Supplementary Data

Kinetic Snapshots of Human DNA Polymerases λ and β during Gap-Filling DNA Synthesis

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Supplementary Table 1. Kinetic parameters for nucleotide incorporation into gapped or recessed DNA catalyzed by dPol λ at 37 °C.

dNTP	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)	Efficiency ratio[a]	Fidelity[b]
21-19/41mer (1-nucleotide gap)					
dGTP	3.1 ± 0.1	1.7 ± 0.2	1.8	-	
dCTP	0.00135 ± 0.00007	1.9 ± 0.4	7.1×10^{-4}	-	3.9×10^{-4}
dATP	0.00066 ± 0.00007	1.8 ± 0.7	3.7×10^{-4}	-	2.0×10^{-4}
dTTP	0.00130 ± 0.00009	7 ± 1	1.9×10^{-4}	-	1.0×10^{-4}
21-19/42mer (2-nucleotide gap)					
dGTP	2.80 ± 0.05	1.24 ± 0.09	2.3	1	
dCTP	0.0208 ± 0.0005	0.85 ± 0.09	2.4×10^{-2}	34 ↑	1.1×10^{-2}
dATP	0.00031 ± 0.00002	3.0 ± 0.6	1.0×10^{-4}	4 ↓	4.6×10^{-5}
dTTP	0.00070 ± 0.00004	4.6 ± 0.7	1.5×10^{-4}	1	6.7×10^{-5}
21-19/45mer (5-nucleotide gap)					
dGTP	3.83 ± 0.06	1.57 ± 0.09	2.4	1	
dCTP	0.0060 ± 0.0003	1.5 ± 0.2	4.0×10^{-3}	6 ↑	1.6×10^{-3}
dATP	0.00042 ± 0.00003	3.3 ± 0.6	1.3×10^{-4}	3 ↓	5.2×10^{-5}
dTTP	0.00154 ± 0.00009	7 ± 1	2.2×10^{-4}	1	9.0×10^{-5}
21-19/47mer (7-nucleotide gap)					
dGTP	2.62 ± 0.05	1.28 ± 0.09	2.0	1	
dCTP	0.0072 ± 0.0002	0.77 ± 0.07	9.4×10^{-3}	13 ↑	4.5×10^{-3}
dATP	0.000102 ± 0.000008	2.1 ± 0.5	4.9×10^{-5}	8 ↓	2.4×10^{-5}
dTTP	0.0006 ± 0.0001	6 ± 3	1.0×10^{-4}	2 ↓	4.9×10^{-5}
21-19/50mer (10-nucleotide gap)					
dGTP	0.27 ± 0.01	2.4 ± 0.3	1.1×10^{-1}	16 ↓	
dCTP	0.00019 ± 0.00001	5 ± 1	3.8×10^{-5}	19 ↓	3.4×10^{-4}
dATP	No incorporation				
dTTP	No incorporation				
21/41mer (no gap)					
dGTP	0.109 ± 0.007	1.7 ± 0.3	6.4×10^{-2}	28 ↓	
dCTP	0.00030 ± 0.00002	4.2 ± 0.7	7.1×10^{-5}	10 ↓	1.1×10^{-3}
dATP	No incorporation				
dTTP	No incorporation				

[a]An upward-pointing arrow (↑) indicates the ratio was calculated as $(k_p/K_d)_{\geq 2\text{-nucleotide gap}}/(k_p/K_d)_{1\text{-nucleotide gap}}$; a downward-pointing arrow (↓) indicates the calculation used a reciprocal of the equation as follows: $(k_p/K_d)_{1\text{-nucleotide gap}}/(k_p/K_d)_{\geq 2\text{-nucleotide gap}}$.
[b]Calculated as $(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.

Supplementary Table 2. Kinetic parameters for nucleotide incorporation into gapped or recessed DNA catalyzed by tPol λ at 37 °C.

