Supporting Information: The A-Rule and Deletion Formation During Abasic and Oxidized Abasic Site Bypass by DNA Polymerase θ.

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Active site titration of Pol θ. The active fraction of Pol θ was determined by pre-steady state kinetic analysis. A solution of Pol θ (50 nM) and primer-template **11a** (100 nM) was mixed with dGTP (1 mM) and quenched at defined time points with a solution of 80% formamide and 100 mM EDTA. In a typical experiment, a solution (150 μL) of Pol θ (100 nM) and primer-template (200 nM) was prepared in reaction buffer (10 mM Tris•HCl pH 8.0, 25 mM KCl, 1 mM BME, 10 mM $MgCl_2$). A solution of dGTP (1 mL, 1 mM) was prepared in the same reaction buffer. The two solutions were mixed in the rapid quench instrument to initiate the reaction and were quenched with a solution of 80% formamide and 100 mM EDTA, which had been loaded into the quench syringe. Reactions were collected in a microcentrifuge tube (1.6 mL) containing a solution of 500 mM EDTA with trace bromophenol blue and xylene cyanol. Reaction times were 3, 4, 5, 6, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000 ms. An aliquot (10 μL) from each reaction was removed and counted with a liquid scintillation counter. Radiation in each sample was normalized and an aliquot (5 uL) of each sample was loaded on a 20% denaturing PAGE, which was run at 55 W. The fraction of extended primer was plotted as a function of time and this was fit to the equation $P=A(1-e^{-kt}) + k_{ss}t$ where P is fraction of extended primer, A is the burst amplitude, k is the burst phase rate constant, k_{ss} is the steady-state rate constant, and t is time. The fraction of active enzyme was determined by dividing the experimental burst amplitude by the theoretical burst amplitude (50%) if all of the enzyme were active. This experiment was carried out 3 times and the active fraction was determined to be 47.5 ± 6.6%.

Pol θ Translesion Synthesis Kinetics. Reactions were carried out as described in the manuscript body. The following specific reaction conditions were used. For **1a**: 0.5, 1, 2.5, 5, 10, 25, 50, 100 μM: 20s. For **1b**: dATP: 2.5, 5, 10 μM: 2 min; 15, 20, 25, 50 μM: 1 min. dGTP: 10, 20, 30 μM: 4 min; 50, 75 μM: 2 min; 100, 150, 200 μM: 1 min. For **1c**: dATP: 5, 10, 20, 40 μM: 5 min; 60, 100, 150 μM: 2.5 min. dGTP: 20, 50, 75, 100, 150 μM: 5 min, 250, 500 μM: 3 min. For **1d**: dATP: 10, 20, 40 μM: 5 min; 60, 100 μM: 2 min; 200, 300, 500 μM: 1 min. dGTP: 150, 300, 400, 500 μM: 4 min; 750, 1000 μM: 2 min; 1500 μM: 1 min. For **1e**: dATP: 2.5, 5, 10 μM: 2 min; 20, 30, 40, 60, 100 μM: 1 min. dGTP: 10, 20 μM: 4 min; 30, 50 μM: 3 min, 100, 150, 200, 500 μM: 2 min.

Pol θ Extension Kinetics. Reactions were carried out as described in the manuscript body. The following specific reaction conditions were used. For **3a**: dCTP: 0.25, 0.5, 1 μM: 1 min; 2.5, 5, 10, 25, 50 μM: 30 s. For **4a**: dCTP: 25, 50, 75. 100 μM: 10 min; 200, 300 μM: 5 min; 500, 1000 μM: 3 min. dTTP: 50, 100, 150 μM: 6 min; 200, 300 μM: 4 min; 400, 500, 750 μM: 3 min. For **5a**: dCTP: 50, 100, 150, 200, 250, 300, 400, 500 μM: 15 min. dTTP: 100, 200, 400, 500, 750, 1000, 1500, 2000 μM: 5 min. For **6a**: dCTP: 25, 50, 75, 100 μM: 3 min; 200, 400, 700, 1000 μM: 1 min. dTTP: 25, 50, 75, 100, 200, 400, 700, 1000 μM: 5 min. For **7a**: Identical to **4a**. For **9a**: dATP: 0.5, 1, 2.5, 5, 10, 25, 50, 100 μM: 20s. For **9b**: dATP: 2.5, 5, 10 μM: 5 min; 25, 50 μM: 3 min; 100, 200, 300 μM: 1.5 min. dTTP: 2.5, 5, 10 μM: 5 min; 25, 50 μM: 2.5 min; 100, 200, 300 μM: 1 min. **9c**: dATP: 5, 10, 20, 50, 100, 250, 500, 1000 μM. dTTP: 25, 50, 75, 100 μM: 5 min; 250, 500, 1000, 2000 μM; 1 min. For **10a**: dATP: 0.5, 1, 2.5, 5, 10, 25, 50, 100 μM: 30s. For **10b**: dATP: 50, 100, 200, 300 μM: 10 min; 400, 500, 1000, 2000 μM: 5 min. dTTP: 25, 50 μM: 5 min; 75, 100, 250 μM: 2.5 min; 500, 1000, 2000 μM: 1 min. For **11a**: dGTP: 0.5, 1, 2.5, 5, 10, 25, 50, 100 μM: 15s. **11b**: dATP 5, 10 μM: 4 min; 25, 50 μM: 2 min; 100, 250, 500, 1000 μM: 45 s. For **11c**: dATP: 10, 25, 50 μM: 4 min; 50, 100, 250 μM: 2 min; 500, 1000, 2000 μM: 1 min. For **12a**: dATP: 0.5, 1, 2.5, 5, 10, 25, 50, 100 μM: 20s. **12b**: dTTP: 10, 25, 50 μM: 5 min; 100, 200, 300, 500, 1000 μM: 2 min. For **12c**: dTTP: 5, 10, 25 μM: 2 min; 50, 100 μM: 1 min; 500, 1000, 2000 μM: 45 s.

