SUPPORTING INFORMATION

Mutagenic Potential of 8-Oxo-7,8-dihydro-2′-deoxyguanosine Bypass Catalyzed by Human Y-family DNA Polymerases

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Table S1. Primers used for the addition of unique barcodes and Illumina adapter sequences.

hPolη-Control forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATCCGTCCAACCAAC-3′
hPolη-Damaged forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCGCCGTCCAACCAAC-3′
hPolκ-Control forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTACCGTCCAACCAAC-3′
hPolκ-Damaged forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTTCCGTCCAACCAAC-3′
hPolι-Control forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACTCCGTCCAACCAAC-3′
hPolι-Damaged forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGGCCGTCCAACCAAC-3′
All 4-Control forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAACCGTCCAACCAAC-3′
All 4-Damaged forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTACATCCGTCCAACCAAC-3′
hRev1-hPolκ-Control forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCACCGTCCAACCAAC-3′
hRev1-hPolκ-Damaged forward[a]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCGCCGTCCAACCAAC-3′
HT-SOSA reverse	5′-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCTGGACGGCATTGGATC-3′
Illumina PCR 1[b]	5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′
Illumina PCR 2[b]	5′-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT-3′
Illumina sequencing[b]	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′

[a]Underlined nucleotides represent the unique barcode sequences that were used to identify each lesion bypass product origin.

Table S2. Number of sequences analyzed by HT-SOSA

Enzyme(s)	DNA substrate	Barcode	Sequences Analyzed	% of total
hPolη	13-mer/40-mer-ctl	GGAT	2,275,445	6.8
hPolη	13-mer/40-mer-8oxoG	TTCG	2,574,720	7.7
hPolκ	13-mer/40-mer-ctl	CCTA	3,525,689	10.6
hPolκ	13-mer/40-mer-8oxoG	CGTT	3,135,229	9.4
hPolι	13-mer/40-mer-ctl	AACT	4,710,203	14.1
hPolι	13-mer/40-mer-8oxoG	TAGG	3,383,766	10.2
All 4 Pols	13-mer/40-mer-ctl	GTAA	3,277,891	9.9
All 4 Pols	13-mer/40-mer-8oxoG	ACAT	3,241,632	9.7
hRev1 + hPolκ	16-mer/40-mer-ctl	ATCA	3,840,351	11.5
hRev1 + hPolκ	16-mer/40-mer-8oxoG	TGCG	3,292,062	9.9

Table S3. Error rates of human Y-family DNA polymerases calculated by HT-SOSA analysis.

