SI: Materials and Methods

Chemicals. Ligation sequencing kit (SQK-LSK 114) and flow cell wash kit (EXP-WSH 004) were purchased from Oxford Nanopore Technologies (Cambridge, UK). QIAquick PCR purification kit, QIAprep spin miniprep kit, QIAquick gel extraction kit and Ni-NTA agarose resin were purchased from Qiagen (Hilden, Germany). AccuRapid cloning kit, AccuPower PCR PreMix and DEPC-DW were purchased from Bioneer (Daejeon, Korea). Formaldehyde solution, glycine and kanamycin were from Sigma–Aldrich (St. Louis, MO). Ampicillin was purchased from BIO BASIC (Ontario, Canada). SYTOX Orange were from ThermoFisher Scientific (Waltham, MA). Biotin was from Kisan Bio (Seoul, Korea). AMPure XP magnetic beads were from Beckman Coulter (Brea, CA). DNA polymerase I was from Roche Applied Sciences (Indianapolis, IN). Biotin-16-dUTP was from Jena Bioscience (Jena, Germany). The following reagents were purchased from New England Biolabs (Ipswich, MA): λ DNA, NDE I, Xma I, Nb.BssSI, T4 DNA Liagase, PreCR repair mix, NEBNext FFPE DNA repair mix, NEBNext Ultra II End repair/dA-tailing module, NEBNEXT quick ligation module, Blunt/TA ligase master mix, ThermoPol buffer, 10×buffer 3, 10×buffer 3.1, dATP, dTTP, dGTP, dCTP and streptavidin magnetic beads.

Preparation of Cro-mNG-biotin. The Cro gene was obtained from λ DNA by PCR amplification. For the Cro part, a forward primer (5'-GCG GCC TGG TGC CGC GCG GCA GCC ATA TGA TGG AAC AAC GCA TAA CCC TGA AAG ATT ATG C-3') and a reverse primer (5'-TAT CCT CCT CGC CCT TGC TCA CCA TCC CGG GGC CGC CAG AGC CGC CTG CTG TTG TTT TTT TGT TAC TCG GGA AGG-3') were used in 50 μL of AccuPower PCR PreMix. The mNeonGreen (mNG) gene was obtained from AddGene (plasmid #124220),[1] and AviTag (GLNDIFEAQKIEWHE) was added at the C-terminus of the mNG gene. The AccuRapid cloning kit was used to ligate the Cro gene and the mNG-AviTag gene. The constructed Cro-mNG-AviTag plasmid (pET-15b) was transformed into the *E. coli* DH5α strain using the heat shock method. The transformed cells were recovered in fresh LB medium without antibiotics for 1 h and then spread on an LB agar plate with ampicillin overnight. A single colony was picked and the Cro-mNG-AviTag gene was identified by DNA sequencing (Cosmogenetech, Seoul, Korea).

The BirA construct in pET28a (w400-2) was obtained from AddGene (plasmid #26624). The BirA construct in pET28a plasmid and the recombinant Cro-mNG-AviTag plasmid were co-transformed into *E. coli* DH5α using the heat shock method. Transformed cells were recovered in fresh LB medium without antibiotics for 1 h and then spread on an LB agar plate with ampicillin and kanamycin overnight for the selection of co-transformed cells. A single DH5α colony was incubated for 12 h in fresh LB medium supplemented with ampicillin and kanamycin. After saturation, 1 mL of this culture was added to 100 mL of fresh LB medium with corresponding antibiotics and cultured at 37°C until OD600 reached 0.4. Cro-mNG-AviTag and BirA proteins were induced by 1 mM IPTG and 200 μM biotin overnight on a shaker at 20 °C and 200 rpm.[2] The Cro-mNG-biotin (Cro-mNG-b) was produced for BirA to attach a biotin to Cro-mNG-AviTag. The protein

was purified using Ni-NTA His-tag affinity chromatography. SDS–PAGE was performed to confirm the purity of the protein and its biotinylation.

RP-11 BAC 436k12 DNA purification. 100 μL of the *E. coli* cell stock solution was incubated for 16 h in fresh LB medium supplemented with chloramphenicol. After saturation, 1.5 mL of this culture was added to 250 mL of fresh LB medium with corresponding antibiotics and cultured at 37 °C until OD600 reached 2.5. The protocol of Phaseprep BAC DNA Kit from Sigma–Aldrich (St. Louis, MO) was used for a midi prep. The DNA solution was buffer exchanged with 1× TE for 3 times.

Preparation of biotin-labeled DNA by nick translation. The mixture contained 4 μL of 500 ng/μL λ phage DNA, 3 μL of 10× NEB buffer 3.1, 2 μL of Nb.BssSI (2 U/μL), 2 μL of DNA polymerase I (5 U/μL), 2 μL of dNTP mix (0.33 mM dATP, dGTP, dCTP, 0.03 mM dTTP, and 0.03 mM biotin-16-dUTP), and DEPC-DW to a final volume of 30 μL. The reaction mixture was incubated for 1 h at 37°C.