Sequencing of Pol θ Bypass Products of AP. Pol θ (95 nM) was incubated with primer-template complex **13a** or **13b** (400 nM) at room temperature with all four dNTPs (100 μM) in reaction buffer (20 mM Tris•HCl pH 8.0, 25 mM KCl, 10 mM $MgCl_2$, 1 mM BME) at a reaction volume of 20 μL. After 30 min, the solution was mixed with Dynabeads M-270 Streptavidin (20 μg, 20 μL) and incubated at room temperature for 30 min with mixing by pipette every 10 min. Following incubation, magnetic beads were concentrated using a magnetic particle concentrator. The supernatant was removed and the beads were washed five times with washing buffer (5 mM Tris•HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl, 50 μL). The abasic site on the template strand was cleaved by incubation with NaOH (0.1 M, 50 μL) at room temperature for 5 min. The beads were washed once with NaOH (0.1 M, 50 μL), twice with

Tris•EDTA buffer (10 mM Tris•HCl, 1 mM EDTA, pH 7.0, 50 µL) and twice with H_2O (50 µL). The beads were resuspended in H_2O (40 µL). An aliquot (5 µL) of this solution was amplified by PCR using Phusion polymerase according to the manufacturer's protocol. This entailed 30 PCR cycles (98 °C for 10 s, 59 °C for 30 s, 72 °C for 30s) with PCR primers 5'-AGA TGG AAT TCG TTC GAC C and 5'-GTA GGT ACC GAT TAA TCA CAG C. The PCR product was purified by 16% non-denaturing polyacrylamide gel electrophoresis and staining with ethidium bromide. The product band was excised from the gel, crushed manually, and eluted in buffer (100 mM NaCl, 1 mM EDTA, 1 mL) by shaking for 12 hr at room temperature. Gel pieces were removed by Poly-Prep column and a Nucleotide Removal Kit was used to remove ethidium bromide from the sample. The purified PCR product was digested with Acc65I (1 µL, 10 units) and EcoRI (1 µL, 20 units) in NEB Buffer 3.1 at 37 °C for 4 h. The reaction was purified using a Nucleotide Removal Kit. pBlueScript SK- plasmid (5 µg) was digested with Acc65I and EcoRI (identical conditions as for the PCR product) and purified by 1% agarose gel electrophoresis. The linearized plasmid was excised from the gel and purified using a Gel Extraction Kit. The digested PCR product (75 fmol) was ligated into linearized pBlueScript SK- plasmid (25 fmol) using a Quick Ligase Kit. An aliquot (3 µL) of the ligation reaction was immediately transformed into DH5α cells. Cells were plated on LB media containing ampicillin (100 µg/ mL). After growth at 37 °C for 18 h, the plates were sent to GeneWiz for colony sequencing using the Genewiz primer M13(-47). All commercially available kits used in this procedure were used according to the manufacturer's protocol.

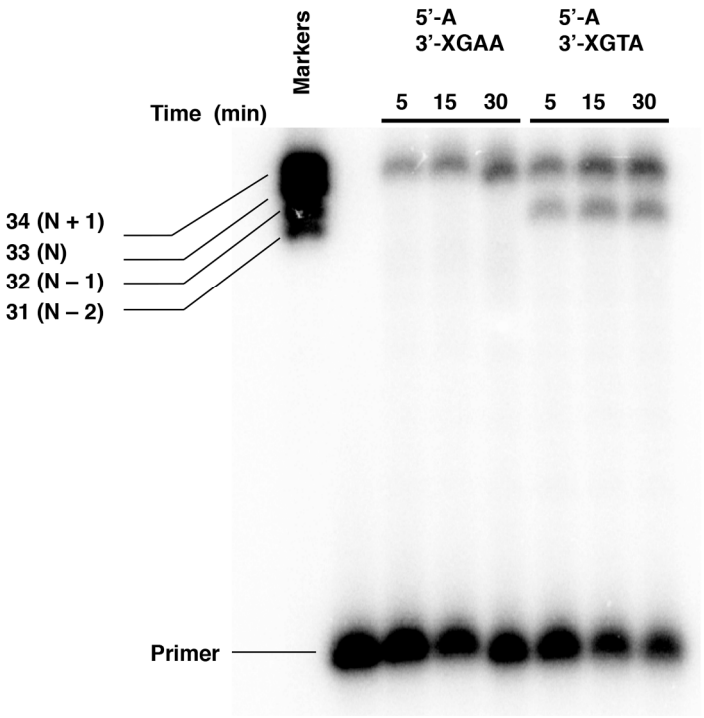

Figure S1. Qualitative primer extension by Pol θ using DNA complexes (**2** and **4a**) containing AP in the template. Reactions were conducted with Pol θ (11.9 nM), primer-template (25 nM), and dNTPs (500 μM). Blunt-end addition of one nucleotide is observed in both reactions. The N+1 product for **2** (left) corresponds to correct bypass (N), while N+1 and N-1 products for **4a** (right) correspond to correct bypass and 2-nucleotide deletion products, respectively.

