Supporting Information.

Mutants of the Base Excision Repair Glycosylase, Endonuclease III: DNA CT as a First Step in Lesion Detection

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EndoIII Mutagenesis Primers. Mutations are emphasized in bold, capital lettering.

H140A

5'- ccgactattgctgtcgacacg**GCC**attttccgcgtttgtaatcg- 3' (forward)

5'- cgattacaaacgcggaaaat**GGC**cgtgtcgacagcaatagtcgg- 3' (reverse)

L81C

5'- gggtgaaaacctatatcaaaacgattggg**TGT**ataacagcaaagc- 3' (forward)

5'- gctttgctgttata**ACA**cccaatcgttttgatataggttttcaccc- 3' (reverse)

Y82S

5'- ggggtgaaaacctatatcaaaacgattgggctt**TCT**aacagcaaagc – 3' (forward)

5'- gctttgctgttagaaagcccaatcgttttgatataggttttcacccc-3' (reverse)

Y82W

5' - ggggtgaaaacctatatcaaaacgattgggctt**TGG**aacagcaaagc – 3' (forward)

5'-gctttgctgttccaaagcccaatcgttttgatataggttttcacccc-3' (reverse)

W178A

5'-gtcgactgccaccat**GCG**ttgatcctgcacgggcg-3' (forward)

5'- cgcccgtgcaggatcaa**CGC**atggtggcagtcgac- 3' (reverse)

Y82F

5'-ggtgaaaacctatatcaaaacgattgggctt**TTT**aacagcaaagc-3' (forward)

5'-gctttgctgtt**AAA**aagcccaatcgttttgatataggttttcacc-3' (reverse)

Y82C

5’-ggtgaaaacctatatcaaaacgattgggctt**TGT**aacagcaaagc-3’ (forward)

5’-gctttgctgtt**ACA**aagcccaatcgttttgatataggttttcacc-3’ (reverse)

Y185A

5’-cctgcacgggcgt**GCT**acctgcattgcccgcaagccccgc-3’ (forward)

5’-gcggggcttgcgggcaatgcaggt**AGC**acgcccgtgcagg-3’ (reverse)

F30A

5’- ccgagcttaatttcagttcgcct**GCT**gaattgctgattgccgtactgc- 3’ (forward)

5’- gcagtacggcaatcagcaattc**AGC**aggcgaactgaaattaagctcgg- 3’ (reverse)

Y55A

5’- gcgacggcgaaactc**GCC**ccggtggcgaatacgcctgcagc -3’(forward)

5’- gctgcaggcgtattcgccaccgg**GGC**gagtttcgccgtcgc –3’(reverse)

Y75A

5’-gaaggggtgaaaacc**GCT**atcaaaacgattgggctttataacagc-3’ (forward)

5’-gctgttataaagcccaatcgttttgat**AGC**ggttttcacccttc-3’ (reverse)

Figure S1: Glycosylase Assay results of EndoIII variants. For this assay, 1 μM, 100 nM, or 10 nM protein sample was incubated with 100 nM of 5'-radiolabeled 35-mer duplex DNA containing 5-hydroxy-uracil. Reactions were incubated for 15 minutes at 37°C and quenched with 1 M NaOH. Reactions were then examined by denaturing gel electrophoresis. Cleavage of the DNA results in a 14-mer. The representative gel below shows the results of WT EndoIII (blue) compared to Y75A (purple) and Y185A (black). Enzyme-free control reactions were loaded into the final two lanes. The reactions with 1 μM protein were used to compare the glycosylase activities of EndoIII variants (Table 2).

Figure S2. Synthesis of DNA with site-specific CA mismatch. Two synthesized phosphorylated primers, one incorporating a 2'-O-methyl ribonucleotide (black and gray, respectively), are utilized to amplify plasmid sequences via PCR. A nucleotide is incorporated within the synthesized primer containing the 2'-O-methyl ribonucleotide (green) that will result in a mismatched or matched final product. As the vector is amplified, polymerase stops at the ribonucleotide, resulting in a large single-strand overhang. Upon performing two separate PCR reactions (red and blue) with two primers each, the resulting duplexes (2.2 or 1.6 kbp) with 14 bp single strand overhangs can be annealed/ligated together in good yield to produce long duplexes with or without a mismatch.

