

SI: Materials and Methods

Viral Suspension. *Escherichia coli* bacteriophages λ (ATCC 23724-B2) obtained from ATCC (Manassas, VA) were used in this study. *Escherichia coli* bacteriophages λ were propagated in bacterial host *Escherichia coli* C600 (ATCC 23724) in a TNT broth (Tryptone, Sodium chloride and Thiamine media) to a titer of 10^6 PFU/mL.¹ Cell culture and phage propagation followed the ATCC protocol.

Chemicals. Purified λ DNA (48.5 kb), PDG (pyrimidine dimer glycosylase), and dNTPs were purchased from New England Biolabs (Ipswich, MA). DNA Polymerase I was purchased from Roche Applied Sciences (Indianapolis, IN). *N*-trimethylsilylpropyl-*N,N,N*-trimethyl ammonium chloride and vinyl trimethoxy silane were purchased from Gelest (Tullytown, PA). High purity ethanol (99.5 %) was purchased from Samchung Chemical (Seoul, Korea). SU-8 and its developer were purchased from Microchem (Newton, MA). Polydimethyl siloxane (PDMS) and its curing agents were purchased from Dow Chemical (Midland, MI). Low melting point agarose (LMP agarose) was purchased from Lonza (Basel, Switzerland). Alexa Fluor 647-labeled dUTPs and dCTPs were purchased from Invitrogen (Carlsbad, CA). All the other chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

Glass Surface Preparation. Positively charged glass surfaces were prepared following the references.² Briefly, glass coverslips (22 x 22 mm) were racked in custom-made Teflon racks, cleaned by boiling in piranha solution (sulfuric acid and hydrogen peroxide 4:1) for 50 min, and rinsed extensively with high-purity water (18.2 M Ω) until pH became neutral. Each coverslip was rinsed three times in high purity ethanol (99.5 %). Then, they were stored in high purity ethanol in a polypropylene container at room temperature. For surface derivatization, 22 glass surfaces (22 mm x 22 mm cover slips) were placed in a Teflon block holder in a clean container and allowed to dry for 10 min at room temperature. The derivatization solution was prepared by mixing 60 μ L of *N*-trimethylsilylpropyl-*N,N,N*-trimethyl ammonium chloride and 3 μ L of vinyl trimethoxy silane into 250 mL high-purity water. The solution was poured into the container of 22 glass slides and incubated at 60°C with 50 rpm of continuous shaking overnight. Finally, the surfaces were rinsed three times with high-purity water and once with ethanol and then stored in high-purity ethanol.

PDMS Channel Preparation. PDMS microchannels were prepared using soft lithography as previously described.² First, a photoresist (SU-8 2005) template was created on the silicon wafer with each channel having dimensions of 100 μ m (width) x 5 μ m (height) x 1 cm (length). The mixture of PDMS and curing agent in a 10:1 ratio was poured onto the microchannel template on a silicon wafer and incubated for 3 hours at 65 °C. After peeling off from the template, the PDMS microchannels were oxidized in air plasma conditions for 30 sec (CuteBasic, Femto, Korea). Then, PDMS was washed and stored in high-purity water.

DNA Mounting. A PDMS device was mounted on the positively charged surface. Then, the solution of DNA molecules (2 ng/ μ L) was loaded onto the entrance of the microfluidic channels. While the solution moved through the microchannels by

capillary action, DNA molecules were elongated and deposited on the positively charged surface. To prevent DNA desorption from the surface during UV irradiation and subsequent chemical reactions, a thin layer of acrylamide (3.3%) was added to the surface and cured. After curing, the surface with the acrylamide layer was washed with Tris EDTA buffer (pH 8.0, 10 mM Tris and 1 mM EDTA; 1xTE).