Separation of biotin-labeled samples into positive and negative solutions. Biotinylated samples were isolated using streptavidin magnetic beads. DEPC-DW was prewarmed in a 70°C bath, and low salt buffer (20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.15 M NaCl) was placed in an ice bath in advance. Then, 100 μL of streptavidin magnetic beads was resuspended in 100 μL of wash/binding buffer (20 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 0.5 M NaCl) in a tube and placed on a magnetic separation rack (MagListo-2, Bioneer) for 30 sec. After the supernatant was removed, 30 μL of the solution of biotinylated and fragmented DNA was added to a single tube; the sample was resuspended, and incubated for 10 min at RT. The tube was placed on a magnetic separation rack, and the supernatant (negative) was removed from the tube with a pipette and stored in a new tube. The DNA molecules in the tube on the magnetic separation rack were washed 10 times with wash/binding buffer and 2 times with low salt buffer. The 25 μL of prewarmed DEPC-DW was added; the mixture was resuspended, and the samples were incubated to elute the biotinylated DNA molecules for 2 min at 70°C. The tube was again placed on a magnetic separation rack. The supernatant was removed from the tube with a pipette and stored in a new tube. The previous step was repeated with the remaining beads. Thus, two tubes containing positive and negative solutions were obtained.

Preparation of Cro-mNG-b bound DNA. λ DNA (2 ng/μL) was incubated with Cro-mNG (0.37 μM) in 200 mM NaCl added 1×TE buffer for 30 min at RT. For crosslinking Cro on DNA, the mixture was incubated with formaldehyde (1% v/v) for 7 min at RT. After crosslinking DNA and protein, glycine (125 mM) was added and incubated for 5 min to neutralize formaldehyde with occasional shaking. The solution was dialyzed using a 25 nm MCE membrane. The sample was sonicated by a WUC-A02H ultrasonic cleaner (Daihan Scientific, Korea). The sonication was continuously performed for 20 min at a maximum power of 161 W for DNA fragmentation.

Preparation of DNA damaged by Nb.BssSI. The mixture contained 1 μg of RP-11 436k12 BAC DNA, 3 μL of NEB 10× buffer 3.1, 3 μL of Nb.BssSI (2 U/μL), and DEPC-DW to a final volume of 30 μL. The mixture was incubated for 5 min at

37 °C. The solution was dialyzed using a 25 nm MCE membrane. The sample was sonicated by a WUC-A02H ultrasonic cleaner (Daihan Scientific, Korea). The sonication was continuously performed for 20 min at a maximum power of 161 W for DNA fragmentation.

Nanopore sequencing. DNA sample was prepared for nanopore sequencing using a ligation sequencing kit (SQK-LSK 114) following a protocol from community.nanoporetech.com. In brief, each library was subjected to DNA repair and end-prep by a NEBNext FFPE DNA Repair Mix and Ultra II End-prep Enzyme mix. After a purification step using AMPure XP magnetic beads, the sequencing adapters were ligated to both ends of the libraries by NEBNext Quick T4 DNA Ligase and Ligation Adaoter (LA). After a final product clean-up using the Short Fragment Buffer (SFB), each sequencing library was loaded into an R10.4.1 version flow cell (FLO-MIN114, ONT). DNA sequencing was performed on a MinION sequencer (MK1B, ONT).

Sequence analysis. The sequencing device control, data acquisition, and real-time base calling were carried out by the MinKNOW software (ONT). The translocation speed, pore occupancy, cumulative output, and N50 of the read length were continuously checked during the sequencing run. Minimap2[3] was used to generate the SAM files, and then samtools was used for conversion to the sorted BAM files. The command "samtools depth" was used to obtain the coverage depth profiles. The result was then displayed in the IGV browser.[4]

Imaging protein bound DNA molecules. A microscopy system consisted of an inverted microscope (Olympus IX70, Japan) equipped with 100× Olympus UPlanSApo oil immersion objectives and an illuminated LED light source (SOLA SM 2 light engine, Lumencor, OR). Fluorescence images were captured by a scientific complementary metal-oxide-semiconductor (sCMOS) camera (PRIME; Photometrics, AZ) and stored in a 16-bit TIFF format generated by Micro-manager software. Cro-mNG-bound DNA was stained with SYTOX Orange. Biotin-labeled λ DNA stained with 100 nM of tTALE-mTurquoise2 and 20 nM of streptavidin-mScarlet. DNA was loaded on a positively charged surface through the PDMS microchannel. These DNA molecules were imaged on a fluorescence microscope using two filter sets and the color images were constructed using merge channels in ImageJ.

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