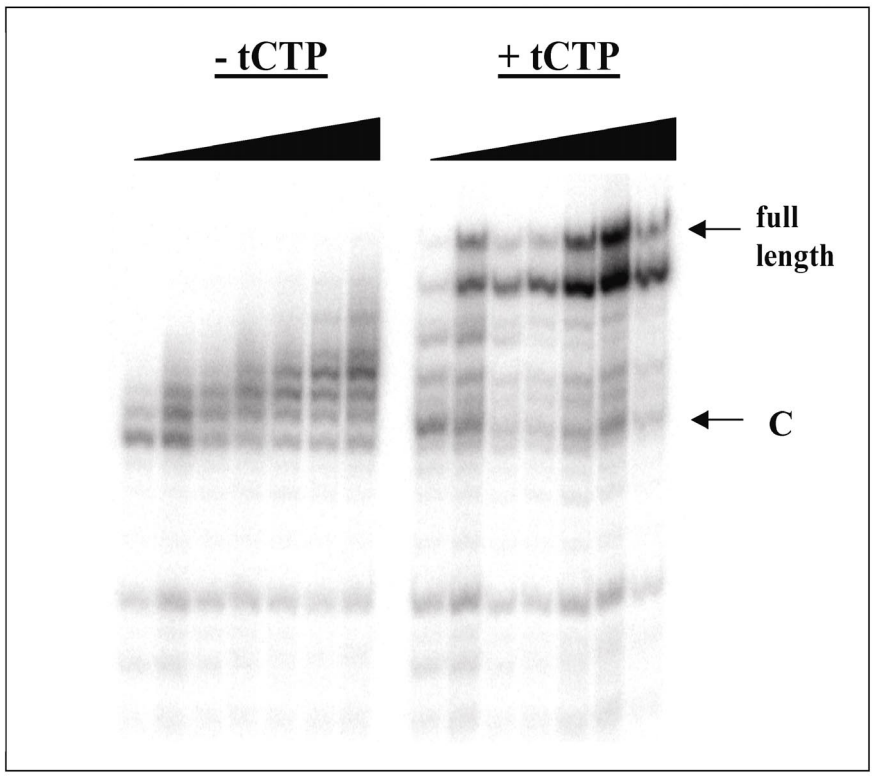
5’- GGGTACGACTCACTATAGGGAGAGG –3’

Template Sequence:

5’-TTCTTCCGTCTTTCTCTCCCTCTCCCTATAGTGAGTCGTACCC –3’

Time points:

10 min, 20 min, 30 min, 45 min, 75 min, 120 min, 180 min



C. tDTP Fidelity

Primer Sequence:

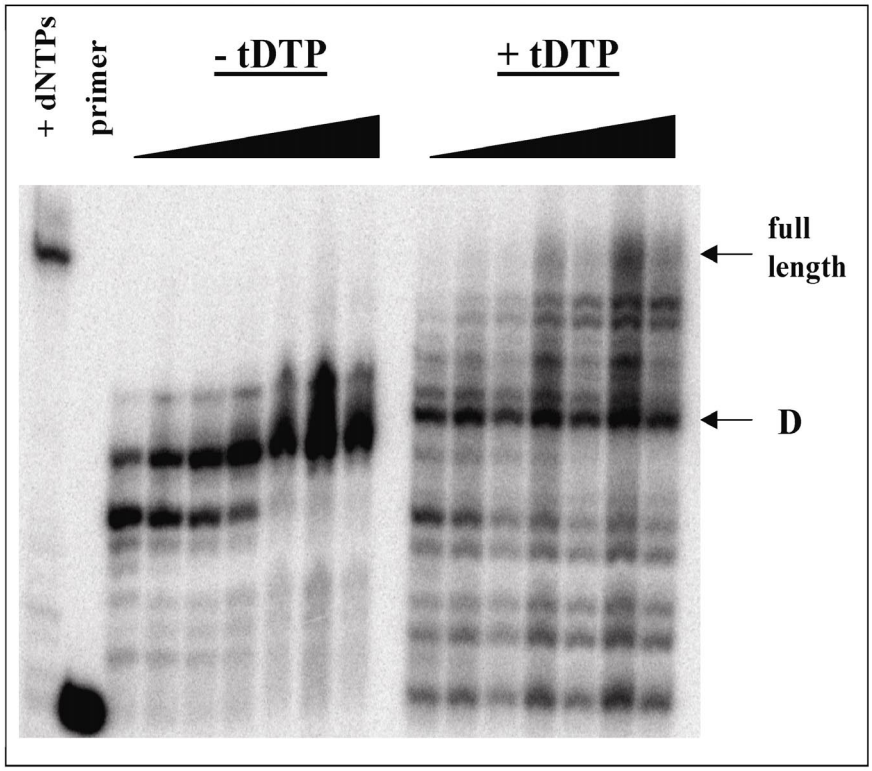
5’- GGGTACGACTCACTATAGGGAGAGG –3’