dNTP	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)	Efficiency ratio[a]	Fidelity[b]
21-19/41mer (1-nucleotide gap)[c]					
dGTP	4.1 ± 0.2	1.9 ± 0.4	2.2	-	
dCTP	0.0098 ± 0.0002	1.5 ± 0.2	6.5×10^{-3}	-	3.0×10^{-3}
dATP	0.0046 ± 0.0001	1.4 ± 0.3	3.3×10^{-3}	-	1.5×10^{-3}
dTTP	0.0065 ± 0.0001	4.7 ± 0.5	1.4×10^{-3}	-	6.4×10^{-4}
21-19/42mer (2-nucleotide gap)					
dGTP	3.7 ± 0.2	2.3 ± 0.3	1.6	1	
dCTP	0.081 ± 0.001	1.12 ± 0.07	7.2×10^{-2}	11 ↑	4.3×10^{-2}
dATP	0.0019 ± 0.0002	2.4 ± 0.6	7.9×10^{-4}	4 ↓	4.9×10^{-4}
dTTP	0.0030 ± 0.0009	6 ± 3	5.0×10^{-4}	3 ↓	3.1×10^{-4}
21-19/45mer (5-nucleotide gap)					
dGTP	5.1 ± 0.2	3.3 ± 0.4	1.5	1	
dCTP	0.0123 ± 0.0003	1.4 ± 0.1	8.8×10^{-3}	1	5.7×10^{-3}
dATP	0.0011 ± 0.0002	2 ± 1	5.5×10^{-4}	6 ↓	3.6×10^{-4}
dTTP	0.006 ± 0.002	9 ± 4	6.7×10^{-4}	2 ↓	4.3×10^{-4}
21-19/47mer (7-nucleotide gap)					
dGTP	3.78 ± 0.08	2.5 ± 0.2	1.5	1	
dCTP	0.028 ± 0.002	3.5 ± 0.8	8.0×10^{-3}	1	5.3×10^{-3}
dATP	0.00035 ± 0.00002	1.6 ± 0.4	2.2×10^{-4}	15 ↓	1.4×10^{-4}
dTTP	0.0027 ± 0.0002	11 ± 2	2.5×10^{-4}	6 ↓	1.6×10^{-4}
21-19/50mer (10-nucleotide gap)					
dGTP	1.43 ± 0.05	5.1 ± 0.4	2.8×10^{-1}	8 ↓	
dCTP	0.00067 ± 0.00006	5 ± 1	1.3×10^{-4}	49 ↓	4.8×10^{-4}
dATP	0.000350 ± 0.000009	3.6 ± 0.3	9.7×10^{-5}	34 ↓	3.5×10^{-4}
dTTP	No incorporation				
21/41mer (no gap)					
dGTP	0.68 ± 0.02	2.0 ± 0.2	3.4×10^{-1}	6 ↓	
dCTP	0.0007 ± 0.0001	5 ± 2	1.4×10^{-4}	47 ↓	4.1×10^{-4}
dATP	No incorporation				
dTTP	No incorporation				

[a]An upward-pointing arrow (↑) indicates the ratio was calculated as $(k_p/K_d)_{\geq 2\text{-nucleotide gap}}/(k_p/K_d)_{1\text{-nucleotide gap}}$; a downward-pointing arrow (↓) indicates the calculation used a reciprocal of the equation as follows: $(k_p/K_d)_{1\text{-nucleotide gap}}/(k_p/K_d)_{\geq 2\text{-nucleotide gap}}$.
[b]Calculated as $(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.
[c]Kinetic parameters are from reference [1].

Supplementary Table 3. Kinetic parameters for nucleotide incorporation into gapped DNA catalyzed by Pol λ at 37 °C.