Enzyme	DNA substrate	Event	Substitution Error Rate[a]	Substitution Error Ratio[b]	Insertion Error Rate[a]	Insertion Error Ratio[b]	Deletion Error Rate[a]	Deletion Error Ratio[b]
hPolη	13-mer/ 40-mer-ctl	Opposite dG[c]	2.5×10^{-2}		7.4×10^{-4}		4.6×10^{-3}	
		Upstream[d]	1.5×10^{-2}		6.6×10^{-4}		3.7×10^{-3}	
		Downstream[e]	1.8×10^{-2}		4.9×10^{-4}		3.0×10^{-3}	
		Total[f]	1.8×10^{-2}		6.0×10^{-4}		3.6×10^{-3}	
hPolη	13-mer/ 40-mer-8oxoG	Opposite 8-oxoG[c]	4.4×10^{-1}	17.6	7.1×10^{-4}	1.0	1.3×10^{-2}	2.8
		Upstream[d]	1.7×10^{-2}	1.1	1.0×10^{-3}	1.5	3.4×10^{-3}	0.9
		Downstream[e]	2.0×10^{-2}	1.1	6.3×10^{-4}	1.3	4.8×10^{-3}	1.6
		Total[f]	7.9×10^{-2}	4.4	8.2×10^{-4}	1.4	5.3×10^{-3}	1.5
hPolκ	13-mer/ 40-mer-ctl	Opposite dG[c]	4.8×10^{-2}		4.0×10^{-4}		9.3×10^{-3}	
		Upstream[d]	8.1×10^{-3}		4.5×10^{-4}		6.8×10^{-3}	
		Downstream[e]	1.1×10^{-2}		4.2×10^{-4}		8.1×10^{-3}	
		Total[f]	1.5×10^{-2}		4.3×10^{-4}		7.7×10^{-3}	
hPolκ	13-mer/ 40-mer-8oxoG	Opposite 8-oxoG[c]	8.0×10^{-1}	16.7	1.2×10^{-4}	0.3	1.8×10^{-2}	1.9
		Upstream[d]	1.4×10^{-2}	1.7	1.6×10^{-3}	3.6	5.0×10^{-3}	0.7
		Downstream[e]	1.7×10^{-2}	1.5	4.0×10^{-4}	1.0	1.4×10^{-2}	1.7
		Total[f]	1.3×10^{-1}	8.7	8.8×10^{-4}	2.0	1.1×10^{-2}	1.4
hPolι	13-mer/ 40-mer-ctl	Opposite dG[c]	3.1×10^{-2}		1.8×10^{-4}		7.0×10^{-2}	
		Upstream[d]	2.7×10^{-1}		1.1×10^{-3}		2.4×10^{-2}	
		Downstream[e]	6.4×10^{-2}		6.5×10^{-4}		1.1×10^{-2}	
		Total[f]	1.5×10^{-1}		7.9×10^{-4}		2.5×10^{-2}	
hPolι	13-mer/ 40-mer-8oxoG	Opposite 8-oxoG[c]	2.7×10^{-1}	8.7	2.9×10^{-4}	1.6	7.3×10^{-2}	1.0
		Upstream[d]	2.9×10^{-1}	1.1	3.0×10^{-3}	2.7	2.3×10^{-2}	1.0
		Downstream[e]	2.7×10^{-2}	0.4	1.7×10^{-3}	2.6	3.3×10^{-2}	3.0
		Total[f]	1.7×10^{-1}	1.1	2.0×10^{-3}	2.5	3.4×10^{-2}	1.4
All 4 Pols	13-mer/ 40-mer-ctl	Opposite dG[c]	3.4×10^{-2}		5.1×10^{-4}		6.6×10^{-3}	
		Upstream[d]	1.4×10^{-2}		4.9×10^{-4}		6.6×10^{-3}	
		Downstream[e]	1.2×10^{-2}		4.1×10^{-4}		6.2×10^{-3}	
		Total[f]	1.6×10^{-2}		4.6×10^{-4}		6.4×10^{-3}	
All 4 Pols	13-mer/ 40-mer-8oxoG	Opposite 8-oxoG[c]	5.5×10^{-1}	16.2	6.1×10^{-3}	12.0	4.3×10^{-2}	6.5
		Upstream[d]	3.4×10^{-2}	2.4	7.3×10^{-4}	1.5	1.6×10^{-2}	2.4
		Downstream[e]	1.7×10^{-2}	1.4	3.3×10^{-4}	0.8	1.8×10^{-2}	2.9
		Total[f]	1.0×10^{-1}	6.3	1.3×10^{-3}	2.8	2.1×10^{-2}	3.3
hRev1+ hPolκ	13-mer/ 40-mer-ctl	Opposite dG[c]	1.0×10^{-2}		1.2×10^{-2}		2.9×10^{-3}	
		Downstream[e]	2.7×10^{-2}		8.0×10^{-4}		4.1×10^{-2}	
		Total[f]	2.3×10^{-2}		3.7×10^{-3}		3.2×10^{-2}	
hRev1+ hPolκ	13-mer/ 40-mer-8oxoG	Opposite 8-oxoG[c]	7.0×10^{-2}	7.0	1.9×10^{-4}	0.02	7.1×10^{-3}	2.4
		Downstream[e]	1.3×10^{-2}	0.5	8.2×10^{-4}	1.0	1.3×10^{-2}	0.3
		Total[f]	2.7×10^{-2}	1.2	6.7×10^{-4}	0.2	1.2×10^{-2}	0.4

[a]Calculated using Σ(specific mutation type)/[(number of sequences)×(number of bases in event)].

