Supporting information

Sequencing of DNA lesions facilitated by site-specific excision via base excision repair DNA glycosylases yielding ligatable gaps

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Table of contents: Page

EXPERIMENTAL

Materials

Uracil-DNA glycosylase (UDG), formamidopyrimidine-DNA glycosylase (Fpg), T4-polynucleotide kinase (T4-PNK), Klenow (exo-) DNA polymerase, T4-DNA ligase, and OneTaq DNA polymerase were purchased from New England Biolabs. Apurinic/Apyrimidinic (Abasic) Endonuclease I (APE1) was a donation from Prof. Sheila S. David at the University of California at Davis. Other chemicals were molecular biology grade.

DNA preparation and purification procedure

DNA was prepared from commercially available phosphoramidites (Glen Research, VA) by the DNA/peptide Core Facility at the University of Utah. The DNA was cleaved and deprotected following the manufacturer's protocol, followed by purification using an ion-exchange HPLC column running a linear gradient of B from 20% to 100% over 30 min while monitoring UV absorbance at 260 nm (A = 20 mM NaP_i, 1 M NaCl, pH 7 in 10% CH_3CN/90% ddH_2O, B = 10% CH_3CN/90% ddH_2O, flow rate = 3 mL/min). Oligonucleotides were dialyzed against ddH_2O followed by concentration via lyophilization.

Site-specific conversion of uracil and AP lesions to deletions. The reaction mixture (50 μL) containing *KRAS*-U (5, 7, or 9) (1 pmol), reaction buffer (25 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl, 1 mM DTT, 1 mM EDTA) and uracil-DNA glycosylase (1 U) was incubated at 37 °C for 30 min. Then AP endonuclease (1 μL, 150 nM solution) was added to the reaction mixture, which was incubated at 37 °C for another 30 min and heated to 95 °C for 10 min. Next, 5 μL of DMSO and 1 μL of 3 mM ATP and 2 μL (800 U) of T4-DNA ligase were added to the reaction and held at 25 °C overnight (16 h).

^{32}P-Postlabeling. Aliquots (3 μL) after each reaction step (deglycosylation, cleavage, and gap ligation) were taken out of the reaction mixture. The samples after ligation were purified by QIAquick® Nucleotide removal kit from QIAGEN to remove residual ATP that interferes in ^{32}P labeling and concentrated to 3 μL. Then 1 μL of 10 x polynucleotide kinase buffer, γ-^{32}P ATP (0.5 μCi, 2 μCi for ligation experiments), T4-polynucleotide kinase (0.5 μL) and ddH_2O to bring the total volume to 10 μL were added to the samples, and heated at 37 °C for 1 h. The samples

were mixed with Ambion gel loading buffer II in a ratio of 1:2 and analyzed by denaturing 20% PAGE (acrylamide/bisacrylamide 19:1, 35% urea, 27 mA, 3 h) using TBE buffer.

PCR and sequencing of the *KRAS* duplex. The processed *KRAS* duplex was PCR amplified using 1 μM primers, 50 pM template, 200 μM dNTPs, 2 U One Taq DNA polymerase in 20 μL volume reaction. The PCR procedure consisted of initial denaturation at 95 °C for 2 min, followed by PCR (20 cycles). Each cycle consisted of three steps - denaturation at 95 °C for 45 s, annealing at 50 °C for 30 s, and extension at 68 °C for 1.5 min and the final extension for 5 min. The PCR products were purified by agarose 3.5% gel electrophoresis using TAE buffer. DNA sequencing was conducted by the DNA Core Facility at the University of Utah using BigDye Terminator v3.1 Cycle Sequencing Kit. The sequencing reaction mixtures contained 0.1 pmol of analysed DNA and 4 pmol of sequencing primer.