UV Irradiation for Double Strand Breaks. A UV-irradiation instrument of UVP (Upland, CA) with a multi-wavelengths UV lamp (model: 3UV-36) was used to irradiate ultraviolet to immobilized DNA on the surface (Figure 1a). Ultraviolet radiation of 254 nm (UV-C) was exposed at 1.29 mW/cm² for 5 min (300sec, 389 mJ/cm² for UV light). The intensity of UV light was measured with a UV Radiometer (Giltron, Seoul, Korea). After UV irradiation, T4 Endonuclease V (Pyrimidine Dimer Glycosylase: PDG) was added to remove pyrimidine dimers. On each cover glass, 200 μL of enzyme solution (80 units in NEB buffer 2) was added and incubated in a humidified chamber at 37°C for 20 min. After PDG digestion, the surface was washed three times with 200 μL of 1xTE buffer solution and then stained with 12 μL of 0.2 μM YOYO-1 with 4% β-mercaptoethanol of an anti-bleaching agent.

UV Irradiation on Bacteriophage λ. Bacteriophage λ (10⁶ pfu) was exposed to 254 nm (UV-C) light for 30 sec (63.9 mJ/cm²) in a tube. After irradiation, the irradiated bacteriophage suspension was combined with the melted 1% low melting point agarose (1:19 ratio by volume). The molten agarose mixture was sprinkled on the petri dish by 30 μL droplets and solidified at 4 °C for 10 min. To extract DNA from the λ phage, agarose plugs were incubated for 2 hours at 37°C with 70 μL proteinase K solutions (50 units proteinase K in 1xTE). After DNA extraction, agarose plugs were washed with 1 mL of 1xTE buffer for 1 hour with gentle agitation. After rinsing the plugs, 70 μL PDG solutions (10 units T4 PDG in 1x T4 PDG reaction buffer) were added to remove the pyrimidine dimers.

Nick Translation on Labeling Single Strand Breaks. DNA polymerase I was used for labeling SSB sites. On each agarose plug, 70 μL of labeling solution (10 units polymerase I with 100 μM Alexa 647-labeled dUTPs, 100 μM Alexa 647-labeled dCTPs, 100 μM, dTTPs, 100 μM dCTPs, 1 mM dATP and 1 mM dGTP in polymerase I reaction buffer; 50 mM Tris-HCl, 1 mM dithioerythritol, 10 mM MgCl₂, pH 7.5) was added and incubated in a humidified chamber at 37°C for 1 hour. After nick translation, agarose plugs were washed with 1 mL of 1xTE buffer for 1 hour with gentle agitation. After washing, agarose plugs were melted by increasing the temperature up to 65°C for 10 min in 70 μL of 1xTE. Then, DNA molecules were stained with 10 μL of 0.2 μM YOYO-1 with 4% β-mercaptoethanol. For the control experiment in *SI* Figure S2, nick translations with or without nicking enzymes (Nt. BbvCI) were also performed with λ DNA extracted from phage particles.

Microscope. The microscopy system consisted of an inverted microscope (Zeiss Observer A1, AG, Germany) equipped with a 63 × Zeiss Plan-Neofluar oil immersion objective illuminated by a solid-state laser (Coherent Sapphire 488, Santa Clara, CA). The laser light was focused into the multimode optical fiber (BFH-22-550, Thorlabs, Newton, NJ) and passed through a holographic notch filter for 488 nm (NamilOptical Components Corp, Incheon, Korea) that was installed to prevent 488 nm

laser light from reaching an electron multiplying charge-coupled device digital camera (EMCCD: Evolve, Roper Scientific, Tucson, AZ). Fluorescence images were captured by EMCCD and stored in 16-bit TIFF format generated by RS Image (Roper Scientific). For image processing and length measurement, homemade ImageJ application was utilized in “FIJI” software.³

1. Campbell A, *Virology*, 1961, **14**, 22-32.
2. E. T. Dimalanta, A. Lim, R. Runnheim, C. Lamers, C. Churas, D. K. Forrest, J. J. de Pablo, M. D. Graham, S. N. Coppersmith, S. Goldstein and D. C. Schwartz, *Anal Chem*, 2004, **76**, 5293-5301.
3. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat Methods*, 2012, **9**, 676-682.

