Supplementary Data for

Characterization of interstrand DNA-DNA cross-links derived from abasic sites using bacteriophage ϕ29 DNA polymerase

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Figure S1

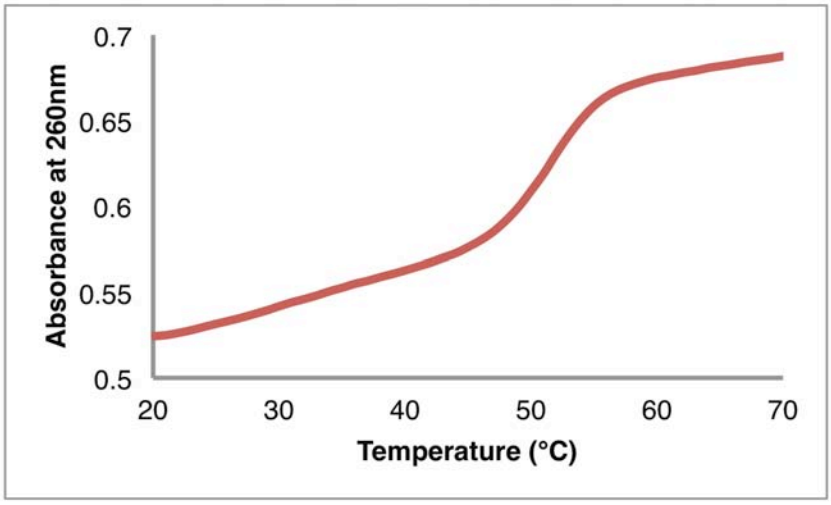

Figure S1. UV melting curve of duplex B (without the primer). The sample contained approximately 1 μM duplex DNA, 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 4 mM DTT, and 0.1 mg/mL bovine serum albumin. The absorbance at 260 nm was monitored as a function of temperature, increasing from 20 °C to 70 °C, at a rate of 0.5 °C/min.

Figure S2

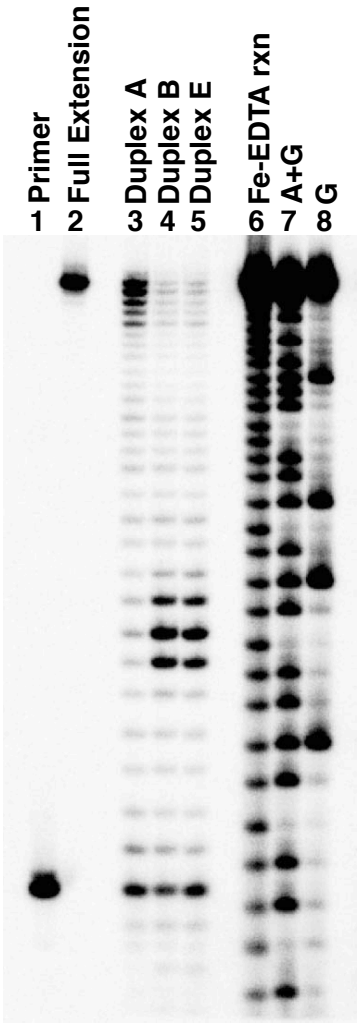

Figure S2. Primer extension by T7 DNA polymerase is blocked near the single-strand-duplex junction in duplexes B and E. The ^{32}P-labeled primers were extended by incubation of the DNA substrates with T7 DNA polymerase (10 units) and the four dNTPs (0.5 mM in each) in Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), $(NH_4)_2SO_4$ (10 mM), DTT (4 mM), and bovine serum albumin (0.1 mg/mL) for 8 min at 24 °C. After reaction work-up, the primer extension products were analyzed by electrophoresis on a 20% denaturing polyacrylamide gel. Lane 1 is the 15 nt, 5'-^{32}P-labeled primer, lane 2 is the 5'-^{32}P-labeled full-length extension product size marker, lane 3 is primer extension on the single-stranded substrate A, lane 4 depicts the results of primer extension on the un-cross-linked duplex-containing substrate B, lane 5 depicts the results of primer extension on the cross-linked duplex-containing substrate E, lane 6 is an iron-EDTA cleavage reaction on a synthetic standard of the full-length extension product (5'-^{32}P-GAT CAC AGT GAG TAC AAT AGA ATA GAT GAA CTA AGA CAT ATA), lanes 7 and 8 are Maxam-Gilbert A+G- and G-reactions on the full-length 5'-^{32}P-labeled extension product. The intense bands in lanes 4 and 5 correspond to extension of the primer to the –8, –9, and –10 locations on templates B and E.