[b]Calculated using $[\Sigma(\text{specific mutation type})/[(\text{number of sequences})\times(\text{number of bases in event})]]_{\text{40-mer-8oxoG}}/[\Sigma(\text{specific mutation type})/[(\text{number of sequences})\times(\text{number of bases in event})]]_{\text{40-mer-ctl}}$.

[c]Opposite dG and opposite 8-oxoG events include all events at position 0 in Fig. 2.

[d]Upstream events include all events that occurred at positions -3 to -1, before an enzyme encountered the lesion.

[e]Downstream events include all events that occurred at positions +1 to +3, after an enzyme encountered the lesion.

[f]Total events include all events from position -3 to +3 in Fig. 2.

Table S4. Relative number of nucleotide incorporations, deletions and insertions generated by hPolη as a function of template position with the control template

	Template Base and Position						
	A	G	T	G	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.84	0.27	98.19	0.57	0.64	0.89	0.37
dT	97.60	0.71	0.23	1.20	0.26	96.09	0.32
dC	0.50	98.25	0.34	97.01	0.07	2.29	98.69
dG	0.46	0.32	0.97	0.68	98.82	0.32	0.17
Deletion	0.53	0.37	0.22	0.46	0.18	0.34	0.38
Insertion	0.07	0.07	0.05	0.07	0.03	0.06	0.06

Table S5. Relative number of nucleotide incorporations, deletions and insertions generated by hPolη as a function of template position with the damaged template

	Template Base and Position						
	A	G	T	Y	C	A	G
	-3	-2	-1	0	1	2	3
dA	1.01	0.56	98.31	41.70	1.02	0.81	0.59
dT	97.48	0.70	0.31	1.32	0.16	96.11	0.64
dC	0.55	97.81	0.37	54.47	0.08	2.07	97.87
dG	0.52	0.41	0.64	1.19	98.36	0.41	0.23
Deletion	0.36	0.42	0.22	1.25	0.32	0.57	0.56
Insertion	0.08	0.10	0.13	0.07	0.05	0.04	0.10

Table S6. Relative number of nucleotide incorporations, deletions and insertions generated by hPolκ as a function of template position with the control template

	Template Base and Position						
	A	G	T	G	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.23	0.17	98.97	1.31	0.34	0.20	0.37
dT	98.53	0.83	0.16	1.05	0.12	98.87	1.64
dC	0.05	97.90	0.09	94.19	0.01	0.21	96.22
dG	0.12	0.38	0.38	2.48	99.10	0.06	0.32
Deletion	1.03	0.67	0.35	0.93	0.41	0.61	1.40
Insertion	0.04	0.04	0.05	0.04	0.02	0.05	0.05

Table S7. Relative number of nucleotide incorporations, deletions and insertions generated by hPolκ as a function of template position with the damaged template

	Template Base and Position						
	A	G	T	Y	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.67	0.63	98.57	78.01	1.66	0.37	0.35
dT	97.85	1.17	0.30	1.42	0.17	97.19	1.55
dC	0.28	97.25	0.20	18.51	0.04	0.44	96.25
dG	0.32	0.48	0.30	0.22	97.09	0.33	0.21
Deletion	0.83	0.42	0.25	1.82	1.03	1.63	1.58
Insertion	0.05	0.06	0.38	0.01	0.02	0.04	0.06

Table S8. Relative number of nucleotide incorporations, deletions and insertions generated by hPolι as a function of template position with the control template

	Template Base and Position						
	A	G	T	G	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.04	0.26	29.47	0.99	0.78	0.09	0.55
dT	99.45	17.68	18.65	2.02	10.28	98.95	6.32
dC	0.02	81.68	0.63	89.86	0.98	0.14	92.27
dG	0.05	0.13	44.41	0.09	85.93	0.02	0.08
Deletion	0.34	0.19	6.65	7.03	1.99	0.69	0.72
Insertion	0.10	0.05	0.19	0.02	0.04	0.11	0.05

