SUPPLEMENTARY FIGURES AND TABLES

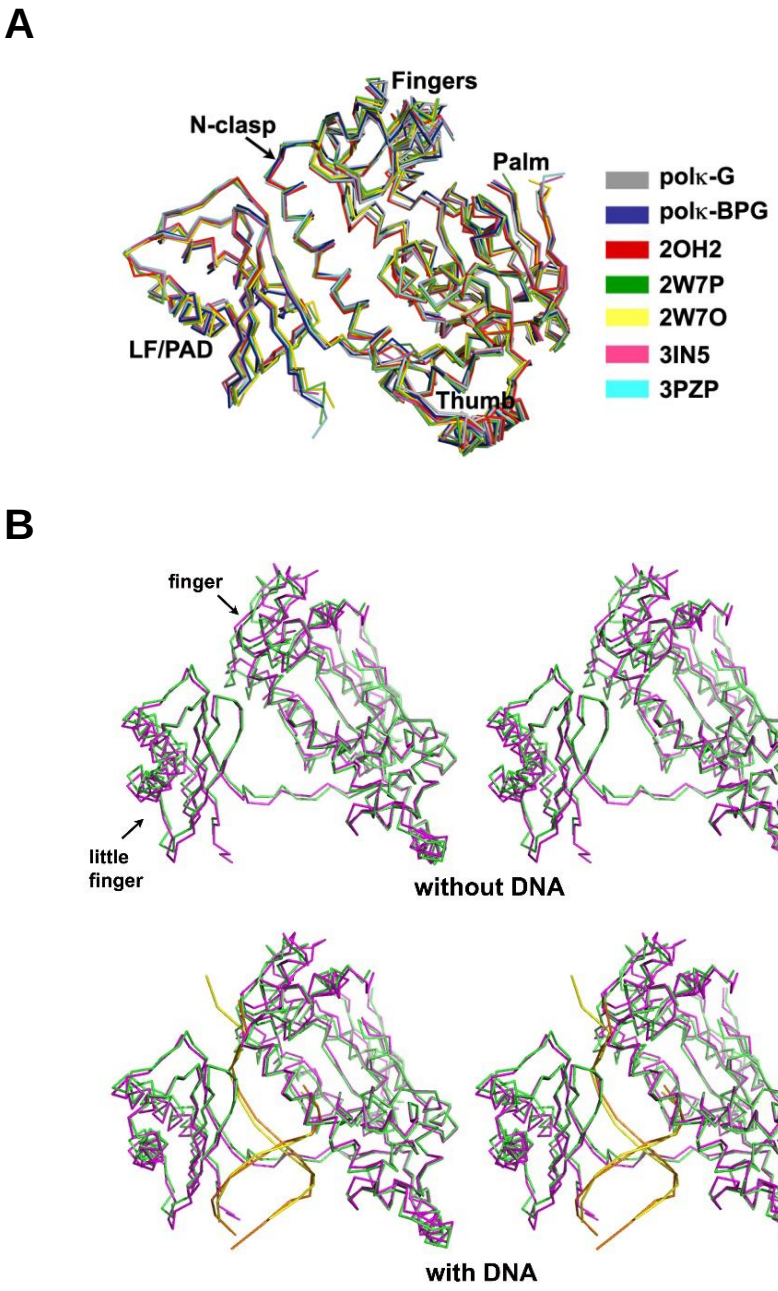

Figure S1. (**A**) The superposition of polκ structures is shown. Polκ-G and polκ-BPG are from this work. 2OH2, 2W7P, 2W7O, 3IN5 and 3PZP are the polκ structures from previously published ternary complexes of polκ with either unmodified DNA (2OH2) or DNA containing 8-oxy-G. (**B**) Stereo views are shown for polκ-G (magenta) and a previous polκ structure (2OH2, green). No significant conformational change in polκ is seen when comparing different polκ-DNA-dNTP ternary complexes. The root-mean-squared deviations (rmsd) are 0.7-0.9 Å over ~423 Cα atoms in pair-wise comparisons among the polκ structures.

