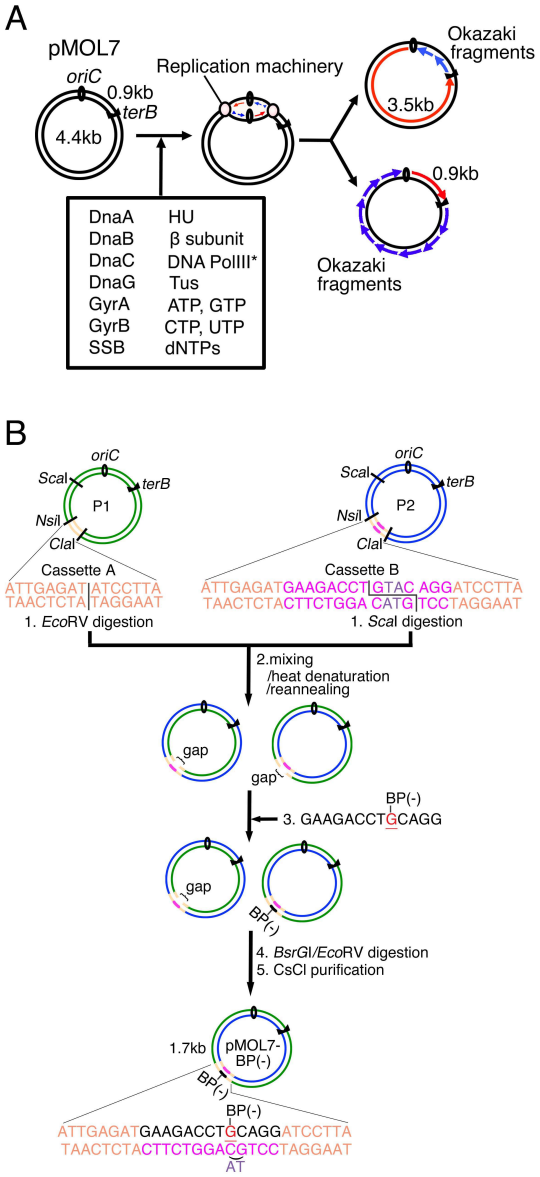
Supplemental Figure



Supplemental Figure Legend

(A) ***Diagram showing the reconstitution of semi-bidirectional DNA replication of an oriC-terB plasmid.*** Reconstituted replication forks proceed bidirectionally with concurrent strand synthesis. Black lines indicate template DNA strands. Red and blue lines with arrowheads indicate leading- and lagging-strand synthesis, respectively. Tus protein blocks the clockwise replication fork at the *terB* sequence. To reconstitute the replication fork, the *oriC-terB* plasmid pMOL7 was incubated with replicative proteins in the presence of dNTPs. The reaction mixtures (15 μl) contained 40 mM Hepes-KOH (pH 7.6), 10 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 10 mM magnesium acetate, 4 mM creatine phosphate, 2 mM ATP, 1 mM each of GTP, CTP, and UTP, 0.1 mM each of dATP, dGTP, dCTP, and dTTP, 30 ng template DNA (0.67 nM), 40 μg/ml creatine kinase, 50 μM NAD, 174 ng single-strand DNA binding protein (SSB), 30 ng β-subunit, 5 ng HU, 108 ng DNA gyrase A subunit, 216 ng DNA gyrase B subunit, 90 ng DnaB, 194 ng DnaG, 55 ng DnaC, 67 ng Tus, 90 ng DnaA, and 14.4 units of Pol III*. The reaction was initiated by the addition of DnaA and incubated at 30°C for the indicated time.

(B) ***Strategy for the construction of template plasmids pMOL7-BP(−).*** Plasmids containing a single dG-BP(−) lesion are prepared by the gapped-duplex strategy. 1. parental plasmids P1 and P2 were digested by *Eco*RV and *Sca*I, respectively. The sequences of these plasmids were identical to that of pMOL7 except that the fragments between *Nsi*I and *Cla*I site were replaced by cassette A containing an *Eco*RV site (P1) or cassette B containing a *Bsr*GI site (P2). 2. Equimolar quantities of the linearized plasmids were mixed, heat denatured, and re-annealed to produce two types of double-stranded intermediates with a 15-mer ssDNA gap. 3. The 13-mer oligonucleotides containing the dG-BP(−) lesion (5´-GAAGACCT$\underline{G}^{BP(-)}$CAGG-3´) were ligated with one type of the gapped-duplex. The resulting covalently closed circular plasmid, called pMOL7-BP(−), contained a 2-nt mismatch across from the dG-BP(−) adduct. 4. The restriction enzyme treatments linearized contaminating parental plasmids, while pMOL7-BP(−) remained circular. 5. pMOL7-BP(−) was separated from other species by CsCl density gradient centrifugation in the presence of

ethydium bromide. To introduce negative supercoils to plasmids, 1.2 μg of plasmid DNA was treated with 4.8 units of gyrase AB (New England Biolabs) for 30 min at 37˚C and then purified for *in vitro* replication assay. The control plasmid, called pMOL7-control, was prepared by the same procedure but using the 13-mer oligonucleotides without adducts.