Supporting Information

Horowitz et al. 10.1073/pnas.0914172107

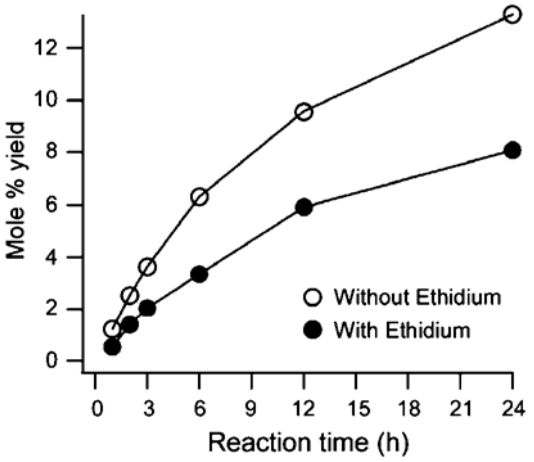

Fig. S1. Cyclization kinetics of d(pCCTA) in the presence and absence of ethidium.Reactions contained 200 μM d(pCCTA), 5 mM $MnCl_2$, 10 mM triethylammonium MES (pH 6), 600 μM ethidium (when present), and 25 mM *N*-cyanoimidazole. The reactions were incubated at 4 °C. At each time point, a 10 μL aliquot was removed and diluted in 90 μL 22 mM EDTA to quench the reaction. The aliquot was then immediately chromatographed (Agilent 1100, 4.6 mm × 250 mm Phenomenex Luna C_{18}, ambient temperature). Gradient: Solvent A = 100 mM triethylammonium acetate, pH 7. Solvent B = Acetonitrile. 0–12 min, 7.5% B. 12–20 min, 7.5–20% B. 20–25 min, 20–70% B. 25–30 min, 70% B.

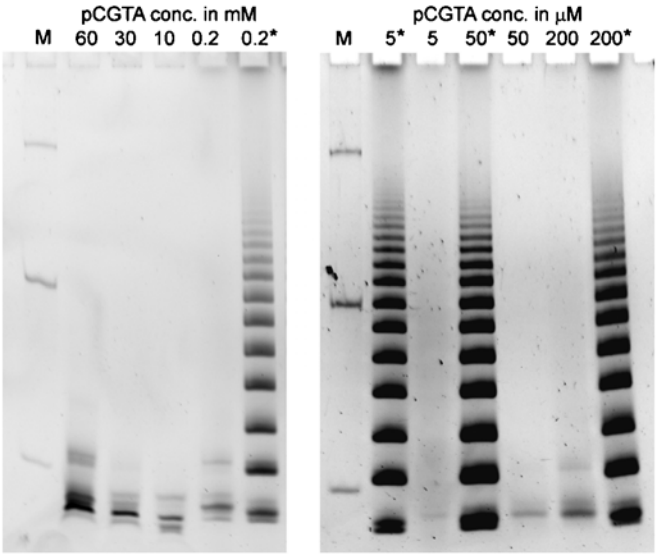

Fig. S2. Oligonucleotide concentration and ethidium dependence of d(pCGTA) polymerization. Reactions contained 200 μM d(pCGTA), 5 mM $MnCl_2$, 10 mM triethylammonium MES (pH 6), 600 μM ethidium (when present, noted with an asterisk above gel lanes), and 250 mM *N*-cyanoimidazole. All reactions were incubated for 72 h at 4 °C. Reactions ≤200 μM in tetranucleotide were ethanol precipitated with linear polyacrylamide carrier before loading to facilitate nucleic acid recovery. The molecular weight marker, labeled as lane M, contains DNA oligonucleotides of length 10, 32, and 110 nt.

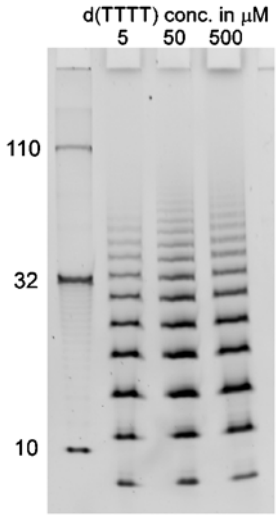

Fig. S3. Tolerance of ethidium-mediated d(pCGTA) polymerization to the presence of non-pairing, non-polymerizable oligonucleotides. Reactions contained 5 μM d(pCGTA), 5 mM $MnCl_2$, 10 mM triethylammonium MES (pH 6), 600 μM ethidium (when present, noted with an asterisk above gel lanes), 250 mM *N*-cyanoimidazole, and the indicated concentration of d(TTTT). All reactions were incubated for 72 h at 4 °C. Reactions ≤200 μM in tetranucleotide were ethanol precipitated with linear polyacrylamide carrier before loading to facilitate nucleic acid recovery. The molecular weight marker contains DNA oligonucleotides of length 10, 32, and 110 nt. Reactions were ethanol precipitated with linear polyacrylamide carrier before loading.