Table S9. Relative number of nucleotide incorporations, deletions and insertions generated by hPolι as a function of template position with the damaged template

	Template Base and Position						
	A	G	T	Y	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.11	0.53	26.78	21.74	1.23	0.73	0.63
dT	99.09	17.71	28.72	4.81	0.73	95.43	3.30
dC	0.09	80.92	0.66	65.75	0.28	0.78	92.24
dG	0.20	0.22	37.32	0.40	93.98	0.18	0.12
Deletion	0.32	0.54	5.91	7.26	3.72	2.71	3.45
Insertion	0.19	0.09	0.61	0.03	0.07	0.17	0.26

Table S10. Relative number of nucleotide incorporations, deletions and insertions generated by the combination of hRev1 and hPolκ as a function of template position with the control template

	Template Base and Position			
	G	C	A	G
	0	1	2	3
dA	0.07	0.20	0.22	0.19
dT	0.52	0.16	91.90	1.18
dC	97.46	4.22	1.25	97.66
dG	0.44	89.69	0.65	0.06
Deletion	0.29	5.66	5.92	0.80
Insertion	1.22	0.07	0.06	0.11

Table S11. Relative number of nucleotide incorporations, deletions and insertions generated by the combination of hRev1 and hPolκ as a function of template position with the damaged template

	Template Base and Position			
	Y	C	A	G
	0	1	2	3
dA	6.59	0.67	0.26	0.24
dT	0.33	0.10	96.78	1.29
dC	92.32	0.06	0.98	96.93
dG	0.04	98.28	0.10	0.13
Deletion	0.71	0.73	1.86	1.36
Insertion	0.02	0.16	0.03	0.06

Table S12. Relative number of nucleotide incorporations, deletions and insertions generated by the combination of all four Y-family polymerases as a function of template position with the control template

	Template Base and Position						
	A	G	T	G	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.21	0.14	97.44	0.94	0.33	0.26	0.26
dT	97.77	0.58	0.21	0.85	0.12	98.22	1.27
dC	1.07	98.63	0.99	95.91	0.25	0.81	97.32
dG	0.09	0.21	0.54	1.59	98.99	0.09	0.08
Deletion	0.81	0.38	0.77	0.66	0.30	0.56	1.00
Insertion	0.04	0.06	0.05	0.05	0.02	0.04	0.06

Table S13. Relative number of nucleotide incorporations, deletions and insertions generated by the combination of all four Y-family polymerases as a function of template position with the damaged template

	Template Base and Position						
	A	G	T	Y	C	A	G
	-3	-2	-1	0	1	2	3
dA	0.52	0.27	97.61	73.09	1.19	0.41	0.34
dT	95.84	0.84	0.34	0.99	0.11	96.98	1.45
dC	2.30	97.64	0.50	23.35	0.06	0.97	96.51
dG	0.32	0.46	0.88	0.44	98.27	0.33	0.15
Deletion	0.98	0.73	0.50	2.12	0.35	1.28	1.50
Insertion	0.04	0.07	0.18	0.02	0.02	0.03	0.05

