

Table 4. SFR1-SFR3 kinetic data

		5' - TAATACGACTCACTATAGGGAGA				5' - TAATACGACTCACTATAGGGAGAN				
		3' - ATTATGCTGAGTGATATCCCTCTXGCTAGGTTACGGCAGGATCGC				3' - ATTATGCTGAGTGATATCCCTCTXGCTAGGTTACGGCAGGATCGC				
		rNTP or dNTP								
		Single incorporation of dNTP				Single incorporation of rNTP				
Template		$k_{cat}$ , min <sup>-1</sup>	$K_M$ , $\mu$ M	$k_{cat}/K_M$ , min <sup>-1</sup> ·M <sup>-1</sup>	Relative to wild type	$k_{cat}$ , min <sup>-1</sup>	$K_M$ , $\mu$ M	$k_{cat}/K_M$ , min <sup>-1</sup> ·M <sup>-1</sup>	Relative to wild type	dNTP/rNTP
<b>Wild type SF</b>	dT	2.09 ± 0.1	7.54 ± 3.2	2.77 × 10 <sup>5</sup>	1	0.004 ± 0.001	483 ± 175	8.3	1	33,500
	dG	4.08 ± 0.9	0.92 ± 0.5	4.42 × 10 <sup>6</sup>	1	0.26 ± 0.05	213 ± 59	1.2 × 10 <sup>3</sup>	1	3,700
	dC	2.78 ± 0.6	0.92 ± 0.1	3.03 × 10 <sup>6</sup>	1	0.39 ± 0.07	257 ± 129	1.5 × 10 <sup>3</sup>	1	2,010
	dA	2.79 ± 0.1	3.29 ± 0.7	8.5 × 10 <sup>5</sup>	1	0.004 ± 0.001	525 ± 90	8.0	1	106,000
<b>SFR1</b>	dT	9.27 ± 0.1	152 ± 22	6.1 × 10 <sup>4</sup>	0.22	20.7 ± 0.2	146 ± 5	1.4 × 10 <sup>5</sup>	17,100	0.43
	dG	10.2 ± 1.3	36.4 ± 6.3	2.8 × 10 <sup>5</sup>	0.06	21.4 ± 0.3	30.5 ± 8.3	7.0 × 10 <sup>5</sup>	586	0.40
	dC	11.0 ± 0.6	45.9 ± 3.3	2.4 × 10 <sup>5</sup>	0.08	21.6 ± 1.0	32.5 ± 3.4	6.7 × 10 <sup>5</sup>	441	0.36
	dA	12.9 ± 0.2	88.0 ± 10	1.5 × 10 <sup>5</sup>	0.17	15.1 ± 0.1	144 ± 2.5	1.0 × 10 <sup>5</sup>	13,100	1.39
<b>SFR2</b>	dT	7.63 ± 0.2	247 ± 8.0	3.1 × 10 <sup>4</sup>	0.11	15.6 ± 2.1	197 ± 5	7.9 × 10 <sup>4</sup>	9,570	0.39
	dG	10.9 ± 1.2	43.8 ± 10	2.5 × 10 <sup>5</sup>	0.06	23.7 ± 0.6	41.5 ± 4.7	5.7 × 10 <sup>5</sup>	478	0.43
	dC	9.07 ± 1.6	53.0 ± 4.0	1.7 × 10 <sup>5</sup>	0.06	22.4 ± 2.1	59.4 ± 10	3.8 × 10 <sup>5</sup>	250	0.45
	dA	9.54 ± 0.3	95.9 ± 5.0	1.0 × 10 <sup>5</sup>	0.12	14.6 ± 0.1	249 ± 1	5.9 × 10 <sup>4</sup>	7,320	1.70
<b>SFR3</b>	dT	9.30 ± 0.4	203 ± 45	4.6 × 10 <sup>4</sup>	0.17	19.3 ± 0.3	134 ± 10	1.4 × 10 <sup>5</sup>	17,400	0.32
	dG	18.7 ± 7.1	37.6 ± 14	5.0 × 10 <sup>5</sup>	0.11	31.0 ± 1.8	23.6 ± 1.3	1.3 × 10 <sup>6</sup>	1,100	0.38
	dC	21.5 ± 7.8	32.2 ± 11	6.7 × 10 <sup>5</sup>	0.22	29.1 ± 1.3	23.0 ± 0.2	1.3 × 10 <sup>6</sup>	839	0.53
	dA	14.6 ± 0.1	91.5 ± 9.4	1.6 × 10 <sup>5</sup>	0.19	21.8 ± 0.3	225 ± 0.1	9.7 × 10 <sup>4</sup>	12,100	1.65

Assay conditions were as follows: 40 nM template-primer duplex, 0.11–1.34 nM enzyme, 50 mM Tris (pH 7.5), 10 mM MgCl<sub>2</sub>, 1  $\mu$ M DTT, 50  $\mu$ g/ml BSA. The reactions were initiated by adding the DNA–enzyme mixture to an equal volume (5  $\mu$ l) of a 2 $\times$  dNTP or rNTP stock solution, incubated at room temperature for 1–10 min, and quenched by the addition of 20  $\mu$ l loading buffer (95% formamide/20 mM EDTA). A 5- $\mu$ l portion of the reaction mixture was then analyzed by 15% PAGE containing 8 M urea. Data are the average of three independent determinations.