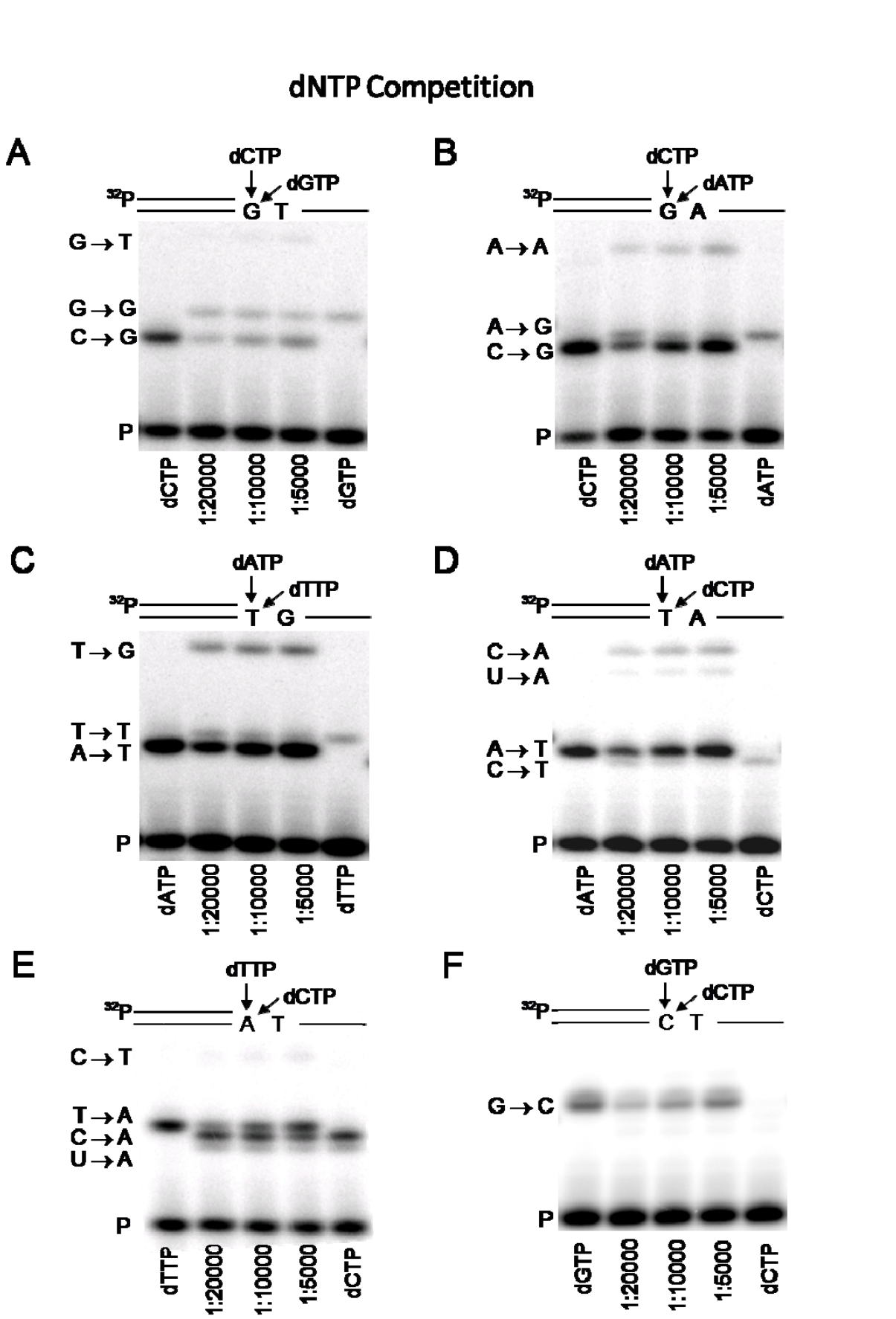

Supporting Information, Figure 1S

Supporting Information, Figure 1S: Additional data obtained by direct competition measurement of DNA polymerase fidelity. Radiolabeled primer and extended primer bands are shown separated by denaturing gel electrophoresis for a wrong dNTP competing with the right dNTP for insertion at the same target site on primer-template DNA, as illustrated in the sketches above each gel. In each case (A-F), the concentration of primer-template is 50 nM and KF^- is 20 nM. The outer lanes show the bands obtained with right dNTP alone (lane 1) and wrong dNTP alone (lane 5). Inner lanes 2, 3, and 4 show the bands obtained when right and wrong dNTPs compete at ratios of [dRTP]:[dWTP] = 1:20000, 1:10000, and 1:5000. The band corresponding to "P" is unextended primer. As described in Figure 1, each primer extension band is labeled with the corresponding nucleotide incorporated opposite the template base, e.g., right C→G or wrong G→G. Direct competition misinsertion frequencies are shown in Table 1. (A) dCTP vs. dGTP, competing for insertion opposite template G. (B) dCTP vs. dATP, competing for insertion opposite template G. (C) dATP vs. dTTP, competing for insertion opposite template T. (D) dATP vs. dCTP, competing for insertion opposite template T. Here, an additional faint band appears, denoted as U→A, likely arising from spontaneous deamination of dCTP to dUTP resulting in some U incorporation opposite template A. The C→A band results from a wrong incorporation opposite the next template base, after a right A→T incorporation. Both U→A and C→A are added to band A→T to measure total amount of DNA extended by correct incorporation of A