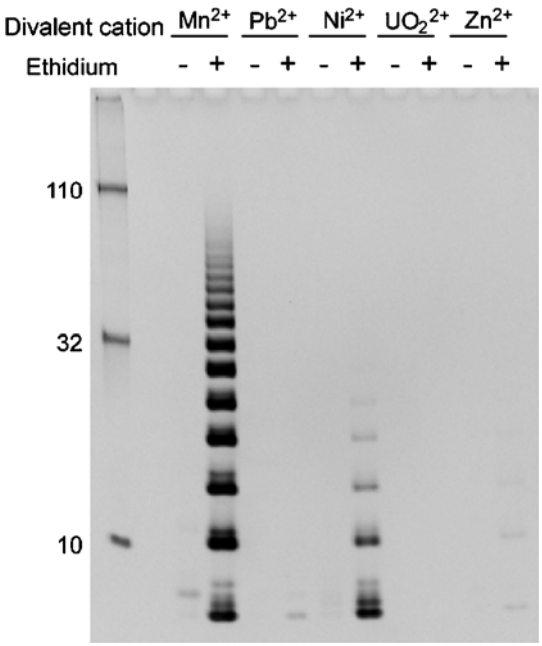

Fig. S4. Divalent metal ion dependence of ethidium-mediated polymerization of d(pCGTA). Reactions contained 200 μM d(pCGTA), 5 mM $MnCl_2$, 10 mM triethylammonium MES (pH 6), 600 or 0 μM ethidium noted with a + or −, respectively, above gel lanes), and 250 mM *N*-cyanoimidazole. All were incubated for 72 h at 4 °C. The molecular weight marker contains DNA oligonucleotides of length 10, 32, and 110 nt.

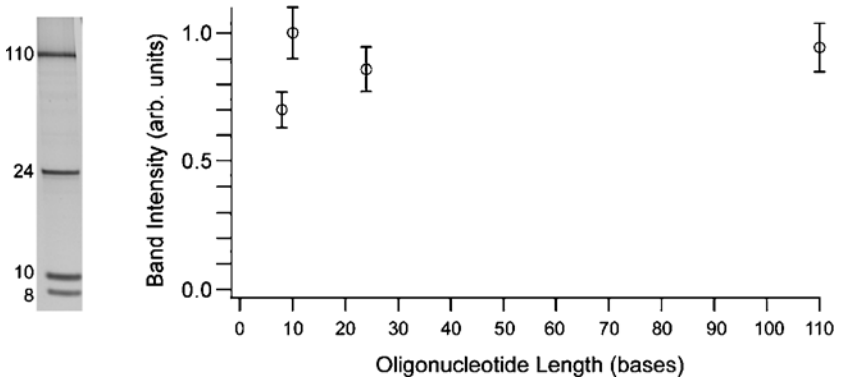

Fig. S5. Staining efficiency of oligonucleotides of varying length. Equal amounts of each oligonucleotide (50 pmoles of nucleotide residues) were separated by denaturing PAGE. The gel was stained with SYBR Gold, and the band intensities were calculated by averaging the integrated intensities of four representative vertical cross-sections of each gel band. Error bars indicate the estimated 10% error in the actual amount of each oligonucleotide loaded onto the gel.

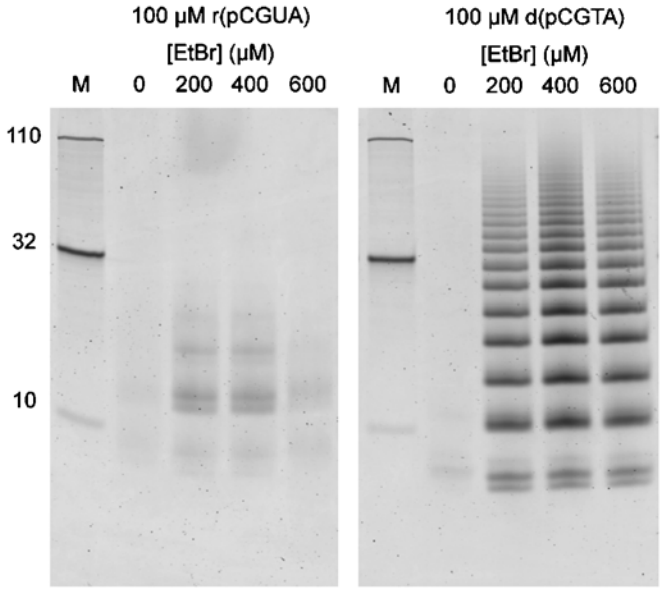

Fig. S6. Comparison of ethidium-mediated polymerization of RNA and DNA tetranucleotides. Reactions contained 100 μM d(pCGTA) or r(pCGUA), 5 mM $MnCl_2$, 10 mM triethylammonium MES (pH 6), 600 μM ethidium (when present, noted with an asterisk above gel lanes), and 250 mM *N*-cyanoimidazole. All were incubated for 72 h at 4 °C. The molecular weight marker, labeled as lane M, contains DNA oligonucleotides of length 10, 32, and 110 nt.