Insertion of the VEGF promoter sequence into the pBR322 plasmid. The pBR322 plasmid (1 μg) was subjected to restriction free (RF) cloning using 5`-CCG CCA GTT GTT TAC CCT CAC AAG AGT CCG GGG CGG GCC GGG GGC GGG GTG AGT CCA TCA CTC GAG CGT TCC AGT AAC CGG GCA TGT T-3` and 5`-AAC ATG CCC GGT TAC TGG AAC GCT CGA GTG ATG GAC TCA CCC CGC CCC CGG CCC GCC CCG GAC TCT TGT GAG GGT AAA CAA CTG GCG G-3` primers (1 μM), dNTPs (200 μM), Phusion® HF DNA polymerase (2 U) in 20 μL volume. Each cycle consisted of initial denaturation at 95 °C for 5 min, followed by 20 cycles. Each cycle consisted of three steps – denaturation at 95 °C for 45 s, annealing at 55 °C for 30 s and extension at 72 °C for 2 min and final extension for 5 min. The product was analyzed by gel electrophoresis agarose 1% using TAE buffer. The plasmid DNA was purified by UltraClean PCR clean-up kit from MO BIO laboratories, Inc. The purified plasmid DNA was transformed to competent *E. coli* then multiplied and isolated by QIAGEN® plasmid maxi kit.

Insertion of the VEGF promoter sequence bearing 8-oxo-7,8-dihydro-2'-deoxyguanosine (OG) into the pBR322 plasmid. Purified plasmid DNA (500 ng) was treated by Nt.Bst.NBI nicking endonuclease (10 U) in 20 μL volume for 1 h. Then the complementary strand (5'-GAT GGA CTC ACC CCG CCC CCG GCC CGC-3') to the target region was added to the reaction mixture and heated to 80 ºC for 3 min and cooled down on ice for 2 min. This cycle was repeated

4 times. The resulting gapped plasmid was purified by Amicon® Ultra 0.5mL centrifugal filters with 100 kDa cut-off washing by T4-DNA ligase buffer. The oligonucleotide 5'-GCG GGC CGG GGG C**O**G GGT GAG TCC ATC-3' bearing an OG nucleotide (**O**) was annealed with the gapped plasmid by heating it to 80 ºC followed by slow cooling. Next, T4-DNA ligase (400 U) was then added to the reaction that progressed for 1 hour. The ligated pBR322 plasmid containing 8-oxoguanine in the *VEGF* promoter sequence was purified by agarose gel (1%) electrophoresis with TAE buffer.

Detection of an OG in pBR322 plasmid by site-specific excision. Reaction mixture (10 μL) containing pBR322 plasmid with an OG lesion (5 ng), reaction buffer (25 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl, 1 mM DTT, 1 mM EDTA), Fpg (5 U), and Endonuclease IV (10 U) was incubated at 37 °C for 30 minutes. Finally, 0.5 μL of 2 mM ATP and 1.5 μL (600 U) of DNA ligase were added to the reaction and kept at 25 °C for 2 h. The processed pBR322 plasmid (2 μL from previous reaction) was PCR amplified using primers (1 μM), dNTPs (200 μM), Phusion® HF DNA polymerase (2 U) in 20 μL volume. The PCR procedure consisted of initial denaturation at 95 °C for 2 min, followed by PCR (20 cycles). Each cycle consisted of three steps - denaturation at 95 °C for 45 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1.5 min and final extension for 5 min. The PCR products were purified by agarose 2 % gel electrophoresis using TAE buffer. DNA sequencing was conducted using BigDye Terminator v3.1 Cycle Sequencing Kit.

Table S1. Oligonucleotides used in this study.

