SUPPLEMENTARY INFORMATION

Toward a Designed Genetic System with Biochemical Function: Polymerase Synthesis of Single and Multiple Size-expanded DNA Base Pairs

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Table S1. Steady-state kinetics for insertion of single nucleotides opposite size-expanded bases by Dpo4 polymerase. [a]

Template Base	dNTP	k_{cat} (min^{-1})	K_m (μM)	$10^6 \times k_{cat}/K_m$ ($min^{-1} \cdot \mu M^{-1}$)	$f_{ins\ (rel)}$
xA	A	0.023±0.004	417±5	5.5×10^{1}	6.0×10^{-2}
xA	C	ND[b]	ND	$<10^{1}$	$<1\times10^{-2}$
xA	G	0.012±0.001	519±29	2.3×10^{1}	2.5×10^{-2}
xA	T	0.49±0.15	514±17	9.2×10^{2}	1
xC	A	0.037±0.003	250±21	1.5×10^{2}	2.0×10^{-1}
xC	C	0.056±0.007	148±31	4.1×10^{2}	5.4×10^{-1}
xC	G	0.44±0.09	574±38	7.6×10^{2}	1
xC	T	0.23±0.02	432±81	5.6×10^{2}	7.4×10^{-1}
xG	A	0.027±0.008	487±139	5.6×10^{1}	2.1×10^{-2}
xG	C	0.25±0.04	92±16	2.7×10^{3}	1
xG	G	0.026±0.007	307±75	8.3×10^{1}	3.1×10^{-2}
xG	T	0.081±0.022	642±208	1.3×10^{2}	4.8×10^{-2}
xT	A	0.53±0.13	307±96	1.8×10^{3}	1
xT	C	0.59±0.07	319±45	1.9×10^{3}	1.1×10^{0}
xT	G	0.041±0.002	791±158	5.8×10^{1}	3.2×10^{-2}
xT	T	1.2±0.2	552±155	2.4×10^{3}	1.3×10^{0}
A	T	4.4±0.3	18±3	2.6×10^{5}	-
C	G	5.5±0.5	21±1	2.6×10^{5}	-
G	C	11±3	8±1	1.3×10^{6}	-
T	A	7.9±0.7	15±2	5.4×10^{5}	-
A	A	0.15±0.02	348±81	4.5×10^{2}	-
G	T	0.28±0.12	328±162	9.8×10^{2}	-
T	G	0.77±0.03	385±30	2.0×10^{3}	-

[a]Conditions: 5 μM template-primer duplex, 40 mM Tris-HCl (pH=8.0), 5 mM $MgCl_2$, 10 mM dithiothreitol, 250 μg/mL bovine serum albumin, and various concentrations of dNTPs, incubated at 37 °C in a reaction volume of 10 μL. Data are averaged from triplicates; standard deviations are shown. V_{max} was normalized to 1 μM enzyme concentration. [b]Not determined due to very low insertion efficiency.

Table S2. Steady-state kinetics data for Kf (exo-)-catalyzed extension of a C-G pair immediately beyond the size-expanded base pairs and mismatches shown.[a]

Template Base	Primer Base	V_{max} ($\mu M \cdot min^{-1}$)	K_m (μM)	$10^6 \times V_{max}/K_m$	$f_{ext\ (rel)}$
xA	A	0.0011±0.0001	141±6	7.7×10^{0}	1.4×10^{-1}
xA	C	ND[b]	ND	<1	$< 2\times10^{-2}$
xA	G	0.00092±0.00003	131±13	7.2×10^{0}	1.3×10^{-1}
xA	T	0.011±0.002	248±87	5.5×10^{1}	1
xC	A	0.00082±0.00003	156±19	5.4×10^{0}	1.1×10^{0}
xC	C	0.011±0.001	94±16	1.2×10^{2}	2.6×10^{1}
xC	G	0.00053±0.00010	119±36	4.6×10^{0}	1
xC	T	0.059±0.030	54±22	9.4×10^{2}	2.0×10^{2}
xG	A	0.0087±0.0010	163±13	5.3×10^{1}	7.0×10^{0}
xG	C	0.0015±0.0002	201±43	7.6×10^{0}	1
xG	G	ND[b]	ND	<1	$< 2\times10^{-2}$
xG	T	0.063±0.002	198±12	3.2×10^{2}	4.2×10^{1}
xT	A	0.0027±0.0002	172±11	1.6×10^{1}	1
xT	C	0.24±0.05	182±71	1.4×10^{3}	8.8×10^{1}
xT	G	ND[b]	ND	<1	$< 2\times10^{-2}$
xT	T	0.038±0.004	85±5	4.4×10^{2}	2.8×10^{1}
A	T	0.44±0.05	0.42±0.09	1.2×10^{6}	-
A	A	0.0025±0.0003	122±13	2.1×10^{1}	-
G	T	0.084±0.030	296±114	2.8×10^{2}	-

[a]Conditions: 0.5 μM template-primer duplex, 100 mM Tris●HCl (pH=7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol and 100μg/mL serum albumin, varied concentrations of dCTP, incubated at 37 °C in a reaction volume of 10 μL. Data are averaged from triplicates; standard deviations are shown. V_{max} was normalized to 0.005 unit/μL enzyme concentration. [b]Not determined due to low extension efficiency.

Table S3. Steady-state kinetics data for Dpo4-catalyzed extension of a C-G pair immediately beyond size-expanded base pairs.[a]

Template Base	Primer Base	k_{cat} (min^{-1})	K_m (μM)	$10^6 \times k_{cat}/K_m$ ($min^{-1} \cdot \mu M^{-1}$)	$f_{ext\ (rel)}$
xA	A	ND[b]	ND	$<10^3$	ND
xA	C	ND[b]	ND	$<10^3$	ND
xA	G	ND[b]	ND	$<10^3$	ND
xA	T	3.5±0.6	15±4	2.3×10^5	1
xC	A	ND[b]	ND	$<10^3$	ND
xC	C	ND[b]	ND	$<10^3$	ND
xC	G	6.6±0.9	163±31	4.1×10^4	1
xC	T	6.3±0.1	80±6	7.9×10^4	1.9×10^0
xG	A	ND[b]	ND	$<10^3$	ND
xG	C	2.4±0.1	68±5	3.5×10^4	1.2×10^0
xG	G	ND[b]	ND	$<10^3$	ND
xG	T	ND[b]	ND	$<10^3$	ND
xT	A	2.5±0.7	89±16	2.7×10^4	1
xT	C	3.1±0.4	106±21	3.0×10^4	1.1×10^0
xT	G	ND[b]	ND	$<10^3$	ND
xT	T	10.7±1.7	84±35	1.5×10^5	5.6×10^0
A	T	18±4	4.5±0.7	4.0×10^6	-
C	G	31±4	7.2±0.1	4.2×10^6	-
G	C	23±4	14±4	1.6×10^6	-
T	A	34±3	6.2±0.6	5.5×10^6	-
G	T	2.9±0.2	140±23	2.2×10^4	-
T	G	6.0±0.5	156±14	3.9×10^4	-

[a]Conditions: 5 μM template-primer duplex, 40 mM Tris-HCl (pH=8.0), 5 mM $MgCl_2$, 10 mM dithiothreitol, 250 μg/mL bovine serum albumin (BSA), and various concentrations of dCTPs, incubated at 37 °C in a reaction volume of 10 μL. Data are averaged from triplicates; standard deviations are shown. V_{max} was normalized to 1 μM enzyme concentration. [b]Not determined due to very low extension efficiency.