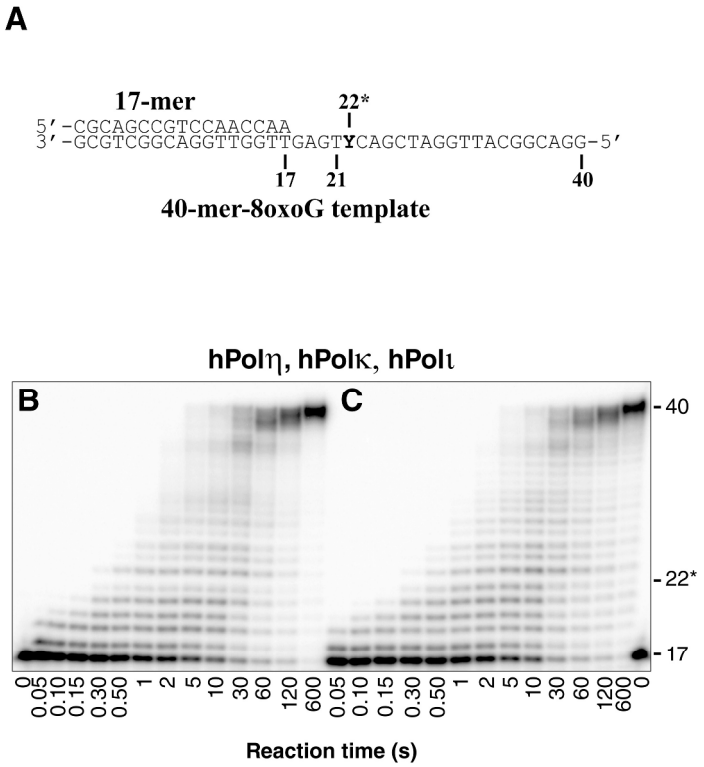

Figure S1. Time-dependent lesion bypass catalyzed by the combination of hPolη, hPolκ and hPolι. (**A**) The damaged 17-mer/40-mer-8oxoG substrate. The position of the 8-oxoG lesion within the template strand is indicated by a "**Y**". A pre-incubated solution containing 25 nM of hPolη, 25 nM of hPolκ and 25 nM of hPolι was first mixed with a solution of 100 nM of 5′-[^{32}P]-labeled (**B**) 17-mer/40-mer or (**C**) 17-mer/40-mer-8oxoG. The enzyme and DNA substrate solution was briefly incubated at 37°C and then rapidly mixed with a solution of all 4 dNTPs (200 μM each). The reaction mixtures were quenched at the indicated times with 0.37 M EDTA and resolved by using denaturing PAGE. The sizes of important products are indicated, and the 22nd position is denoted with an asterisk (*) to indicate an incorporation opposite the 8-oxoG lesion site.