Figure S3

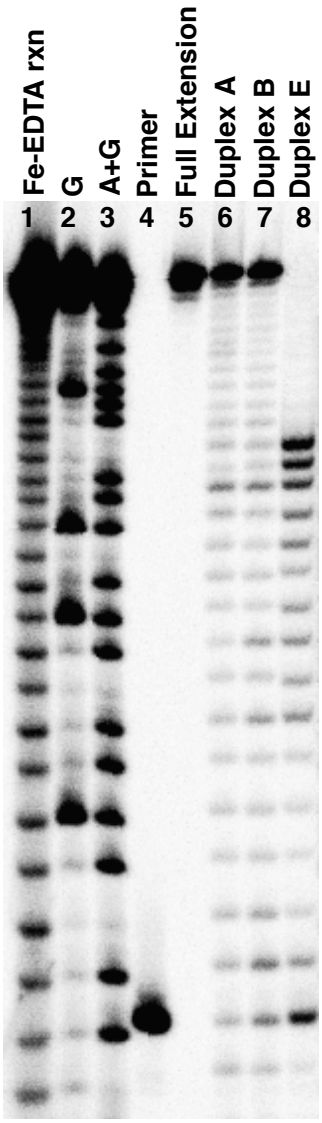

Figure S3. ϕ29 DNA polymerase fully extends the primer on the single-stranded substrate A and the duplex-containing substrate B. The ^{32}P-labeled primers were extended by incubation of the DNA substrates with *ϕ*29 DNA polymerase (10 units) and the four dNTPs (1 mM in each) in Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), $(NH_4)_2SO_4$ (10 mM), DTT (4 mM), and bovine serum albumin (0.1 mg/mL) for 30 min at 24 °C. After reaction work-up, the primer extension products were subjected to electrophoretic analysis on a 20% denaturing polyacrylamide gel. Lane 1 is an iron-EDTA cleavage reaction on a synthetic standard of the full-length extension product (5'-^{32}P-GAT CAC AGT GAG TAC AAT AGA ATA GAT GAA CTA AGA CAT ATA), lanes 2 and 3 are Maxam-Gilbert G- and A+G-reactions carried out on the 5'-^{32}P-labeled full-length extension product, lane 4 is the 15 nt, 5'-^{32}P-labeled primer, lane 5 is a synthetic standard of the 5'-^{32}P-labeled full-length extension product, lane 6 depicts the results of primer extension on the single-stranded substrate A, lane 7 depicts the results of primer extension on the duplex-containing substrate B, and lane 8 depicts the results of primer extension on the cross-linked duplex-containing substrate E.

Figure S4

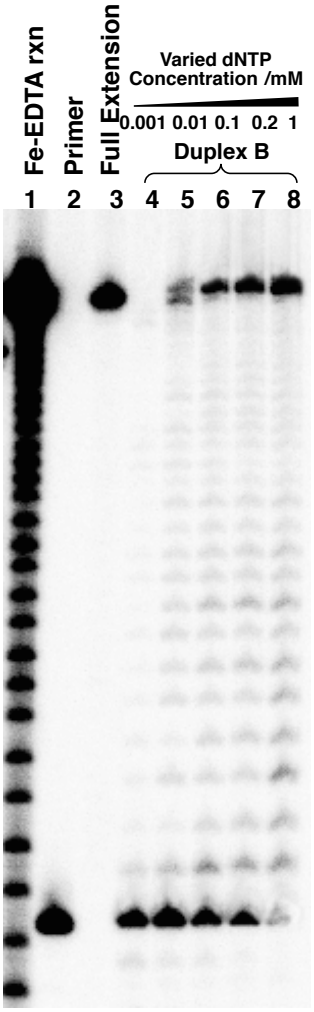

Figure S4. All dNTP concentrations used in this study support ϕ29 DNA polymerase-mediated extension of primer to full length on substrate B. The ^{32}P-labeled primers were extended by incubation of the DNA substrates with ϕ29 DNA polymerase (10 units) and the four dNTPs (0.001-1 mM in each) in Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), $(NH_4)_2SO_4$ (10 mM), DTT (4 mM), and bovine serum albumin (0.1 mg/mL) for 30 min at 24 °C. After reaction work-up, the primer extension products were subjected to electrophoretic analysis on a 20% denaturing polyacrylamide gel. Lane 1 is an iron-EDTA cleavage reaction on a synthetic standard of the full-length extension product (5'-^{32}P-GAT CAC AGT GAG TAC AAT AGA ATA GAT GAA CTA AGA CAT ATA), lane 2 is the 15 nt, 5'-^{32}P-labeled primer, lane 3 is a synthetic standard of the 5'-^{32}P-labeled full-length extension product, and lanes 4-8 depict the results of primer extension on duplex substrate B at the indicated dNTP concentrations.