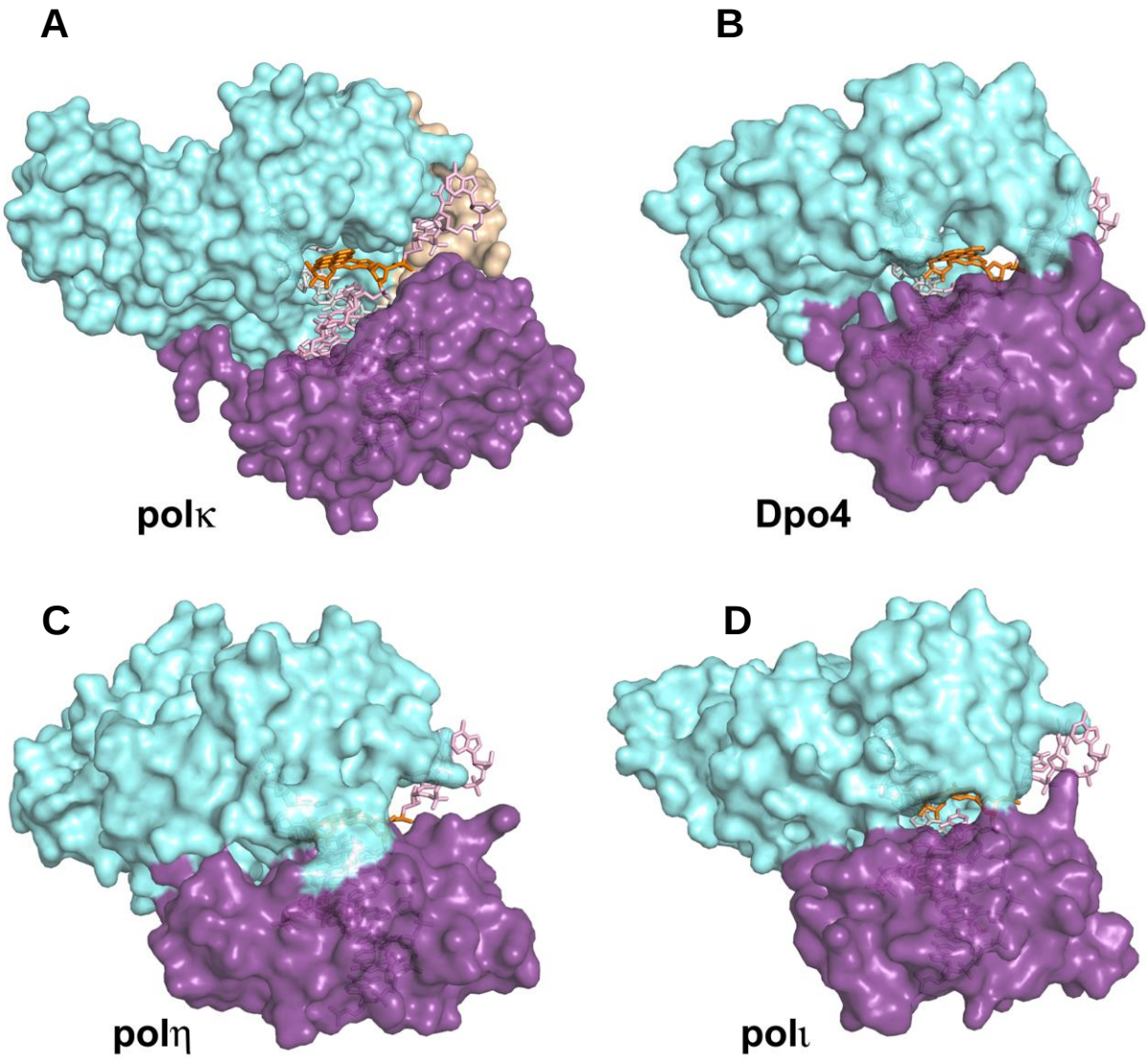

Figure S2. A comparison is shown of the minor groove sides of Y-family DNA polymerases. Core domains are in cyan, LF domains in purple, DNA in sticks, and the BP-dG adduct in orange. All four proteins are placed in the same orientation by superimposing their palm domains. Panels are the back view, with minor grooves of DNA facing toward the reader. (**A**) The polκ-BPG structure from this work. (**B-D**) The Dpo4 (1JX4), polη (3MR2) and polι (3GV8) structures are modelled with the DNA substrate from the polκ-BPG complex. Only polκ (panel A) has BP-dG snuggly packed against the core domains, while the other three Y-pols clash with the bulky lesion at the minor groove side of DNA.