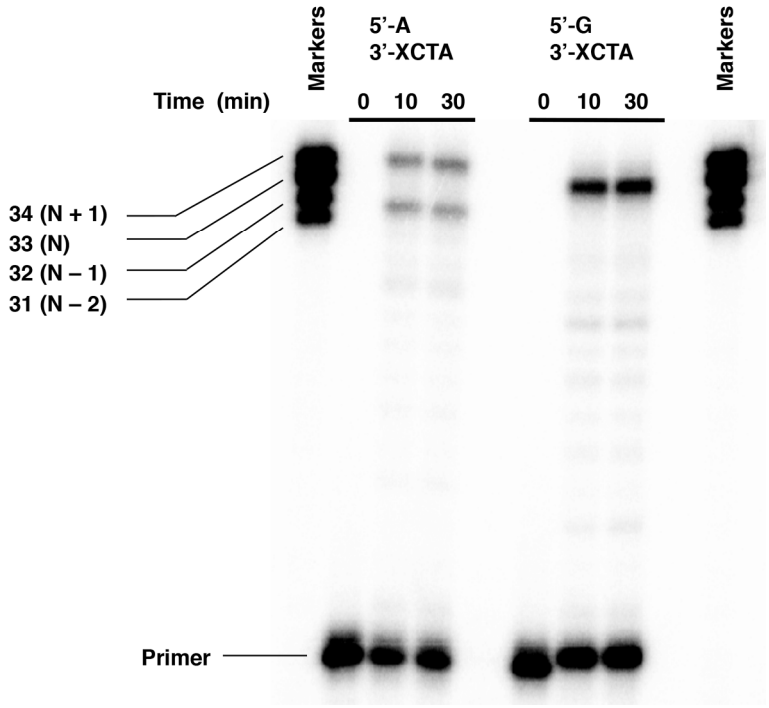

Figure S2. Qualitative primer extension by Pol θ using DNA complexes (**8** and **11b**) containing AP in the template. Reactions were conducted with Pol θ (11.9 nM), primer-template (25 nM), and dNTPs (500 μM). Blunt-end addition of one nucleotide is observed in both reactions. The N+1 and N-1 products for **8** (left) correspond to correct bypass and two-nucleotide deletion, respectively. Full-length (N) product observed for **11b** (right) corresponds to 1-nucleotide deletion.

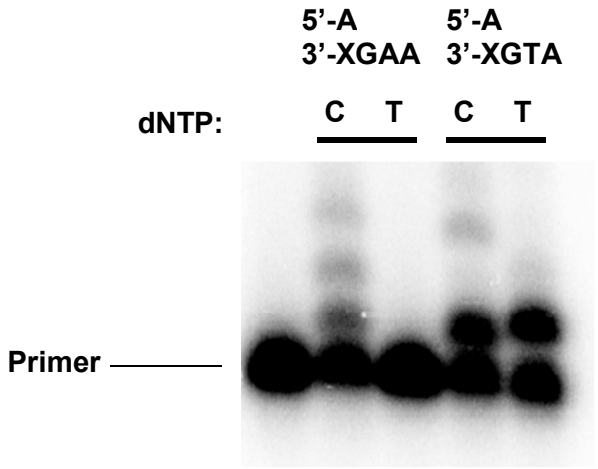

Figure S3. Single nucleotide incorporation on primer-templates **2** and **4a** by Pol θ. Pol θ (11.9 nM) was incubated with primer-template **2** (left) or **4a** (right) (25 nM) and the indicated dNTP (500 μM) for 10 min.

5'-A
3'-XCTA

5'-G
3'-XCTA

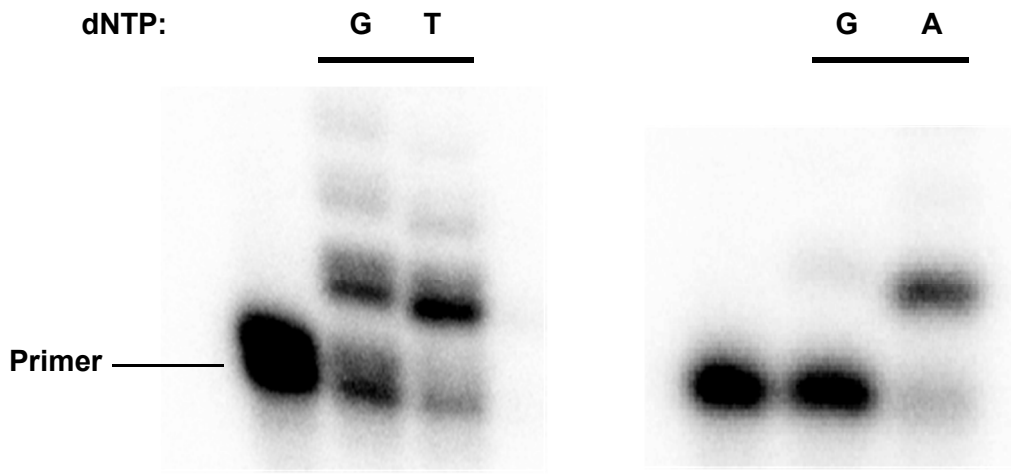

Figure S4. Single nucleotide incorporation on primer-templates **8** and **11** by Pol θ. Pol θ (11.9 nM) was incubated with primer-template **8** (left) or **11** (right) (25 nM) and the indicated dNTP (500 μM) for 5 min.