opposite T. (E) dTTP vs. dCTP competing for insertion opposite template A. The band labeled U→A is correct incorporation of dUTP opposite A and was not included when determining the misinsertion efficiency of A/C. (F) dGTP vs. dCTP competing for insertion opposite template C. Since no band was detected for dCTP misincorporation (lanes 2-5), misinsertion efficiency for C/C was not determined.

Supporting Table 1S: Steady State and Presteady State Kinetic Parameters[a]

Template(N)[b]/dNTP	k_{cat} (s^{-1})	K_m (μM)	k_{pol} (s^{-1})	K_d (μM)
G(T1)/C	0.18 ± 0.02	0.013 ± 0.001	65 ± 2	2.7 ± 0.6
G(T1)/G	0.033 ± 0.007	40 ± 2	0.22 ± 0.01	130 ± 6
G(T1)/T	0.024 ± 0.005	73 ± 7	0.17 ± 0.02	200 ± 69
G(T2)/C	0.56 ± 0.10	0.015 ± 0.004	24 ± 0.1	1.5 ± 0.2
G(T2)/A	0.0075 ± 0.0016	23 ± 3	0.026 ± 0.001	140 ± 5
C(T7)/G	0.16 ± 0.02	0.019 ± 0.001	37 ± 3	1.2 ± 0.1
C(T7)/C	nd[c]	nd[c]	0.0010 ± 0.0001	160 ± 54
C(T7)/A	0.0020 ± 0.0001	12 ± 2	0.030 ± 0.001	100 ± 6
C(T8)/G	0.27 ± 0.04	0.039 ± 0.01	47 ± 2	1.4 ± 0.2
C(T8)/T	0.019 ± 0.005	50 ± 4	nd[c]	nd[c]
T(T5)/A	0.18 ± 0.05	0.040 ± 0.004	96 ± 5	4.0 ± 0.5
T(T5)/C	0.0093 × 0.0017	390 ± 350	0.035 ± 0.004	490 ± 77
T(T5)/G	0.043 ± 0.009	79 ± 13	0.89 ± 0.04	280 ± 49
T(T6)/A	0.15 ± 0.03	0.0094 ± 0.0012	50 ± 1	3.4 ± 0.3
T(T6)/T	0.0019 ± 0.0002	62 ± 11	0.0094 ± 0.0004	66 ± 14
A(T3)/T	0.12 ± 0.01	0.0075 ± 0.0015	89 ± 4	4.9 ± 1.5
A(T3)/C	0.081 ± 0.003	150 ± 21	1.9 ± 0.03	1300 ± 340
A(T3)/G	0.0020 ±0.0004	21 ± 5	0.019 ± 0.002	100 ± 25
A(T4)/T	0.087 ± 0.001	0.013 ± 0.001	85 ± 3	6.4 ± 0.6
A(T4)/A	0.022 ± 0.002	22 ± 1	0.38 ± 0.02	150 ± 18

[a]Values are reported as the mean ± standard error of three replicates. [b]N denotes the template used, see MATERIALS AND METHODS. [c]nd-not detectable.