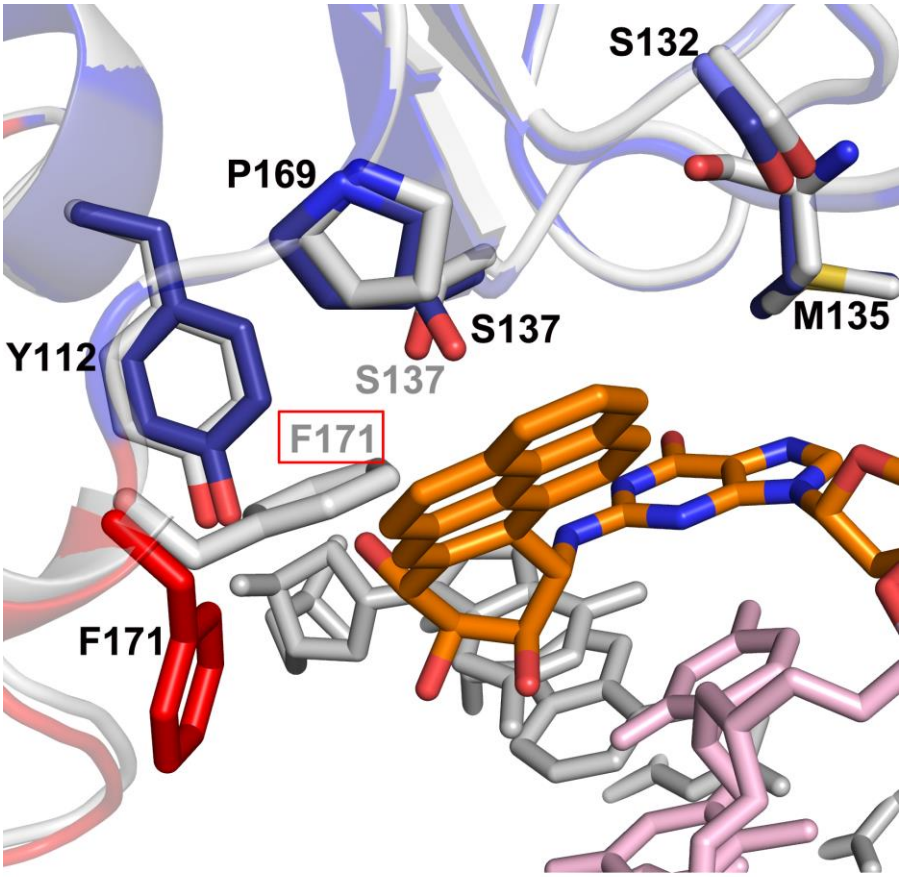

Figure S3. A superposition is shown for the residues surrounding the BPDE rings in polκ-BPG (colored) and those in polκ-G (light grey). Two residues (F171, S137) change conformation. F171 flips to the left to avoid steric conflicts with the BPDE ring (orange) in the polκ-BPG structures. S137 rotates to contact the ring in the BP-dG-modified DNA. DNA is in stick with the primer in grey, template in pink and BP-dG in orange.