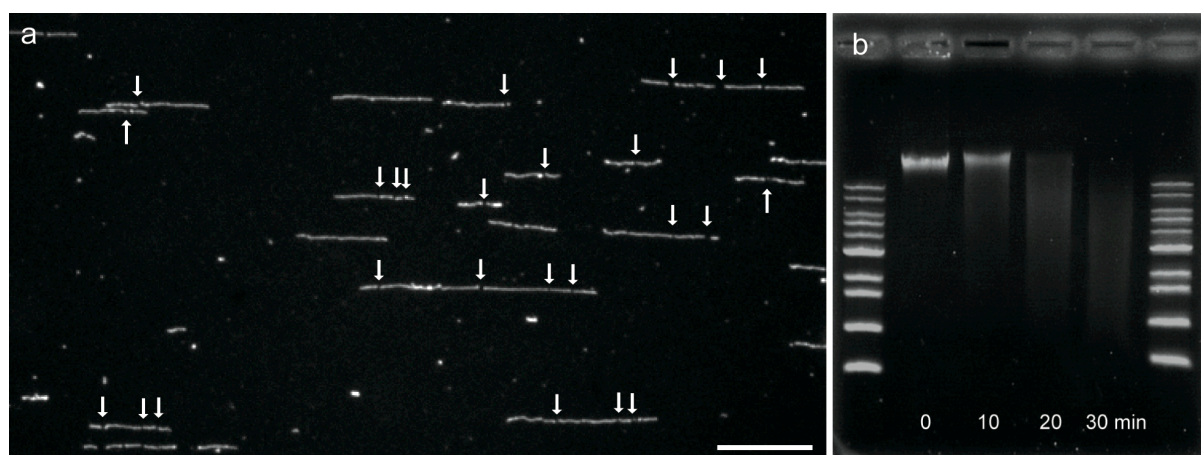


Figure S1. Direct visualization of UV irradiation-induced DSB on single DNA molecules compared with gel electrophoresis results. (a) A fluorescence micrograph of λ DNA concatemer after UV-C (254 nm) irradiation for 5 min followed by T4 PDG (pyrimidine dimer glycosylase) enzyme treatment. Scale bar 20 μ m. (b) Gel electrophoresis results of UV-C damaged DNA in test tubes after T4 PDG treatment. From the left, 1) 1 kb ladder, 2) λ DNA (48.5 kb), 3) λ DNA after 10 min, 4) 20 min, 5) 30 min of UV exposure, 6) 1 kb ladder.