dNTP	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)	Efficiency ratio[a]	Fidelity[b]
21-19/41mer (1-nucleotide gap)					
dGTP	2.7 ± 0.1	1.9 ± 0.2	1.4	-	
dCTP	0.00145 ± 0.00005	1.0 ± 0.1	1.5×10^{-3}	-	1.0×10^{-3}
dATP	0.00047 ± 0.00002	0.9 ± 0.1	5.2×10^{-4}	-	3.7×10^{-4}
dTTP	0.00135 ± 0.00009	2.9 ± 0.6	4.7×10^{-4}	-	3.3×10^{-4}
21-19/42mer (2-nucleotide gap)					
dGTP	1.77 ± 0.02	1.51 ± 0.06	1.2	1	
dCTP	0.0161 ± 0.0004	0.69 ± 0.08	2.3×10^{-2}	16 ↑	2.0×10^{-2}
dATP	0.00037 ± 0.00002	1.2 ± 0.2	3.1×10^{-4}	2 ↑	2.6×10^{-4}
dTTP	0.00070 ± 0.00003	3.8 ± 0.5	1.8×10^{-4}	3 ↑	1.6×10^{-4}
21-19T/42merCGA (2-nucleotide gap)					
dGTP	2.4 ± 0.1	2.4 ± 0.4	1	1	
dCTP	0.0305 ± 0.0007	0.52 ± 0.06	5.9×10^{-2}	40 ↑	5.5×10^{-2}
dATP	0.00069 ± 0.00003	0.6 ± 0.1	1.2×10^{-3}	2 ↑	1.1×10^{-3}
dTTP	0.00075 ± 0.00004	3.3 ± 0.7	2.3×10^{-4}	2 ↓	2.3×10^{-4}
21-19/42merCAG (2-nucleotide gap)					
dGTP	2.9 ± 0.1	1.7 ± 0.3	1.7	1	
dCTP	0.00076 ± 0.00007	1.7 ± 0.5	4.5×10^{-4}	3 ↓	2.6×10^{-4}
dATP	0.00049 ± 0.00002	0.9 ± 0.2	5.4×10^{-4}	1	3.2×10^{-4}
dTTP	0.0025 ± 0.0002	4.4 ± 0.8	5.7×10^{-4}	1	3.3×10^{-4}
21-19/47mer (7-nucleotide gap)					
dGTP	1.86 ± 0.04	1.2 ± 0.1	1.6	1	
dCTP	0.049 ± 0.001	0.9 ± 0.1	5.4×10^{-2}	38 ↑	3.4×10^{-2}
dATP	0.00066 ± 0.00001	0.24 ± 0.02	2.8×10^{-3}	5 ↑	1.8×10^{-3}
dTTP	0.0051 ± 0.0002	1.1 ± 0.2	4.6×10^{-3}	10 ↑	3.0×10^{-3}
21-19/47merCAT (7-nucleotide gap)					
dGTP	3.3 ± 0.2	3.1 ± 0.6	1.1	1	
dCTP	0.0087 ± 0.0001	0.58 ± 0.03	1.5×10^{-2}	10 ↑	1.4×10^{-2}
dATP	0.0045 ± 0.0001	1.2 ± 0.1	3.8×10^{-3}	7 ↑	3.5×10^{-3}
dTTP	0.146 ± 0.002	2.5 ± 0.1	5.8×10^{-2}	125 ↑	5.2×10^{-2}

[a]An upward-pointing arrow (↑) indicates the ratio was calculated as $(k_p/K_d)_{\geq 2\text{-nucleotide gap}}/(k_p/K_d)_{1\text{-nucleotide gap}}$; a downward-pointing arrow (↓) indicates the calculation used a reciprocal of the equation as follows: $(k_p/K_d)_{1\text{-nucleotide gap}}/(k_p/K_d)_{\geq 2\text{-nucleotide gap}}$.
[b]Calculated as $(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.

Supplementary Table 4. Kinetic parameters for nucleotide incorporation into gapped DNA catalyzed by dPol λ at 37 °C.

dNTP	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)	Efficiency ratio[a]	Fidelity[b]
21-19/41mer (1-nucleotide gap)					
dGTP	3.1 ± 0.1	1.7 ± 0.2	1.8	-	
dCTP	0.00135 ± 0.00007	1.9 ± 0.4	7.1×10^{-4}	-	3.9×10^{-4}
dATP	0.00066 ± 0.00007	1.8 ± 0.7	3.7×10^{-4}	-	2.0×10^{-4}
dTTP	0.00130 ± 0.00009	7 ± 1	1.9×10^{-4}	-	1.0×10^{-4}
21-19/42mer (2-nucleotide gap)					
dGTP	2.80 ± 0.05	1.24 ± 0.09	2.3	1	
dCTP	0.0208 ± 0.0005	0.85 ± 0.09	2.4×10^{-2}	34 ↑	1.1×10^{-2}
dATP	0.00031 ± 0.00002	3.0 ± 0.6	1.0×10^{-4}	4 ↓	4.6×10^{-5}
dTTP	0.00070 ± 0.00004	4.6 ± 0.7	1.5×10^{-4}	1	6.7×10^{-5}
21-19T/42merCGA (2-nucleotide gap)					
dGTP	2.57 ± 0.08	0.9 ± 0.1	2.9	1	
dCTP	0.0200 ± 0.0006	0.80 ± 0.07	2.5×10^{-2}	35 ↑	8.7×10^{-3}
dATP	0.00027 ± 0.00002	1.3 ± 0.4	2.1×10^{-4}	2 ↓	7.3×10^{-5}
dTTP	0.00024 ± 0.00002	3.3 ± 0.8	7.3×10^{-5}	3 ↓	2.5×10^{-5}
21-19/42merCAG (2-nucleotide gap)					
dGTP	3.9 ± 0.2	1.6 ± 0.3	2.4	1	
dCTP	0.00116 ± 0.00008	2.3 ± 0.5	5.0×10^{-4}	1	2.1×10^{-4}
dATP	0.00030 ± 0.00003	2.5 ± 0.8	1.2×10^{-4}	3 ↓	4.9×10^{-5}
dTTP	0.0027 ± 0.0002	4.0 ± 0.9	6.8×10^{-4}	4 ↑	2.8×10^{-4}