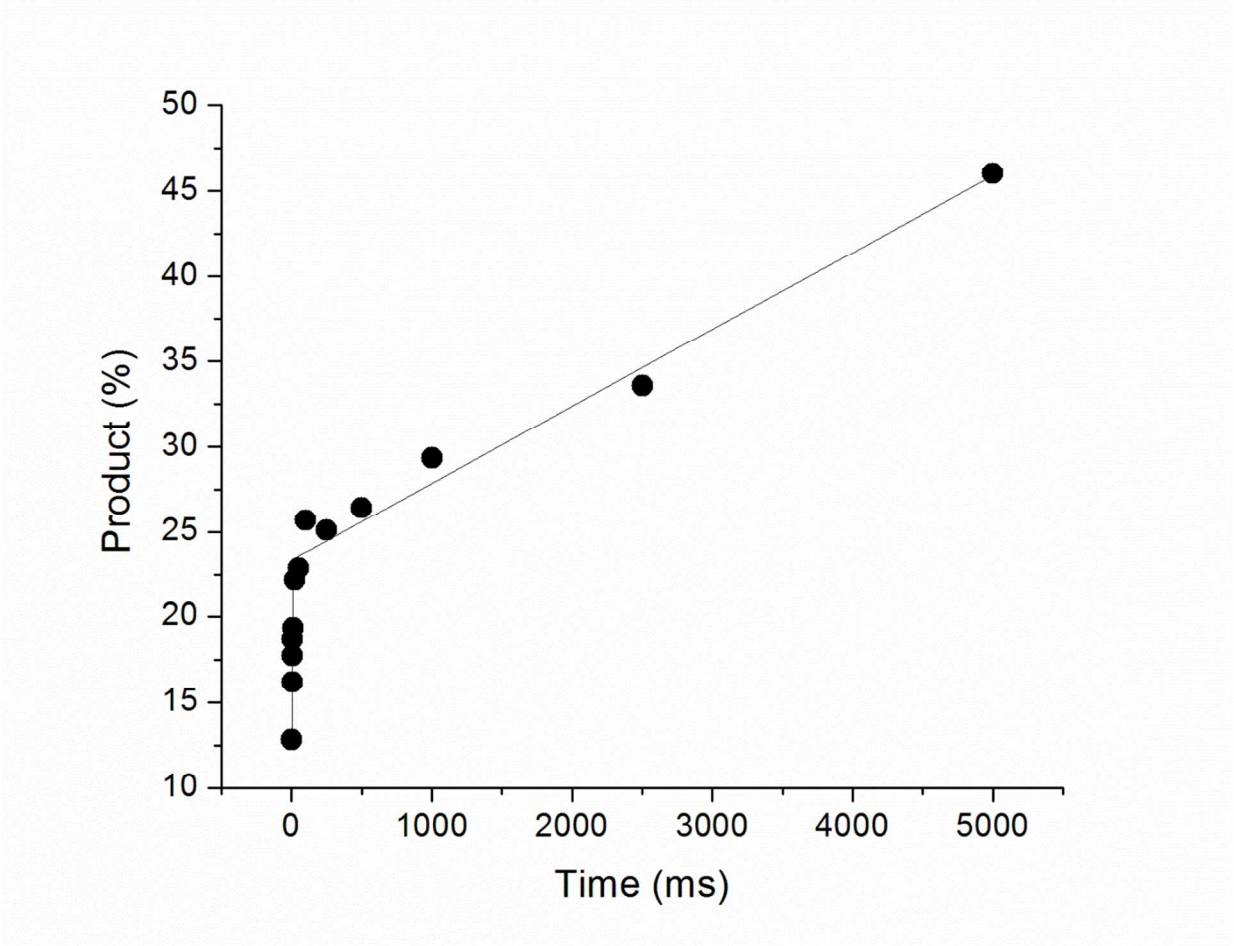

Figure S5. Representative active site titration of Pol θ.