Name	DNA Sequences Studied
KRAS-OG	Primer Region 1: 5'-CAGGAAACAGCTATGAC / 3'-X-T_{10}-GTCCTTTGTCGATACTG; *KRAS* Sequence: ACTCTTGCCTACGCCA**C**GAGCTCCAACTAC / TGAGAACGGATGCGGT**O**CTCGAGGTTGATG; Primer Region 2: ACTGGCCGTCGTTTTAC-3' / TGACCGGCAGCAAAATG-T_{10}-X-5'
KRAS-U	Primer Region 1: 5'-CAGGAAACAGCTATGAC / 3'-X-T_{10}-GTCCTTTGTCGATACTG; *KRAS* Sequence: ACTCTTGCCTACGCCA**U**GAGCTCCAACTAC / TGAGAACGGATGCGGT**G**CTCGAGGTTGATG; Primer Region 2: ACTGGCCGTCGTTTTAC-3' / TGACCGGCAGCAAAATG-T_{10}-X-5'
KRAS-U-7	Primer Region 1: 5'-CAGGAAACAGCTATGAC / 3'-X-T_{10}-GTCCTTTGTCGATACTG; *KRAS* Sequence: ACTCTTGCCTACGCCA**U**CAGCT**U**CAACTAC / TGAGAACGGATGCGGT**G**GTCGA**G**GTTGATG; Primer Region 2: ACTGGCCGTCGTTTTAC-3' / TGACCGGCAGCAAAATG-T_{10}-X-5'
KRAS-U-9	Primer Region 1: 5'-CAGGAAACAGCTATGAC / 3'-X-T_{10}-GTCCTTTGTCGATACTG; *KRAS* Sequence: ACTCTTGCCTACGCCA**U**CAGCTCC**U**ACTAC / TGAGAACGGATGCGGT**G**GTCGAGG**T**TGATG; Primer Region 2: ACTGGCCGTCGTTTTAC-3' / TGACCGGCAGCAAAATG-T_{10}-X-5'
KRAS-U-11	Primer Region 1: 5'-CAGGAAACAGCTATGAC / 3'-X-T_{10}-GTCCTTTGTCGATACTG; *KRAS* Sequence: ACTCTTGCCTACGCCA**U**CAGCTCCAA**U**TAC / TGAGAACGGATGCGGT**G**GTCGAGGTT**G**ATG; Primer Region 2: ACTGGCCGTCGTTTTAC-3' / TGACCGGCAGCAAAATG-T_{10}-X-5'

X = triethylene glycol cap O = 8-oxoguanine

Figure S1. Scheme for the gap ligation method performed on a duplex bearing two uracil lesions at a distance of 5 nucleotides apart.

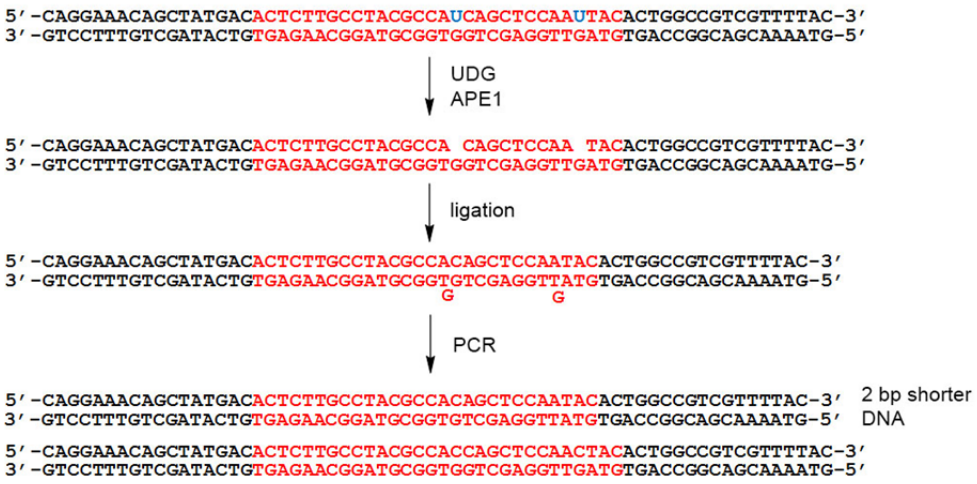

Figure S2. Scheme for the gap ligation method performed on a duplex bearing two uracil lesions at a distance of 9 nucleotides apart.