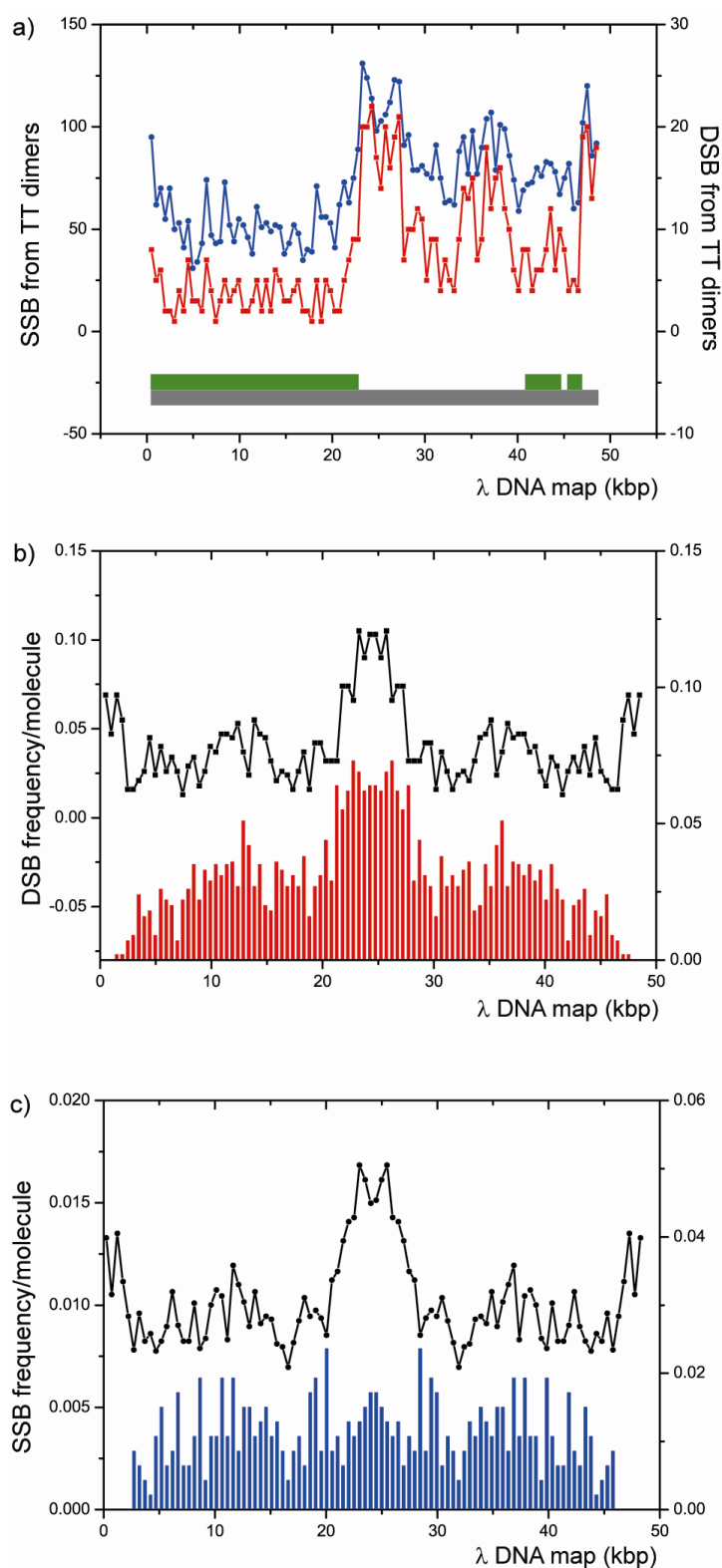


Figure S2. (a) Expected SSB (circle) and DSB (square) patterns in the λ DNA *in silico* map. Green bars present the locations of essential genes. (b) Comparison of TT dimer map and irradiation-damaged DNA patterns overlapped from both directions. DSB of both ends were calculated from λ concatemers. In these graphs, 48,502 bp were divided into 98 segments. (c) Comparison of TT dimer map and irradiation-damaged SSB label patterns. In these graphs, 48,502 bp were divided into 98 segments and each point represents 495 bp.

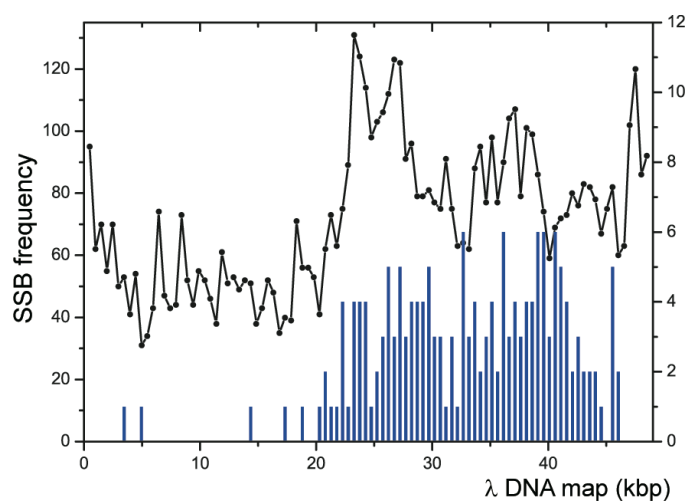


Figure S3. The comparison of the *in silico* TT dimer map with UV-induced SSB DNA patterns from 91 molecules in which SSB labels are located only in half of their backbones with the assumption that the abundance of labels indicates the direction. The whole genome of 48,502 bp is divided into 98 segments and each point represents 495 bp.