[a]An upward-pointing arrow (↑) indicates the ratio was calculated as $(k_p/K_d)_{\geq 2\text{-nucleotide gap}}/(k_p/K_d)_{1\text{-nucleotide gap}}$; a downward-pointing arrow (↓) indicates the calculation used a reciprocal of the equation as follows: $(k_p/K_d)_{1\text{-nucleotide gap}}/(k_p/K_d)_{\geq 2\text{-nucleotide gap}}$.
[b]Calculated as $(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.

Supplementary Table 5. Kinetic parameters for nucleotide incorporation into gapped DNA catalyzed by tPol λ at 37 °C.

dNTP	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)	Efficiency ratio[a]	Fidelity[b]
21-19/41mer (1-nucleotide gap)[c]					
dGTP	4.1 ± 0.2	1.9 ± 0.4	2.2	-	
dCTP	0.0098 ± 0.0002	1.5 ± 0.2	6.5×10^{-3}	-	3.0×10^{-3}
dATP	0.0046 ± 0.0001	1.4 ± 0.3	3.3×10^{-3}	-	1.5×10^{-3}
dTTP	0.0065 ± 0.0001	4.7 ± 0.5	1.4×10^{-3}	-	6.4×10^{-4}
21-19T/42mer (2-nucleotide gap)					
dGTP	3.7 ± 0.2	2.3 ± 0.3	1.6	1	
dCTP	0.081 ± 0.001	1.12 ± 0.07	7.2×10^{-2}	11 ↑	4.3×10^{-2}
dATP	0.0019 ± 0.0002	2.4 ± 0.6	7.9×10^{-4}	4 ↓	4.9×10^{-4}
dTTP	0.0030 ± 0.0009	6 ± 3	5.0×10^{-4}	3 ↓	3.1×10^{-4}
21-19/42merCGA (2-nucleotide gap)					
dGTP	4.8 ± 0.1	2.1 ± 0.2	2.3	1	
dCTP	0.141 ± 0.003	0.78 ± 0.06	1.8×10^{-1}	28 ↑	7.3×10^{-2}
dATP	0.0014 ± 0.0001	1.3 ± 0.3	1.1×10^{-3}	3 ↓	4.7×10^{-4}
dTTP	0.0028 ± 0.0002	5 ± 2	5.6×10^{-4}	2 ↓	2.4×10^{-4}
21-19/42merCAG (2-nucleotide gap)					
dGTP	4.6 ± 0.2	2.6 ± 0.4	1.8	1	
dCTP	0.0064 ± 0.0003	1.3 ± 0.2	4.9×10^{-3}	1	2.8×10^{-3}
dATP	0.002 ± 0.0001	1.3 ± 0.3	1.5×10^{-3}	2 ↓	8.7×10^{-4}
dTTP	0.016 ± 0.001	4.3 ± 0.9	3.7×10^{-3}	3 ↑	2.1×10^{-3}