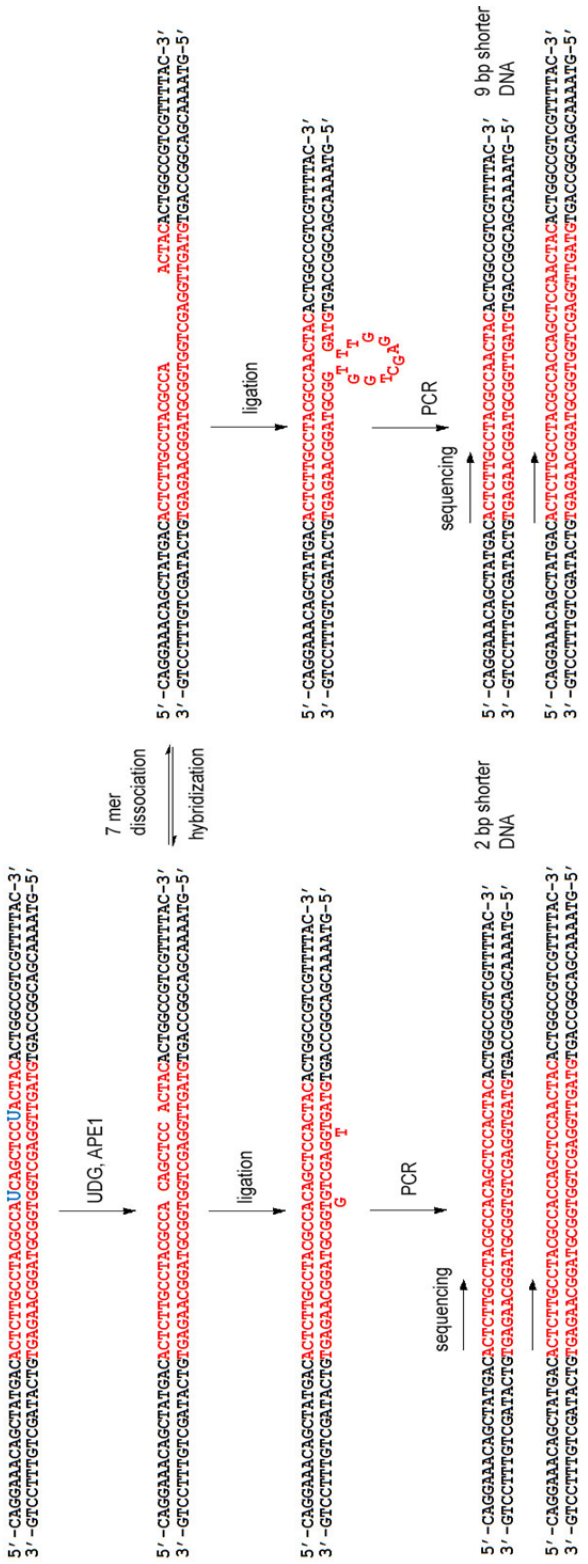

Figure S3. Scheme for the gap ligation method performed on a duplex bearing two uracil lesions at a distance of 7 nucleotides apart.

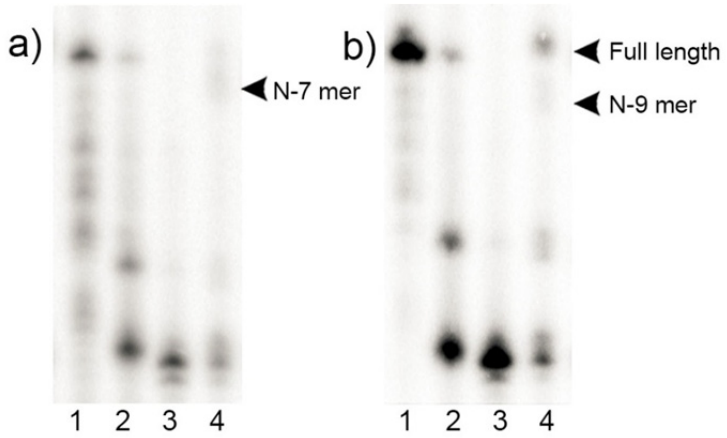

Figure S4. a) A denaturing-PAGE autoradiogram depicting samples isolated at various steps during the site specific removal of two uracil lesions positioned 5 bp apart by single nucleotide gap ligation. The samples depicted are as follows: lane 1, *KRAS*-U; lane 2, UDG treatment; lane 3, APE1 treatment; lane 4, T4-DNA ligase treatment (18% yield). b) A denaturing-PAGE depicting samples isolated at various steps during the site specific removal of two uracil lesions positioned 7 bp apart by single nucleotide gap ligation. The samples depicted are as follows: lane 1, *KRAS*-U9; lane 2, UDG treatment; lane 3, APE1 treatment; lane 4, T4-DNA ligase treatment (26% yield).