Template Sequence:

5’- CCCGCCGTGGCCCGCCGCCCTCTCCCTATAGTGAGTCGTACCC –3’

Time points:

10 min, 20 min, 30 min, 45 min, 75 min, 120 min, 180 min



D. tTTP Fidelity

Primer Sequence:

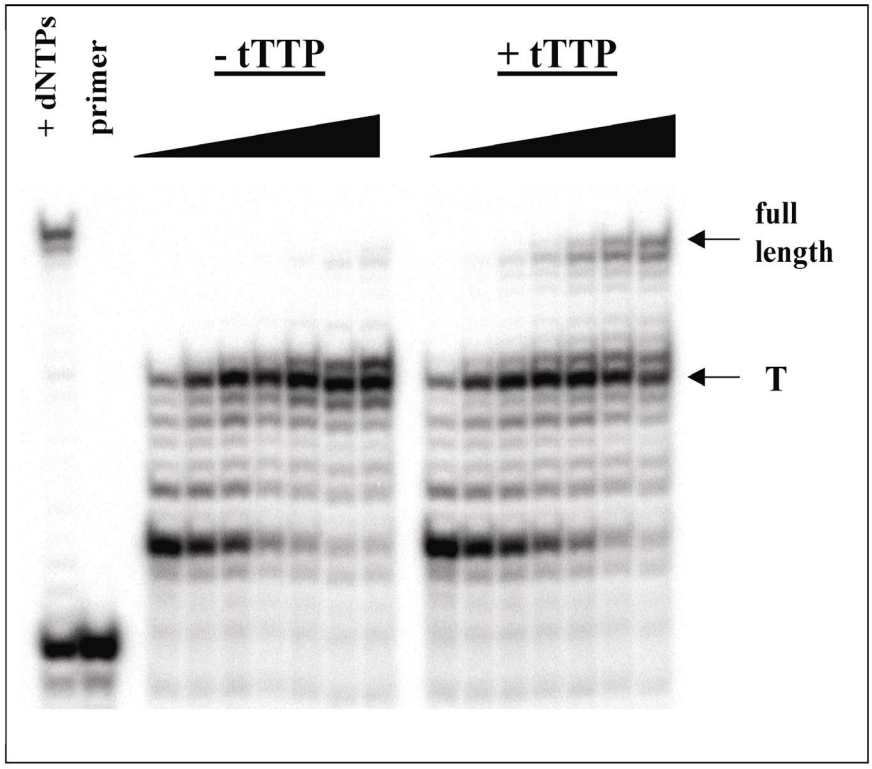
5’- TAATACGACTCACTATAGGGAGA –3’

Template Sequence:

5’- CTTCTCCDTTCTTCGTTTTCTCCCTATAGTGAGTCGTATTA –3’

Time points:

10 min, 20 min, 30 min, 45 min, 75 min, 120 min, 180 min



Supporting Table 1. Minimum apparent fidelity of TNA Polymerization. Gels from Supporting figure 1 were quantified by phosphorimager analysis (ImageQuant v1.2, Molecular Dynamics). Apparent fidelity for each base equals 1-[(% full-length -tNTP)/ (% full-length +tNTP)]. The values shown likely underestimate the true fidelities due to the absence of competition between correct and incorrect nucleotides during the drop-out reactions.

Substrate	tGTP	tCTP	tDTP	tTTP
Apparent Fidelity	> .99	.98	.95	.94

Supporting Table 2. Fraction of Error-free TNA Transcripts. The fraction of full-length TNA strands generated by Therminator polymerase that would be error-free, as a function of length. These calculations assume that the sequences contain an equal number of all four nucleobases and that the full-length products were gel purified from a reaction when 10-20% of the primers were fully extended. These calculations are based upon the apparent fidelities for individual tNTPs listed in Supporting Table 1. Actual fidelities, and thus fraction of error-free TNA strands, may be higher due to competition between tNTPs in reactions containing all four tNTPs.

TNA Product Length	40 nt	50 nt	60 nt
Fraction Error-free	.24	.17	.12