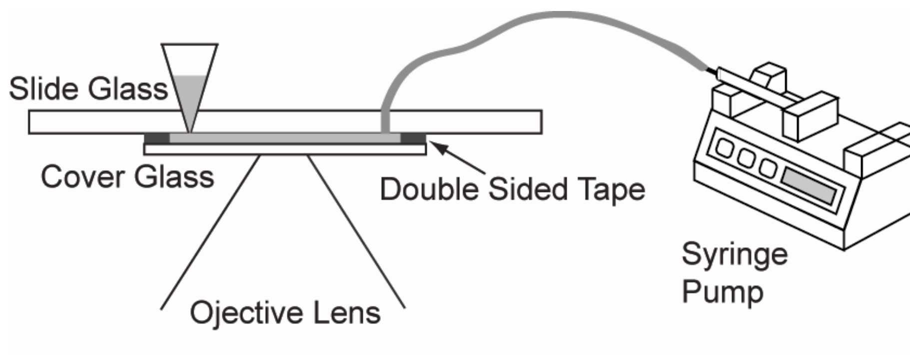
This journal is (c) The Royal Society of Chemistry 2011

Materials and Methods

Chemicals. All enzymes and λ DNA (48.5 kb) were purchased from New England Biolabs (Ipswich, MA). T4 GT7 DNA (165,644 bp) was purchased from Nippon Gene (Tokyo, Japan). Epoxy was from Devcon (Riviera Beach, FL). Biotin-labeled bovine serum albumin (BSA) was from Sigma (St. Louise, MI) and neutravidin was from Pierce (Rockford, IL). Biotin labeled oligonucleotide (5'-pGGGCGGCGACCT-TEG-Biotin) was ordered from Bioneer (Daejeon, Korea).

Flow Cell. Flow cell was prepared by a cover slip on the slide glass with the spacing of 100 μm by double-sided tape. On the microscope slide glass, inlet and outlet holes were drilled through the slide glass using a diamond-coated bit. The glasses were cleaned in piranha solution for 30 min, thoroughly rinsed with water and ethanol and stored in methanol before use. A yellow pipette tip was installed on the inlet port and a tubing line was connected to the outlet port with epoxy bonding: cured at RT for 5 min. Then a cover slip was bound on the slide glass by double-sided 3M tape. The dimension of the flow cell was 3 x 17 x 0.1 mm (L x W x H). The total volume of the flow cell was 5.1 μL. A syringe pump NE-1000 (New Era Pump Systems Inc., Wantagh, NY) was used to control buffer delivery to the flow cell: flow rate was 0.150 mL/min. (see Fig. S1)



Figures S1. Experimental Set-Up on the fluorescent microscope. The volume of flow cell was 5.1 μL and the flow rate of syringe pump was 0.150 mL/min

Surface Preparation The surface of the flow cell was initially coated by biotin-BSA followed by neutravidin. For the coating, biotin-BSA stock solution (10 mg/mL, 10mM Tris, 50mM NaCl, pH8.0) was diluted ten times with 10 mM Tris buffer, and then loaded. After five minutes BSA adsorption, neutravidin solution (0.25 mg/mL 10mM Tris, 50mM NaCl, pH8.0) was loaded into the BSA coated flow cell and kept at RT for 5 min.

DNA Loading and Stretching. In order to dehybridize λ concatemer, DNA solution was heated up to 65℃ for 10 min and quickly put into ice water bath. YOYO-1 stained DNA solution (1/50 of stock solution) was loaded with pH 7.5 Tris buffer by using a syringe pump. After coiled DNA randomly adsorbed on the surface, the flow of pH 8.5 Tris buffer passed through for partial desorption and stretching of DNA molecules for five minutes. Then, the flow of pH 7.5 Tris-HCl buffer immobilized stretched DNA molecules. Alternatively, the

flow of 1 mM $MgCl_2$ was used for DNA immobilization.

Restriction Mapping. On the immobilized T4 DNA molecules, BbvC1 (4 unit/30mL in 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.5) was loaded into the flow cell. After 20 min digestion reaction at RT, 0.2 μM YOYO-1 in (10 mM Tris, pH 7.5) was loaded into the cell for DNA visualization.

Tethered DNA Ligation. After sequential coating of biotin-BSA and neutravidin, biotin linked oligonucleotides (5'-pGGGCGGCGACCT-TEG-Biotin, 1μM) was loaded. After five minutes anchoring reaction, 50 μL λ-DNA solution with T4 DNA ligase (200 units) was loaded. After 30 minute ligation reaction, 0.2 μM YOYO-1 in Tris-HCl buffer (10mM tris, pH 8.3) was loaded into the flow cell for DNA visualization.

Microscope. 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 a holographic notch filter for 488 nm (Namil Optical Components Corp, Incheon, Korea) was installed to prevent 488 laser light from reaching EMCCD camera. The power of a solid-state laser was reduced by an additional optical density filter (NDQ-100-1.00, Korea Electro Optics, Bucheon, Korea) to acquire DNA images without significant photo-damage. Fluorescence images were captured by a electron multiplying charge coupled device digital camera (Evolve EMCCD, Roper Scientific, Tucson, AZ) and stored as 16 bit TIFF format generated by a software of RS Image (Roper Scientific, Tucson, AZ). For image processing and length measurement, ImageJ was utilized with Java plug-in developed in our lab.