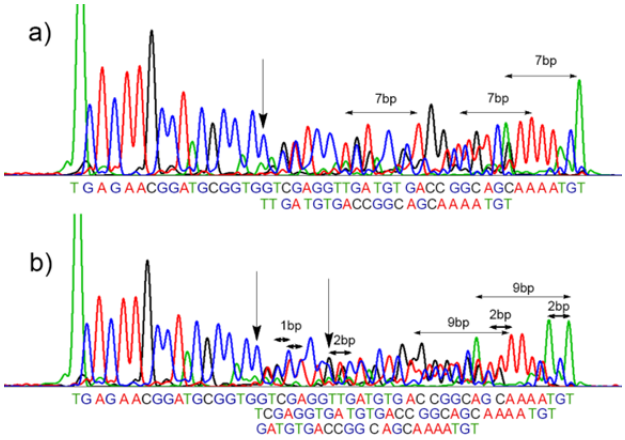

Figure S5. Sequencing of amplified DNA after conversion of a uracil to a deletion after 25 cycles of amplification. (a) Sequencing of the amplified processed duplex DNA bearing two uracil lesions 5 bases apart. (b) Sequencing of the amplified processed duplex DNA bearing two uracil lesions 7 bases apart.

Figure S6. A denaturing-PAGE autoradiogram depicting samples isolated at various steps during the site specific removal of 8-oxoguanine lesion by single nucleotide gap ligation. The samples depicted are as follows: lane 1, *KRAS*-OG; lane 2, FPG treatment; lane 3, endonuclease IV and polynucleotide kinase treatment; lane 4 and 5, incorporation of dNTP verifying full procession of a single nucleotide gap; lane 6, T4-DNA ligase treatment.

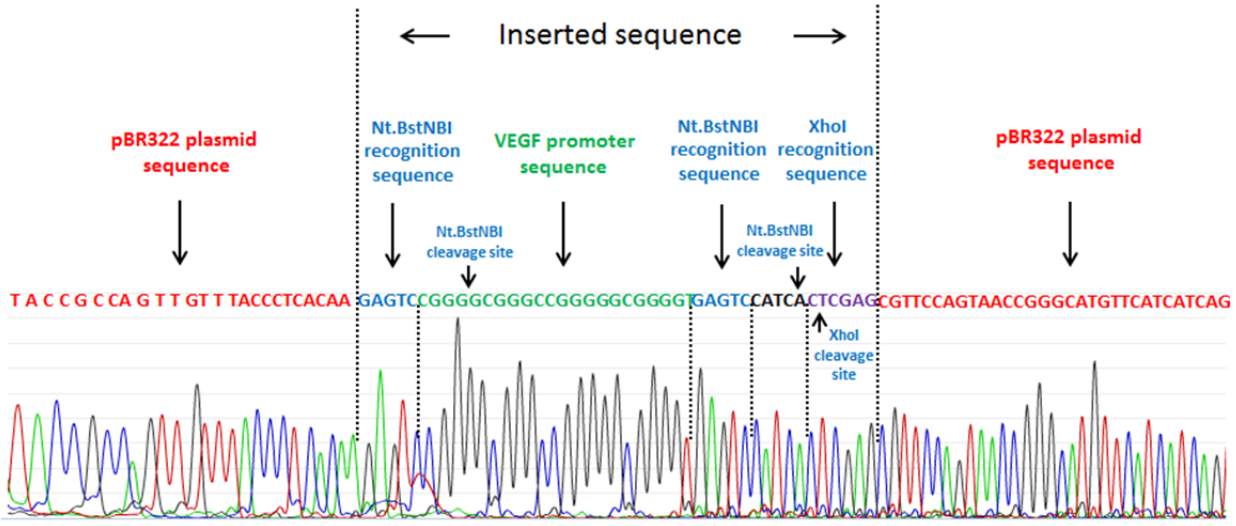

Figure S7. Sequencing of the pBR322 plasmid with the inserted *VEGF* promoter sequence and recognition sequence for Nt.BstNBI nicking endonuclease.