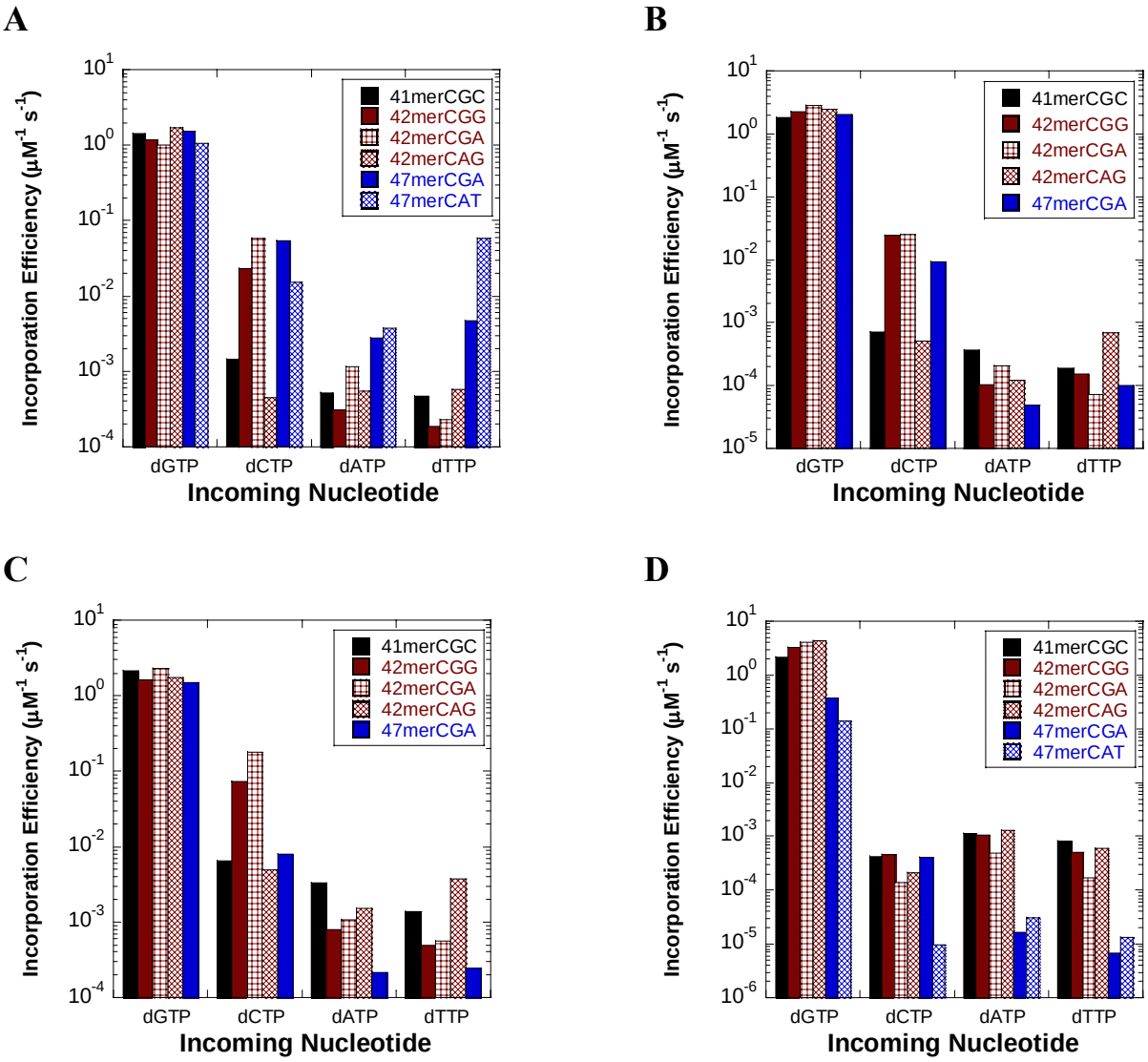
[a]An upward-pointing arrow (↑) indicates the ratio was calculated as $(k_p/K_d)_{\geq 2\text{-nucleotide gap}}/(k_p/K_d)_{1\text{-nucleotide gap}}$; a downward-pointing arrow (↓) indicates the calculation used a reciprocal of the equation as follows: $(k_p/K_d)_{1\text{-nucleotide gap}}/(k_p/K_d)_{\geq 2\text{-nucleotide gap}}$.
[b]Calculated as $(k_p/K_d)_{incorrect}/[(k_p/K_d)_{correct} + (k_p/K_d)_{incorrect}]$.
[c]Kinetic parameters are from Reference [1].