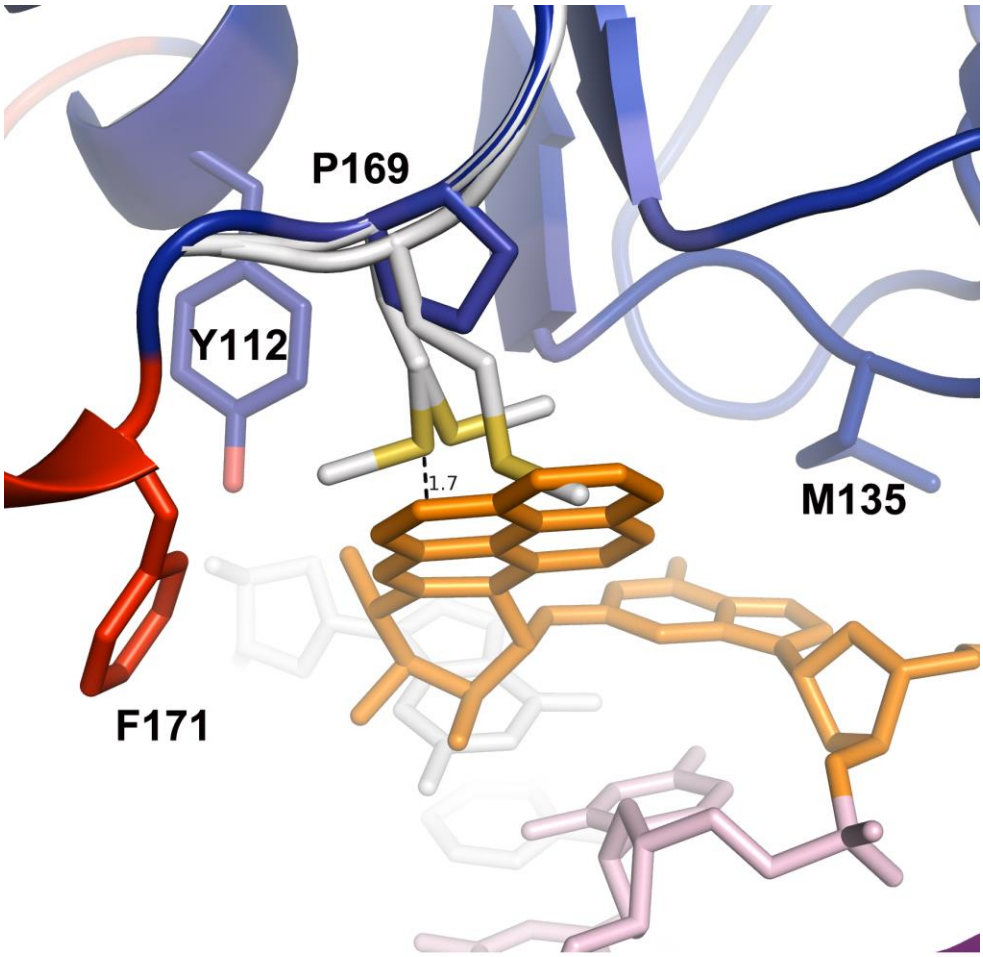

Figure S4. Methionine is modelled at the P169 position. P169 was mutated to methionine. Possible rotamers of methionine that would impose steric clashes with BP-dG are shown in light grey sticks. The side chain of methionine would be too close to the bulky ring of BP (indicated with a dashed line and a 1.7 Å distance). The template strand is in pink, primer strand in grey, and BP-dG in orange. Residues are in the colors of the domains to which they belong.

3′ CCCCCTTCCTAA**G***TACT
5′ GGGGGAAGGATTC

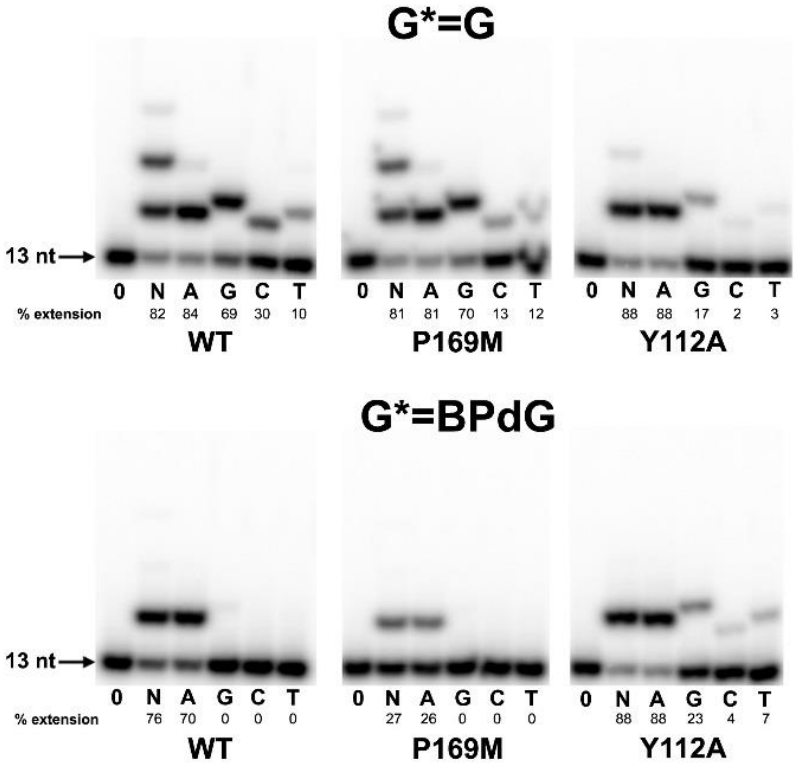

Figure S5. Replication assays are shown for extending primers after unmodified G or BP-dG using mutant polκs (extension stage). The sequence of the DNA substrate is on the top of the gel images. When comparing the ability of wild-type (WT) and mutant polκ to bypass BP-dG (lower panel), less significant difference exists in the extension stage than in the insertion stage (Figure 1B).

