Supplemental Information and Tables

The energetic difference between synthesis of correct and incorrect base pairs accounts for highly accurate DNA replication.

Andrew C. Olson, Jennifer N. Patro, Milan Urban, and Robert D. Kuchta

ABBREVIATIONS

dATP, 9-β-D-2'-deoxyribofuranosyl-(adenine)-5'-triphosphate; dCTP, 1-β-D-2'-deoxyribofuranosyl-(cytidine)-5'-triphosphate; dGTP, 9-β-D-2'-deoxyribofuranosyl-(guanine)-5'-triphosphate; dTTP, 1-β-D-2'-deoxyribofuranosyl-(thymine)-5'-triphosphate; dNTP, deoxynucleoside triphosphate; dsDNA, double stranded deoxyribonucleic acid; exo$^-$, 3'-5'exonuclease deficient; BF, *Bacillus stearothermophilus* Large Fragment; KF, Klenow Fragment (exo$^-$) of DNA polymerase I; $Vent_R$, $Vent_R$ (exo$^-$) DNA Polymerase.

Methods Summary

DNA polymerases: were purchased from New England BioLabs.

Oligonucleotides: DNA oligonucleotides were purchased from Integrated DNA Technologies or were synthesized as previously described[S1,S2]. Primer strands were gel purified, 5'-^{32}P labeled, and annealed to their template counterpart as previously described[4].

Determination of ΔG°: The equilibrium constant (K_{eq}) for the correct incorporation and misincorporation of a nucleotide opposite a templating base was determined using equation 1 and used to calculate $\Delta G^\circ_{incorporation}$ using equation 2. A radio labeled primer annealed to a template strand was incubated with three concentrations of the desired dNTP (1μM, 2μM, and3μM for correct incorporation and 2mM, 3mM, and 4mM for misincorporation) to be incorporated as well as pyrophosphate (PP_i) (4mM), to provide an observable reverse reaction. An exonuclease deficient polymerase (5nM) was used as a catalyst with the primer-template (100nM) in Tris-HCl pH 7.5 (50 mM) and $MgCl_2$ (8mM). Total reaction volume was 30 ul. Reactions were incubated at 37°C in an air incubator to prevent formation of condensation on the sample tube lid. Samples were taken at various time points and quenched with formamide. Products were analyzed on a 20% polyacrylamide gel and quantitated on a Typhoon Phosphorimager using Image Quant software (Molecular Dynamics). Reactions were considered to be at equilibrium when the ratio of DNA_n / DNA_{n+1} did not change significantly over the course of 45 minutes for correct incorporation and 4 hours for misincorporation. Observing similar ΔG° values at each of the three different concentrations of dNTP was required to verify a legitimate ΔG° value. Similar ΔG°s were measured at each dNTP concentration and the reported values are the averages of all measurements for each polymerization event are reported.After the measurements of ΔG° were completed 1mM of the next correct dNTP was added and equilibrium reactions were incubated for an additional hour to completely extend DNA products to DNA_{n+1}. Complete extension ensured that the polymerase was active for the duration of the ΔG° measurements. In the absence of pyrophosphate each primer/template was able to achieve ≥ 95% extension of DNA_n to DNA_{n+1} over the course of the experiment (105 minutes for correct incorporation and 24 hours for misincorporation) for both the correct and incorrect incorporation events. This ensured that in the absence of pyrophosphate each reaction type was able to start at the same point, DNA_n, and proceed to the same endpoint, complete extension of DNA_n to DNA_{n+1} (Figure 1b, lane 6 and Figure S1, lane 2).

Equation 1: $K_{eq} = ([DNA_{n+1}] \bullet [PP_i])/([DNA_n] \bullet [dNTP])$

Equation 2: $\Delta G^\circ = -RT\ln K_{eq}$ where the molar gas constant $R=8.314 m^2\ kg\ s^{-2}\ K^{-1}$, T = temperature in Kelvin, and K_{eq} is the equilibrium constant.

REFERENCES

(S1) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Letters* **1981**, *22*, 1859.

(S2) Mcbride, L. J.; Caruthers, M. H. *Tetrahedron Letters* **1983**, *24*, 245.

Table S1. Modified Primer-Template Sequences

Primer-Template	Incorporation Event	ΔG° kcal/mol	ΔΔG° kcal/mol
Primer C long/DNA_a			
CAGTCCAGCGGTGCAGTCTGCTCACAC	T → A	-4.47±0.06	
GTCAGGTCGCCACGTCAGACGAGTGTG**A**TTCTTATCATCT	C → A	-0.10±0.11	4.37±0.13
Primer C/$DNA_{abasic1}$	C → _	+0.60±0.13	N/A
TCCATATCACAC	T → _	-0.38±0.07	N/A
AGGTATAGTGTG_ATCTTATCATCT	A → _	-2.36±0.09	N/A
	G → _	-1.89±0.11	N/A
Primer C/$DNA_{abasic4}$	C → _	+0.87±0.08	N/A
TCCATATCACAC	T → _	-0.15±0.11	N/A
AGGTATAGTGTG____TTATCATCT	A → _	-2.60±0.05	N/A
	G → _	-2.16±0.06	N/A

Average results of two independent experiments are displayed with the estimated error (±standard deviation). Within each experiment, ΔG° was determined at three different dNTP concentrations in quadruplicate. The underlined base is the templating position. $Vent_R$ (exo-) DNA Polymerase was used as the catalyst.

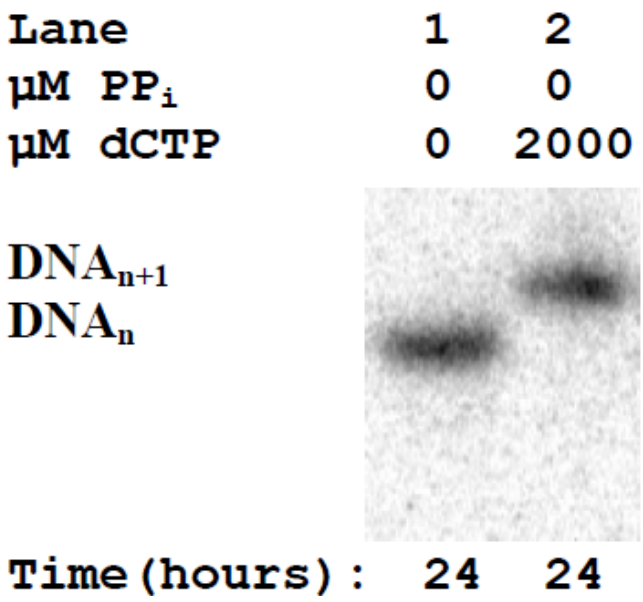

Figure S1. Complete extension of DNA_n to DNA_{n+1} during misincorporation of dCTP into Primer C/DNAt. Reactions contain polymerase, DNA_n, and dNTP where noted. Reactions contained no pyrophosphate.