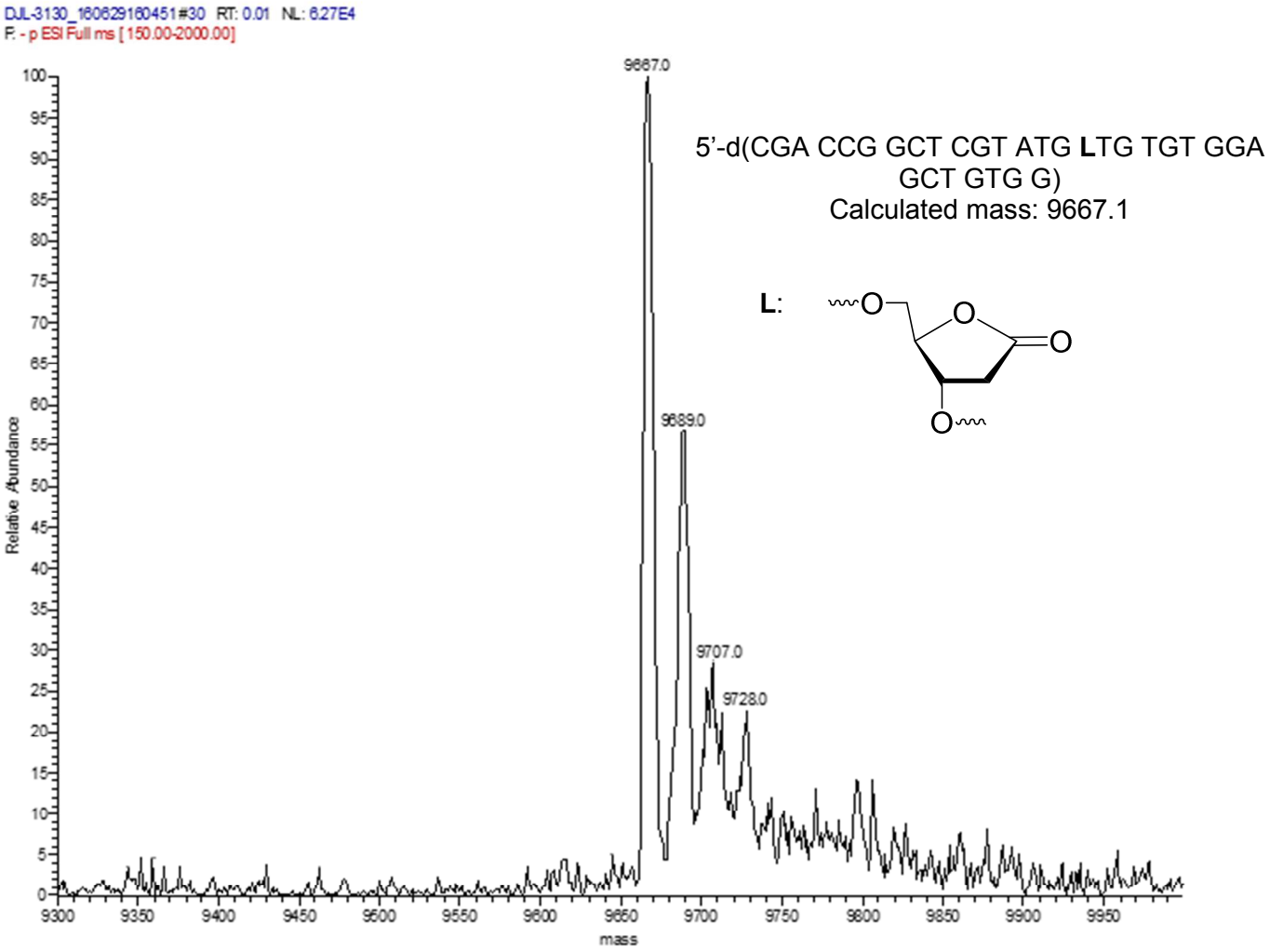

Figure S6. ESI-MS spectrum of precursor to template used in **1d**, **5**.

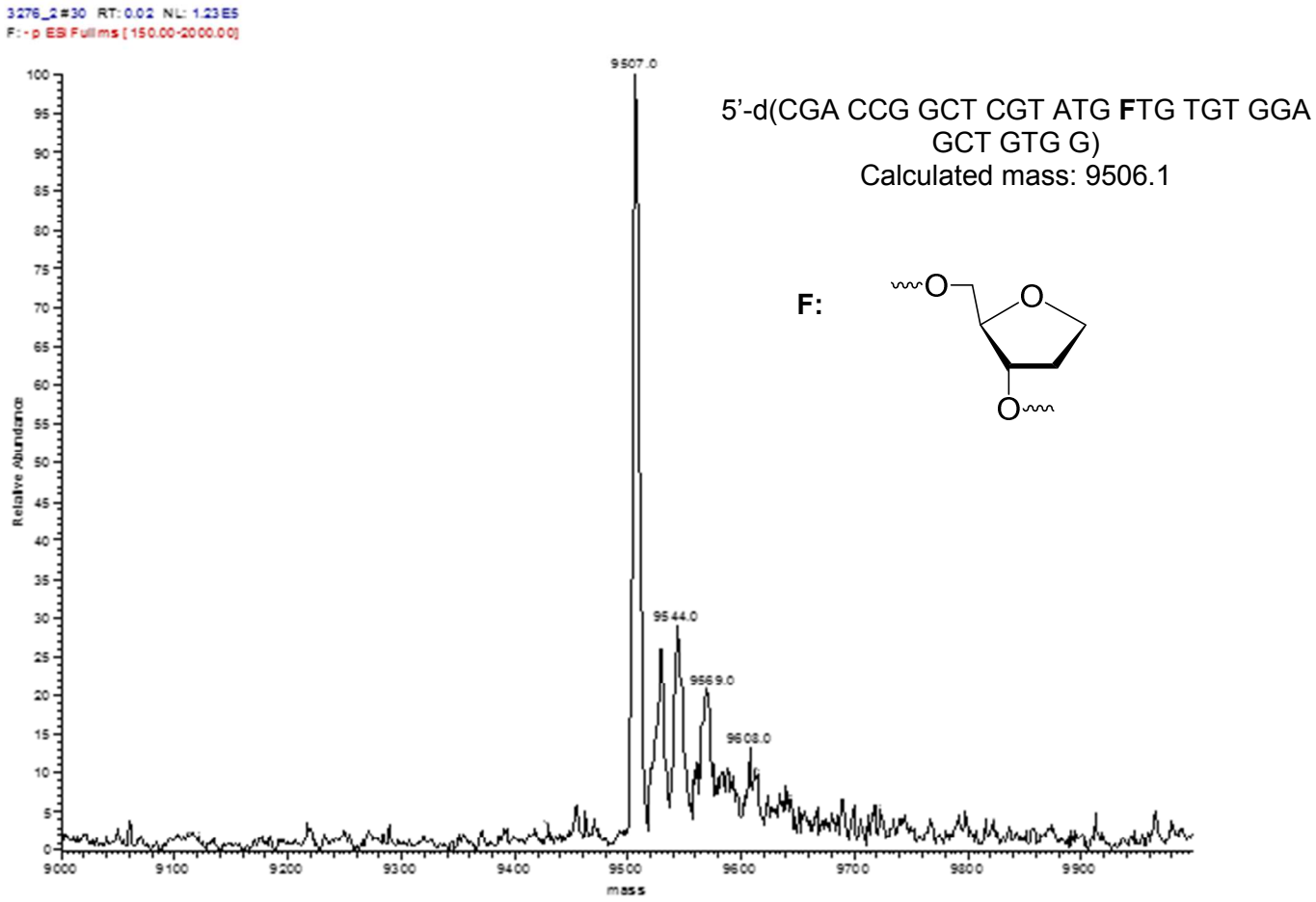

Figure S7. ESI-MS spectrum of precursor to template used in **1e**, **7**.

