Supporting Information

Ortega et al. 10.1073/pnas.1417711112

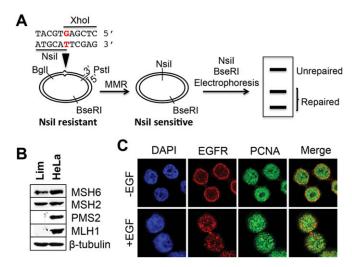


Fig. S1. (A) Principle of in vitro MMR assay. (B) Western blot analysis showing no expression of MLH1 and PMS2 in Lim2405 cells. (C) Immunofluorescence analysis in HeLa cells treated with or without EGF. EGFR molecules translocate into nucleus from cytosol in EGF-treated cells, and some of them colocalize with PCNA.

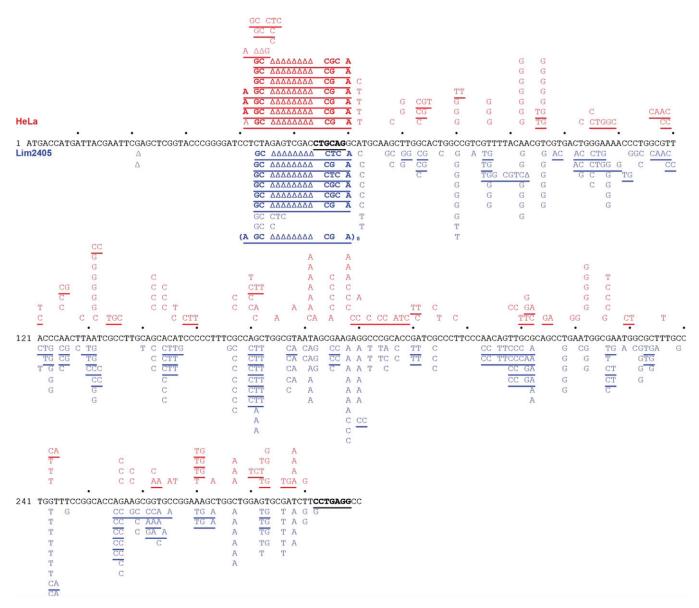


Fig. S2. Mutation spectra induced during gap-filling DNA synthesis by HeLa (red type) and Lim2405 (blue type) extracts. The underlined mutations represent clustered alterations, Δ indicates a deletion, and the underlined bold sequences CTGCAG and CCTGAGG are recognition sequences of PstI and Bsu36I, respectively, which were used to generate single-stranded gap in gapped substrate construction (see Fig. 6A).

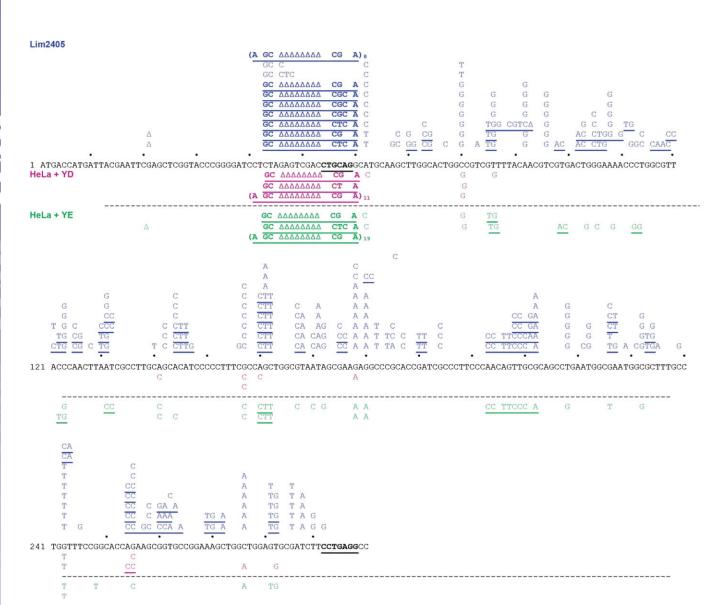


Fig. S3. Mutation spectra induced during gap-filling synthesis by extracts of Lim2405 (blue type) and HeLa supplemented with PCNA-Y211D (purple type) or PCNA-Y211E (green type). The underlined mutations represent clustered alterations, Δ indicates a deletion, and the underlined bold sequences CTGCAG and CCTGAGG are recognition sequences of PstI and Bsu36I, respectively, which were used to generate single-stranded gap in gapped substrate construction (see Fig. 6A).

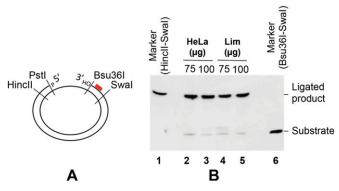


Fig. S4. Analysis of nick ligation after gap-filling DNA synthesis. (*A*) Gapped DNA substrate. (*B*) Ligation analysis. Gapped substrate was incubated with the indicated amount of HeLa or Lim2405 extract at 37 °C for 20 min, and the DNA products were digested with HincII and SwaI and followed by Southern blot analysis, using a ³²P-labeled oligonucleotide probe (red bar) complementary to the synthesized strand between Bsu36I and SwaI. The ligated product (lane 1) and the substrate markers (lane 6) were created by digesting dsM13mp18 with HincII-SwaI and Bsu36I-SwaI, respectively.