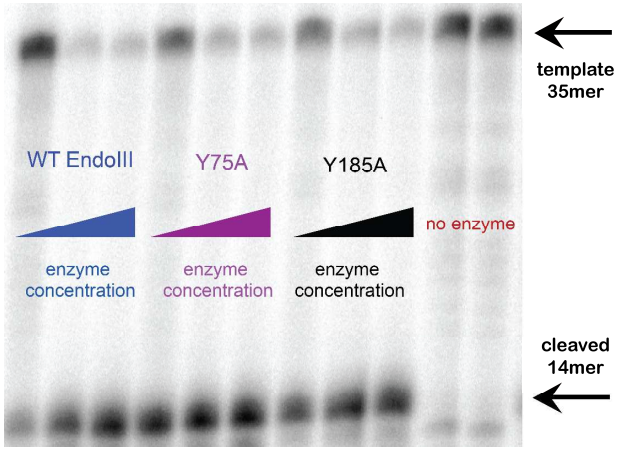
WT EndoIII
Y75A
Y185A
no enzyme
enzyme concentration
enzyme concentration
enzyme concentration
template 35mer
cleaved 14mer

Figure S1.

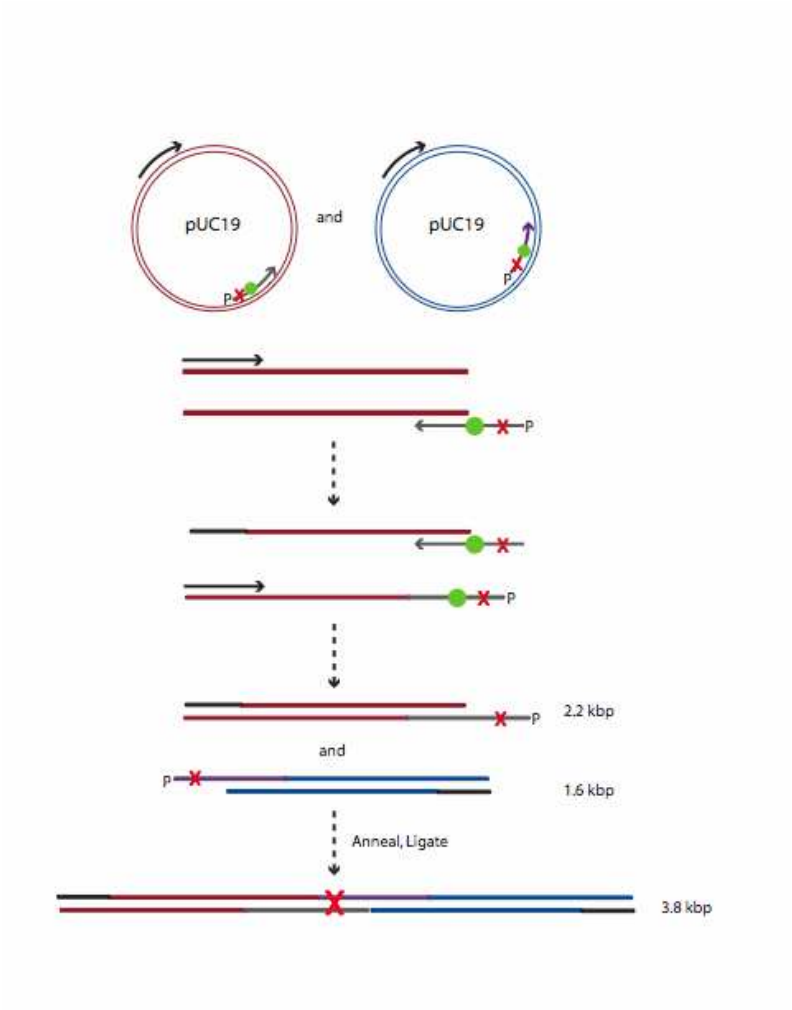

Figure S2.