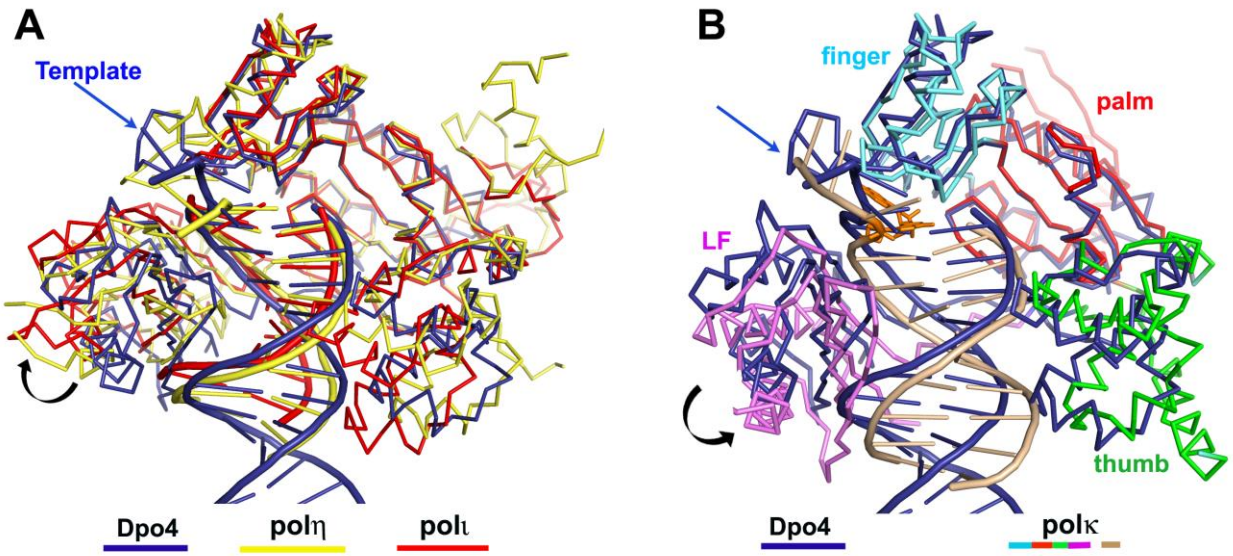

Figure S6. This comparison of Y-family polymerases shows domain and DNA movements relative to those of Dpo4. Proteins are shown in the same orientation (front views) by being superimposed on their palm domains. Green arrows point to the templates, and black curved arrows indicate movements of the LF domains of polη, polι, and polκ relative to the LF of Dpo4. (**A**) The superposition of polι, polη, and Dpo4. The LF domains of polι and polη rotate and closer to the minor groove (the back side) when compared to the LF of Dpo4 (blue). The positions of the template strands are superimposable near the active sites in polι, polη and Dpo4. (**B**) The superposition of polκ and Dpo4. The black arrow indicates the polκ's LF domain (pink) moves towards the major groove side (the front side) of the DNA, in an opposite direction relative to polι and polη, when compared to Dpo4 (blue). The position of the template (beige) in polκ also deviates from that (blue) in Dpo4. The N-clasp domain was removed for clarity.

Supplementary Table 1. Average rmsd of DNA molecules superposed over P atoms in the ss-DNA regions

Structures	Pol-G	Pol-BPG	NMR (1AXO)	2OH2
Pol-G		0.54	3.25	0.98
Pol-BPG	0.54		3.46	1.01
NMR (1AXO)	3.25	3.46		5.25
2OH2	0.98	1.01	5.25	

Supplementary Table 2. B-factors of BPDE ring atoms when refined either in 5′ and 3′ orientations for the two complexes in the asymmetric unit

Group	Atom	Molecule A BPDE refined in		Molecule B BPDE refined in	
		5′ oriented[a]	3′ oriented[b]	5′ oriented[a]	3′ oriented[b]
epoxy	C4′	43.7	67.5	45.7	68.4
	C3′	41.7	76.0	42.0	76.3
	C2′	47.3	77.6	53.8	73.8
	C1′	48.8	92.4	53.2	84.0
	O1	61.3	76.2	61.3	69.4
	O2	53.7	72.5	59.7	70.8
	O3	46.7	69.4	47.5	80.8
pyrene	C61	49.9	75.8	50.6	70.4
	C7	46.8	96.7	51.5	82.6
	C81	54.3	97.8	61.6	87.1
	C8A	60.6	106.0	62.8	97.0
	C5A	60.4	74.3	57.7	64.5
	C5B	61.6	105.0	65.2	91.8
	C51	55.1	63.0	55.7	56.4
	C41	63.1	80.3	57.7	75.6
	C3B	67.7	109.0	68.1	95.9
	C1A	69.7	111.2	69.0	97.9
	C10	60.8	106.8	60.9	98.6
	C9	58.1	100.0	57.3	90.8
	C1	67.6	107.7	64.4	97.4
	C3A	72.5	97.6	67.8	91.7
	C3	78.9	94.0	69.5	93.5
	C21	72.4	101.6	66.9	100.5