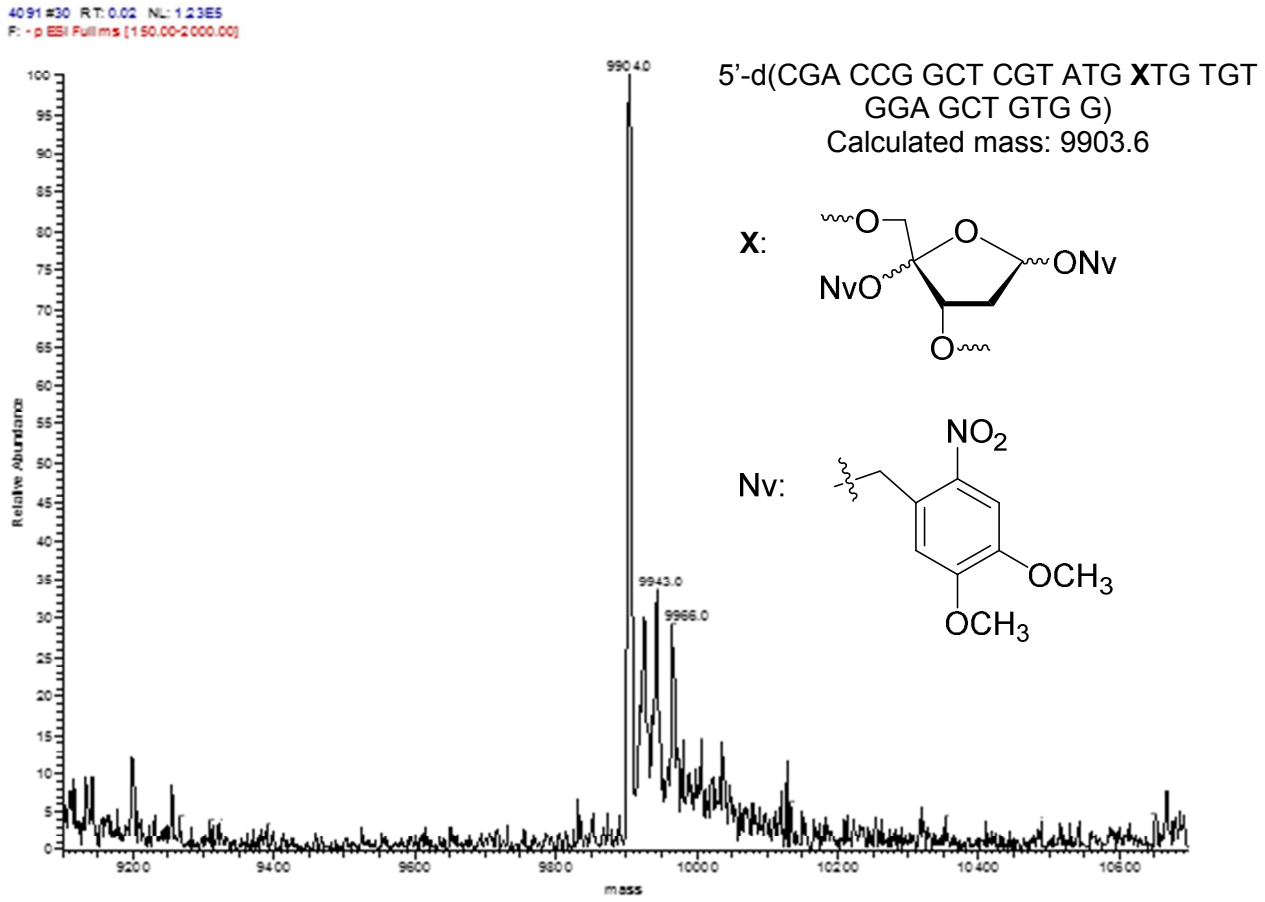

Figure S8. ESI-MS spectrum of precursor to template used in **9c**.