Supplementary Table 6. Kinetic parameters for nucleotide incorporation into gapped DNA catalyzed by Pol β at 37 °C.

dNTP	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)	Efficiency ratio[a]	Fidelity[b]
21-19/41mer (1-nucleotide gap)					
dGTP	18.8 ± 0.4	8.7 ± 0.4	2.2	-	
dCTP	0.059 ± 0.002	140 ± 20	4.2×10^{-4}	-	1.9×10^{-4}
dATP	0.32 ± 0.02	280 ± 60	1.1×10^{-3}	-	5.3×10^{-4}
dTTP	0.27 ± 0.01	330 ± 40	8.2×10^{-4}	-	3.7×10^{-4}
21-19/42mer (2-nucleotide gap)					
dGTP	39 ± 1	12 ± 2	3.3	1	
dCTP	0.0153 ± 0.0002	34 ± 3	4.5×10^{-4}	1	1.4×10^{-4}
dATP	0.212 ± 0.009	200 ± 20	1.1×10^{-3}	1	3.3×10^{-4}
dTTP	0.173 ± 0.009	340 ± 50	5.1×10^{-4}	2 ↓	1.6×10^{-4}
21-19T/42merCGA (2-nucleotide gap)					
dGTP	41 ± 4	10 ± 3	4.1	2 ↑	
dCTP	0.041 ± 0.006	300 ± 100	1.4×10^{-4}	3 ↓	3.3×10^{-5}
dATP	0.094 ± 0.005	190 ± 30	4.9×10^{-4}	2 ↓	1.2×10^{-4}
dTTP	0.26 ± 0.07	1500 ± 600	1.7×10^{-4}	5 ↓	4.2×10^{-5}
21-19/42merCAG (2-nucleotide gap)					
dGTP	44 ± 1	10 ± 1	4.4	2 ↑	
dCTP	0.072 ± 0.004	340 ± 60	2.1×10^{-4}	2 ↓	4.8×10^{-5}
dATP	0.180 ± 0.008	140 ± 20	1.3×10^{-3}	1	2.9×10^{-4}
dTTP	0.0103 ± 0.0003	17 ± 2	6.1×10^{-4}	1	1.4×10^{-4}
21-19/47mer (7-nucleotide gap)					
dGTP	37 ± 5	100 ± 30	3.7×10^{-1}	6 ↓	
dCTP	0.203 ± 0.006	500 ± 40	4.1×10^{-4}	1	1.1×10^{-3}
dATP	0.013 ± 0.002	800 ± 300	1.6×10^{-5}	70 ↓	4.4×10^{-5}
dTTP	0.0096 ± 0.0005	1400 ± 100	6.9×10^{-6}	120 ↓	1.9×10^{-5}
21-19/47merCAT (7-nucleotide gap)					
dGTP	32 ± 4	230 ± 60	1.4×10^{-1}	16 ↓	
dCTP	0.0116 ± 0.0009	1200 ± 200	9.7×10^{-6}	44 ↓	6.9×10^{-5}
dATP	0.04 ± 0.01	1300 ± 700	3.1×10^{-5}	37 ↓	2.2×10^{-4}
dTTP	0.0113 ± 0.0003	850 ± 40	1.3×10^{-5}	62 ↓	9.6×10^{-5}