a. R_{work}/ R_{free} = 0.212/0.252
b. R_{work}/ R_{free} = 0.216/0.256

Supplementary Table 3. Local base-pair/step parameters comparison between different Polκ and NMR DNAs determined by 3DNA analyze program.

B	Buckle				Propeller			
	Polκ-BPG	**Polκ-G**	**2OH2**	**1AXO**	**Polκ-BPG**	**Polκ-G**	**2OH2**	**1AXO**
1[a]	4.38	33.89	8.84	-24.20	18.89	-7.93	-0.03	-19.32
2	29.59	20.02	-3.09	-11.55	1.21	-8.46	-3.49	3.00
3	11.19	7.74	9.57	16.82	-1.93	-3.43	0.91	-14.10
4	4.23	4.51	13.41	20.74	-1.38	-0.87	5.42	-18.57
5	-2.62	6.92	0.52	30.60	-9.31	-3.27	2.82	-30.08
6	-6.63	-1.52	-5.53	-14.49	-7.08	-2.64	1.67	-4.33
7	-8.50	-7.24	-6.66	-0.52	-13.55	-12.89	-4.39	-11.92
8	2.04	-5.72	-2.64	15.77	-6.13	-8.07	-8.66	-13.67
9	-6.13	-7.91	-5.35	18.64	-8.83	-5.84	-4.56	-6.19
10	-6.79	-5.75	-2.52	-4.30	-11.46	-11.16	6.67	16.81
Avg.	2.08	4.50	0.65	4.75	-3.96	-6.46	-0.36	-9.84
s.d.	11.58	13.57	7.24	18.16	9.28	3.91	4.85	13.05

step	Rise				Tilt				Twist			
	PolBPG	**PolG**	**2OH2**	**1AXO**	**PolBPG**	**PolG**	**2OH2**	**1AXO**	**PolBPG**	**PolG**	**2OH2**	**1AXO**
1[b]	4.62	3.22	3.20	2.92	0.16	5.40	1.13	-12.60	33.93	23.96	19.68	43.28
2	3.05	3.04	3.64	2.34	-1.45	4.05	3.80	4.14	24.12	27.04	34.61	19.84
3	3.21	3.26	3.43	3.45	-4.18	-2.15	-2.48	1.82	29.65	27.84	30.52	31.13
4	3.15	3.39	3.11	2.82	3.90	6.22	0.83	7.29	29.09	32.38	28.26	38.32
5	3.24	3.22	3.38	4.87	-2.27	-2.71	-0.59	-3.26	32.78	30.11	35.75	43.64
6	3.28	3.09	3.41	3.38	-1.47	-3.56	-0.80	2.45	35.74	34.27	31.23	30.03
7	3.65	3.44	3.45	2.87	3.48	-2.01	-2.07	-2.06	31.34	35.50	24.35	32.34
8	3.10	3.26	3.35	3.30	0.19	3.66	3.72	4.31	29.04	29.20	38.39	44.64
9	3.25	3.27	3.40	4.06	-5.06	-0.50	4.81	1.47	32.44	34.34	36.49	34.66
Avg.	3.39	3.24	3.38	3.33	-0.74	0.93	0.93	0.40	30.90	30.52	31.03	35.32
s.d.	0.49	0.12	0.15	0.75	3.06	3.85	2.67	5.84	3.41	3.89	6.13	8.08

Minor groove width (based on P-P distances)				
Polκ-BPG	**Polκ-G**	**2OH2**	**1AXO**	**B-DNA***
12.0-14.3	13.2-14.1	13.2-14.5	8.1-11.7	11.7

a base-pair corresponds to the templating base and incoming nucleotide

b base-pairs corresponds to the templating base and incoming nucleotide and -1 base pairs

*B-DNA reconstructed using 3-DNA reconstruction program using the Polκ-G DNA sequence.

All DNAs listed in the table is in B-form as determined by the 3DNA analyze program.