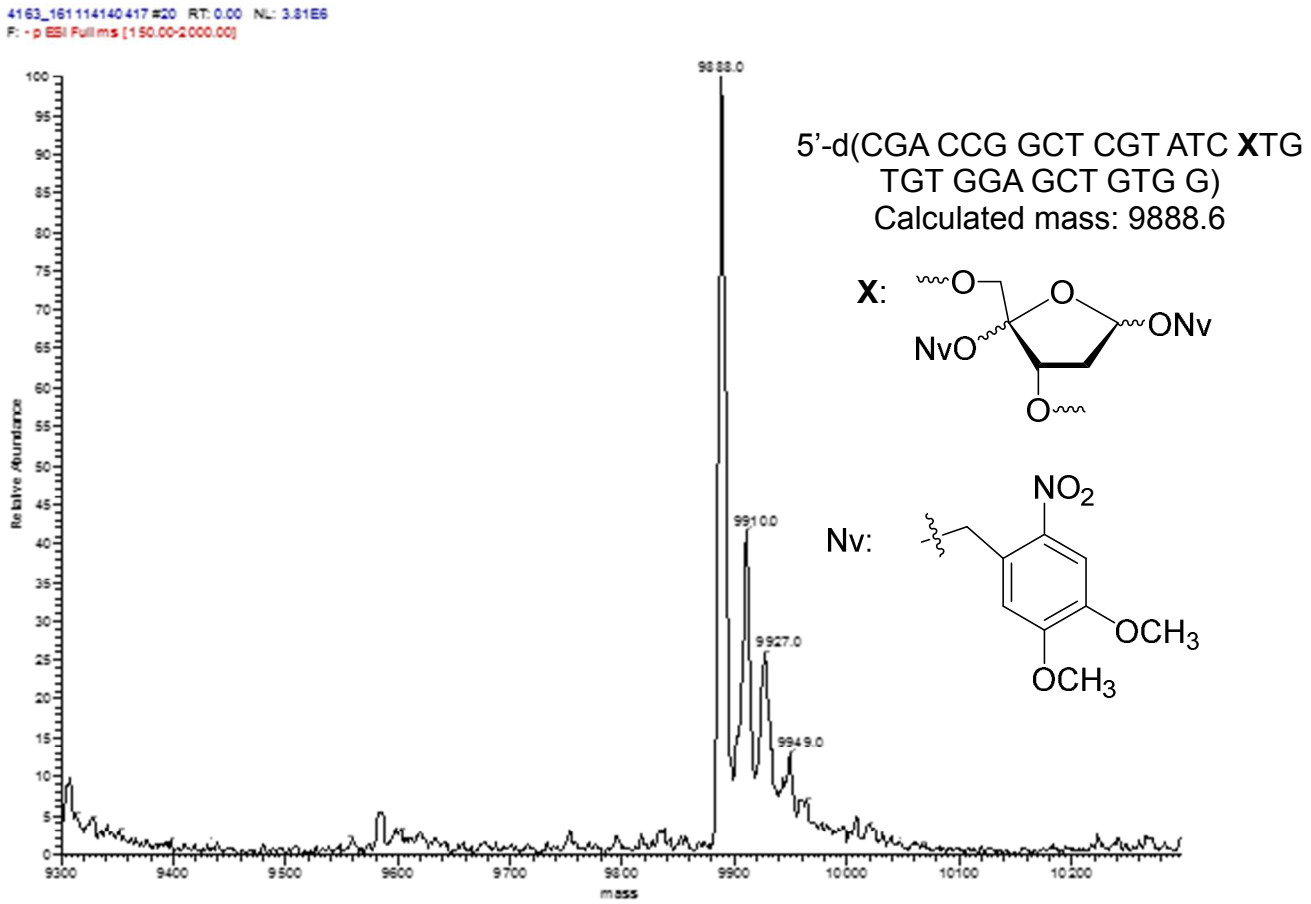

Figure S9. ESI-MS spectrum of precursor to template used in **11c**.

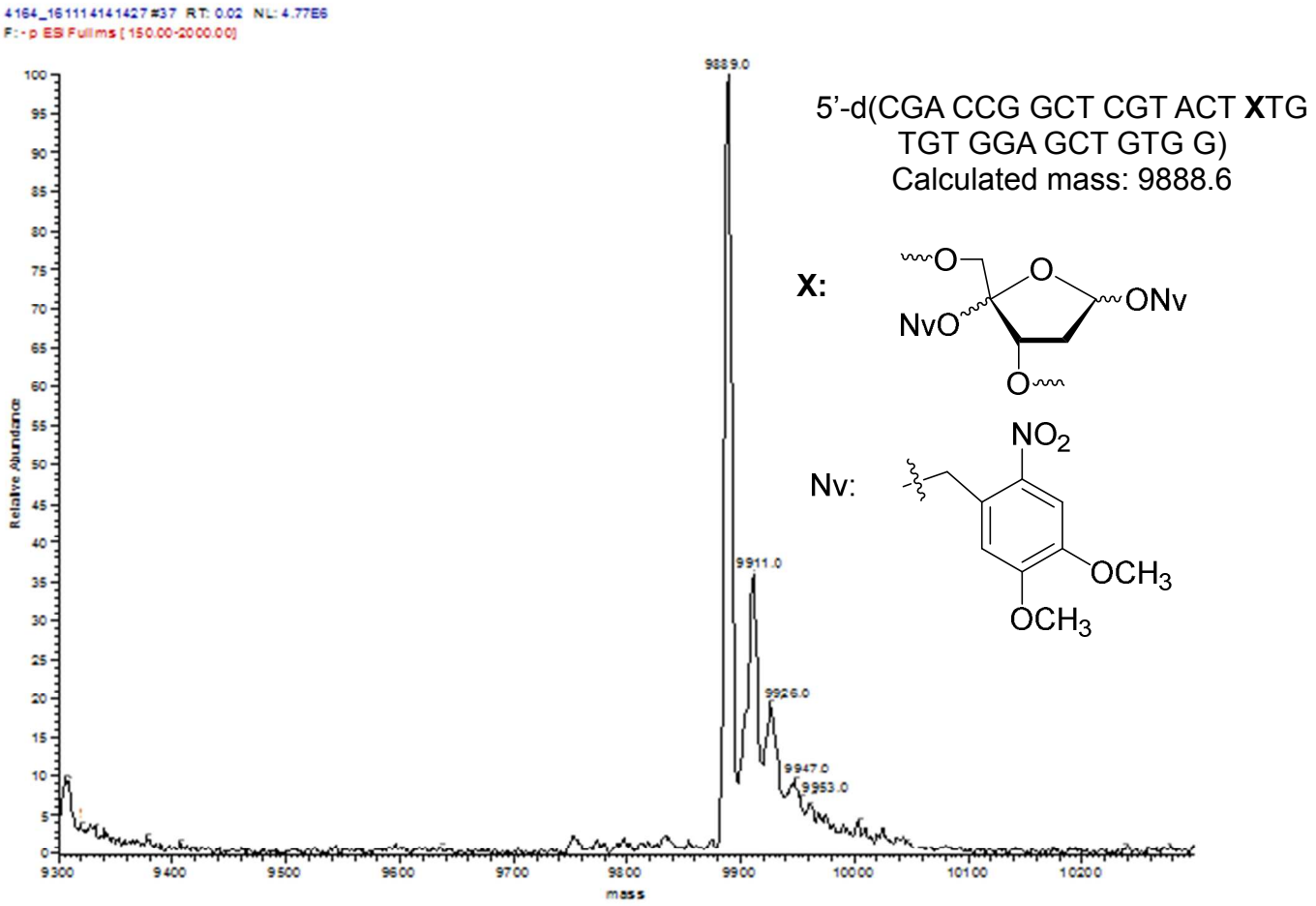

Figure S10. ESI-MS spectrum of precursor to template used in **12c**.