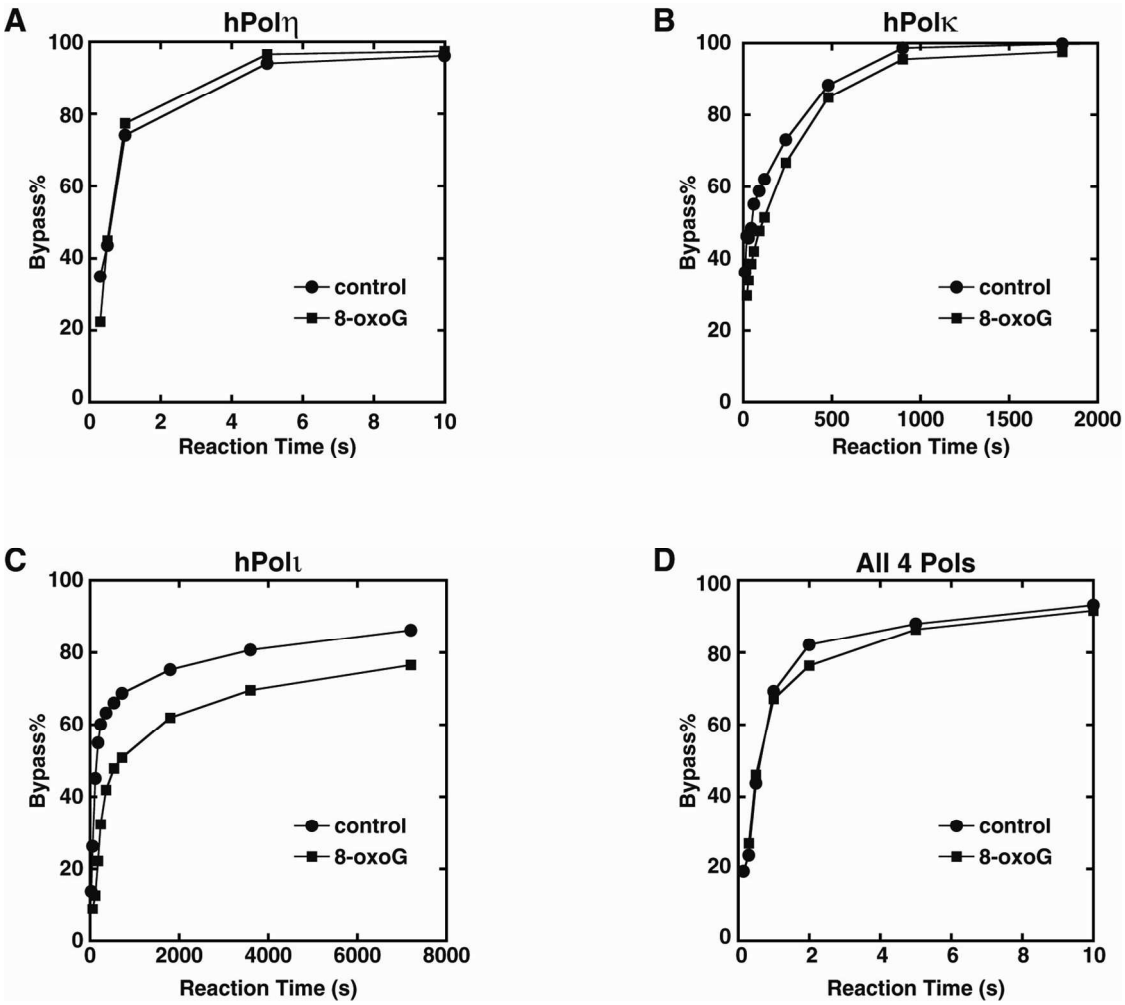

Figure S2. Time-dependent lesion bypass during running start assays. The control 17-mer/40-mer or damaged 17-mer/40-mer-8-oxoG substrates (100 nM) were extended by an individual Y-family polymerase (100 nM) or all 4 Y-family DNA polymerases (25 nM each). The control dG or damaged 8-oxoG bypass% was plotted as a function of reaction time for (A) hPolη, (B) hPolκ, (C) hPolι or (D) the combination of all 4 human Y-family polymerases.

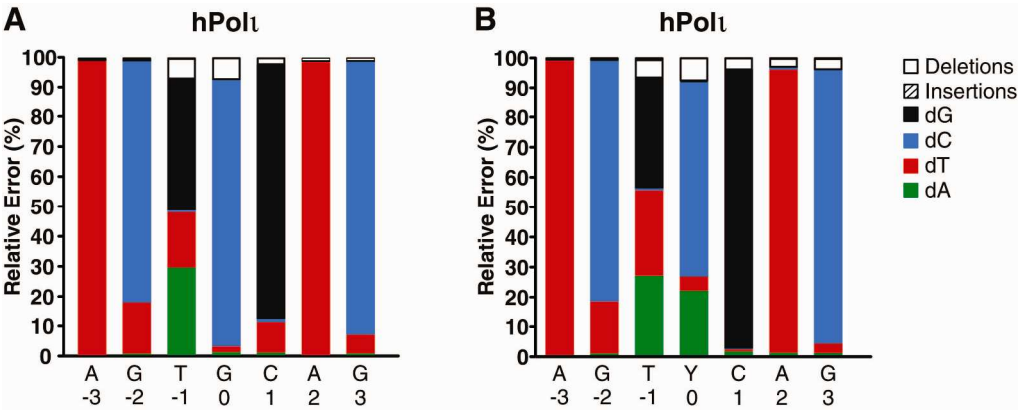

Figure S3. Histogram of nucleotide incorporations, insertions and deletions generated by hPolι as a function of template position. Lesion bypass analysis for hPolι by using either (**A**) the undamaged 17-mer/40-mer substrate or (**B**) the damaged 17-mer/40-mer-8oxodG substrate are shown. The relative number of base deletions (white bar), base insertions (striped bar), or dG (black bar), dC (blue bar), dT (red bar) or dA (green bar) incorporations as a percentage of the total dNTP incorporations are indicated at each template position. The template bases are denoted and the 8-oxodG lesion is represented as "**Y**". The indicated template positions are relative to the 8-oxodG lesion site within the 40-mer-8oxodG template.