Figure S5

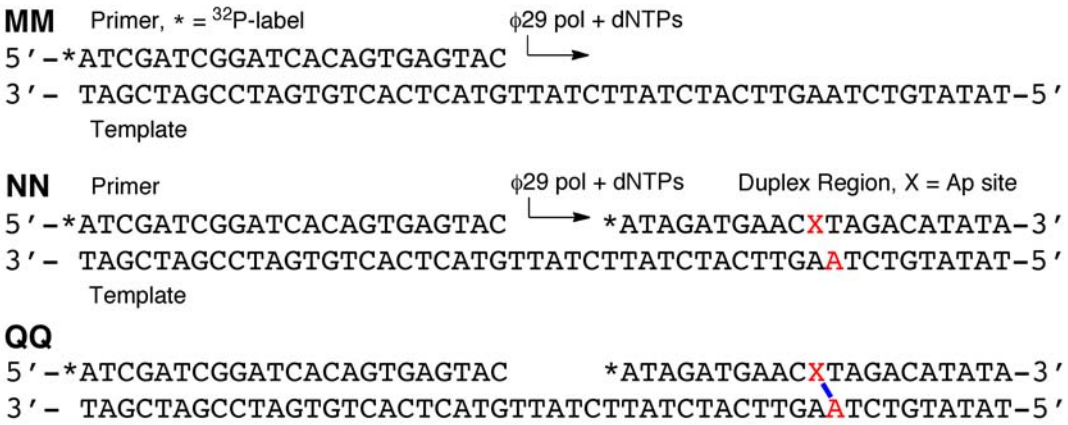

Figure S5. Substrates used in primer extension reactions at 37 °C. A longer primer was used in these substrates to ensure complete hybridization of the primer to the template at 37 °C. Substrates NN and QQ contain a 23 nt ^{32}P-labeled primer *and* a 21 nt ^{32}P-labeled strand in the duplex region of the substrate.

Figure S6

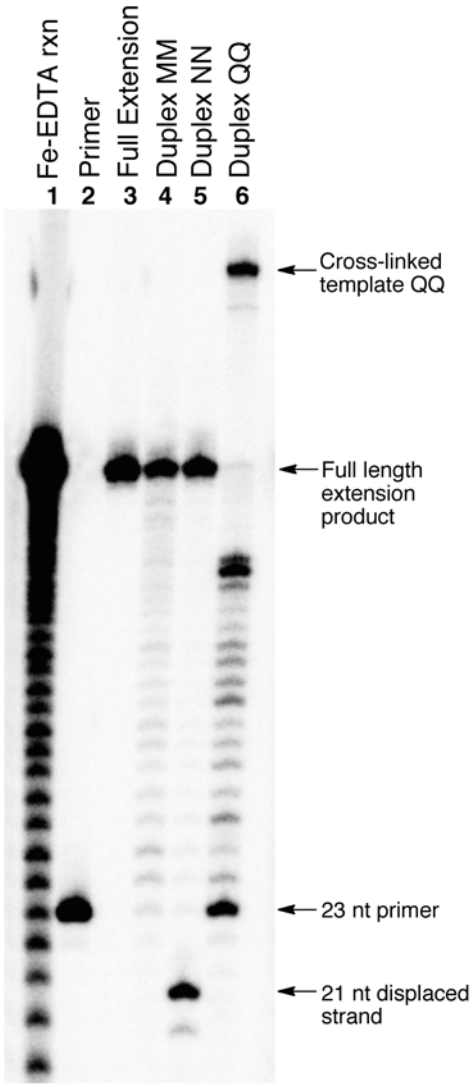

Figure S6. ϕ29 DNA polymerase-mediated primer extension on substrates MM, NN, and QQ at 37 °C was blocked by the dA-Ap cross-link. The ^{32}P-labeled primers were extended by incubation of the DNA substrates with *ϕ*29 DNA polymerase (10 units) and the four dNTPs (1 mM in each) in Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), $(NH_4)_2SO_4$ (10 mM), DTT (4 mM), and bovine serum albumin (0.1 mg/mL) for 60 min at 37 °C. After reaction work-up, the primer extension products were subjected to electrophoretic analysis on a 20% denaturing polyacrylamide gel. Lane 1 is an iron-EDTA cleavage reaction on a synthetic standard of the full-length extension product (5'-^{32}P-ATC GAT GCG ATC ACA GTG AGT ACA ATA GAA TAG ATG AAC TAA GAC ATA TA), lane 2 is the 23 nt, 5'-^{32}P-labeled primer, lane 3 is a synthetic standard of the 5'-^{32}P-labeled full-length extension product, and lanes 4-6 depict the results of primer extension reactions on substrates MM, NN, and QQ. Note that NN and QQ contain a 23 nt ^{32}P-labeled primer *and* a 21 nt ^{32}P-labeled strand in the duplex region of the substrate (Figure S5). The presence of two ^{32}P labels in these substrates gives rise to the "21 nt displaced strand" and the "cross-linked template QQ" band in lanes 5 and 6, respectively.