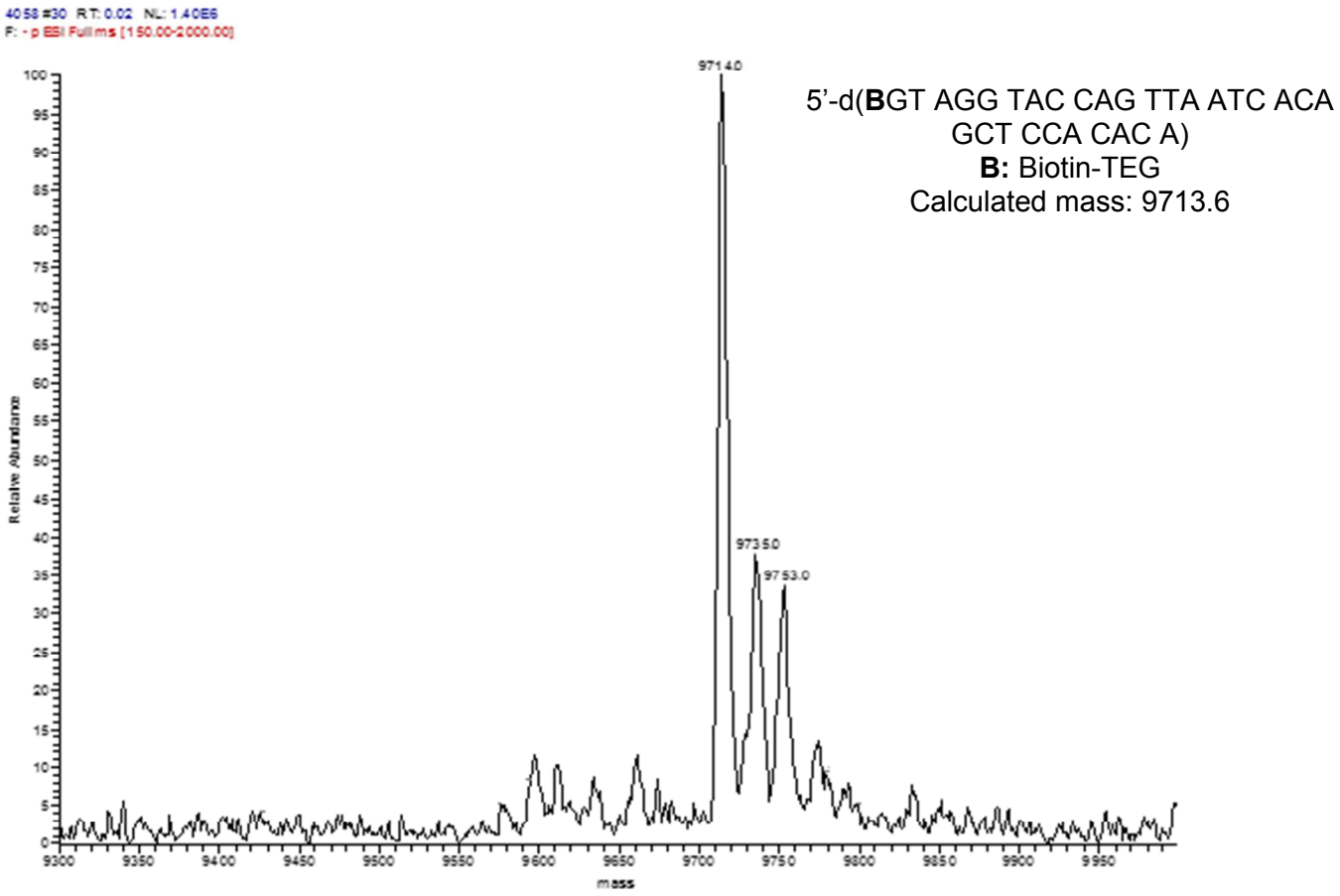

Figure S11. ESI-MS spectrum of primer used in **13**.

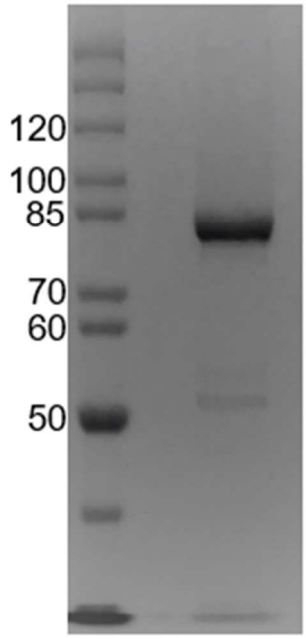

Figure S12. SDS-PAGE analysis of Pol θ. Pol θ (residues 1792-2590) was purified as described previously[1] and subjected to electrophoresis on a 10% polyacrylamide gel and stained with Coommassie blue. The molecular weight (in kDa) of individual proteins in the protein ladder is shown to the left of the image for comparison.

1. Hogg, M.; Seki, M.; Wood, R. D.; Doublié, S.; Wallace, S. S., Lesion Bypass Activity of DNA Polymerase θ (POLQ) Is an Intrinsic Property of the Pol Domain and Depends on Unique Sequence Inserts. *J. Mol. Biol.* **2011,** *405*, 642-652.