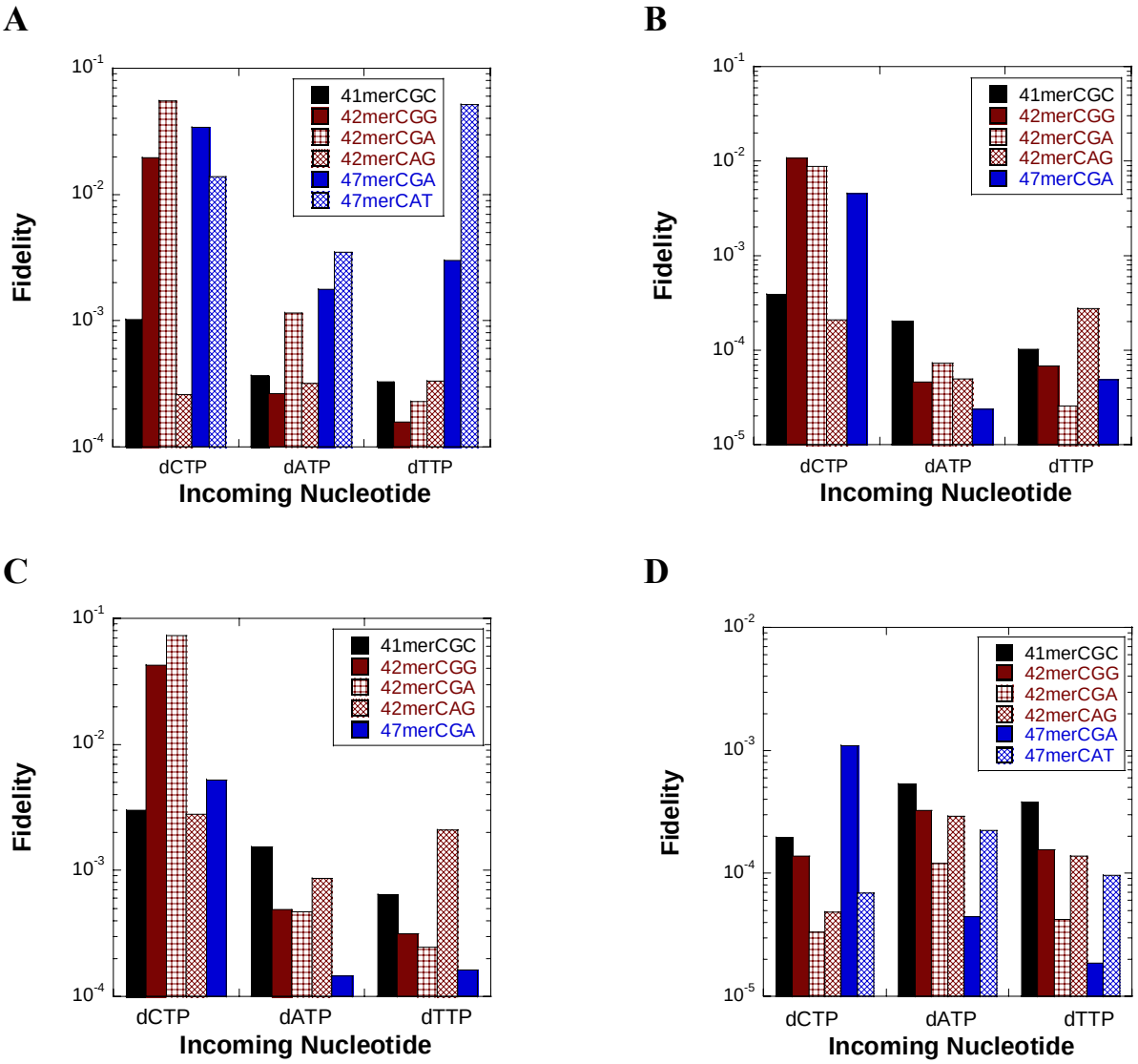
[a]An upward-pointing arrow (↑) indicates the ratio was calculated as $(k_p/K_d)_{\geq 2\text{-nucleotide gap}}/(k_p/K_d)_{1\text{-nucleotide gap}}$; a downward-pointing arrow (↓) indicates the calculation used a reciprocal of the equation as follows: $(k_p/K_d)_{1\text{-nucleotide gap}}/(k_p/K_d)_{\geq 2\text{-nucleotide gap}}$.
[b]Calculated as $(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.

Supplementary Figure 1



Supplementary Figure 1. Effect of DNA sequence on polymerization efficiency. Incorporation efficiency is plotted for each of the incoming nucleotides for (A) Pol λ, (B) dPol λ, (C) tPol λ, and (D) Pol β. Nucleotide incorporation into the different DNA substrates is represented in the legend as follows: solid black bars for 21-19/41merCGC, solid scarlet bars for 21-19/42merCGG, scarlet grid bars for 21-19T/42merCGA, scarlet criss-cross bars for 21-19/42merCAG, solid blue bars for 21-19/47merCGA, and blue criss-cross bars for 21-19/47merCAT.

Supplementary Figure 2



Supplementary Figure 2. Effect of DNA sequence on polymerization fidelity. The base substitution fidelity is plotted for each of the incoming nucleotides for (A) Pol λ, (B) dPol λ, (C) tPol λ, and (D) Pol β. The fidelity was calculated as $(k_p/K_d)_{incorrect}/[(k_p/K_d)_{correct} + (k_p/K_d)_{incorrect}]$. Nucleotide incorporation into the different DNA substrates is represented in the legend as follows: solid black bars for 21-19/41merCGC, solid scarlet bars for 21-19/42merCGG, scarlet grid bars for 21-19T/42merCGA, scarlet criss-cross bars for 21-19/42merCAG, solid blue bars for 21-19/47merCGA, and blue criss-cross bars for 21-19/47merCAT.

REFERENCE

1. Fiala, K. A., Abdel-Gawad, W. & Suo, Z. (2004) Pre-steady-state kinetic studies of the fidelity and mechanism of polymerization catalyzed by truncated human DNA polymerase lambda, *Biochemistry. 43*, 6751-62.