Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Specific and reversible photochemical labeling of plasmid DNA using photoresponsive oligonucleotides containing 3-cyanovinylcarbazole

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General: Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. Irradiation was performed by UV-LED (OMRON, ZUV, 366 nm, 1,600 mW/cm^2) or 15 W transilluminator (Funakoshi, TR-312R/J, 312 nm). HPLC was performed on a Chemcobond 5-ODS-H column (10 × 150 mm, 4.6 × 150 mm) or a Chemcosorb 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. Reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research. Plasmid DNAs PhiX 174 was purchased from NEB, and other plasmid DNA, M13 mp18, M13 mp19 and pUC18 were purchased from Takara.

Preparation of ODNs: ODN sequences were synthesized by the conventional phosphoramidite method using an Applied Biosystems 3400 DNA synthesizer. The coupling efficiency was monitored with a trityl monitor. The coupling efficiency of a crude mixture of ^{CNV}K showed a 97% yield. The coupling time of a crude mixture of ^{CNV}K was 999 sec. For 5' Cy3 and 3' biotin labeling, Cy3 phosphoramidite (Glen Research) and 3'-Biotin TEG CPG (Glen Research) was used, respectively. They were deprotected by incubation with 28% ammonia for 4 h at 65 ˚C and purified on a Chemcobond 5-ODS-H column (10 ´ 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-25% CH_3CN, linear gradient (30 min) at a flow rate of 2.5 mL/min. Preparation of ODNs was confirmed by MALDI-TOF-MS analysis. MALDI-TOF-MS: calcd. 5750.30 for ODN-1 [(M + H)$^+$], found 5759.87; calcd. 5766.30 for ODN-2 [(M + H)$^+$], found 5767.76; calcd. 5775.31 for ODN-3 [(M + H)$^+$], found 5774.81; calcd. 5683.27 for ODN-4 [(M + H)$^+$], found 5682.4; calcd. 4512.97 for ODN-5 [(M + H)$^+$], found 4512.84; calcd. 5741.19 for ODN-6 [(M + H)$^+$], found 5741.62; calcd. 7001.40 for ODN-7 [(M + H)$^+$], found 7002.94; calcd. 4707.00 for ODN-8 [(M + H)$^+$], found 4707.42.

Photochemical labeling/de-labeling of plasmid DNA: A solution containing ODN and plasmid DNA in 50 mM sodium cacodylate buffer (pH 7.0) and 150 mM NaCl was irradiated with a UV-LED (366 nm ± 15 nm light) at a distance of 1.5 cm. The reaction mixture was heated at 90°C for 7 min, and subsequently cooled in an ice bath. To this

solution, 10 mL of loading buffer (a solution of 80% v/v formamide 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue, and 1.8% orange G) was added and 10 mL of water and the samples (1-2 mL) were loaded onto 0.7% agarose gel and electrophoresed at 50 V for 60 min. The Cy3-labeled ODN in the gel was visualized by a LAS-3000 system (Fujifilm).

Atomic force microscopic analysis: Biotin-labeled M13 mp18 was prepared under the same conditions described above and purified by agarose gel electrophoresis using a DNA purification kit (Qiagen). After the addition of streptavidin (1 equiv.), the solution was diluted with THF (1:10000) and then poured onto an amino-modified mica surface. After washing with deionized water, AFM imaging was performed by an AFM instrument (JSPM-4210, JEOL) in tapping mode. A cantilever (OMCL-AC160TS, Olympus) with resonant frequency of 300 kHz and spring constant of 42 N/m was used.

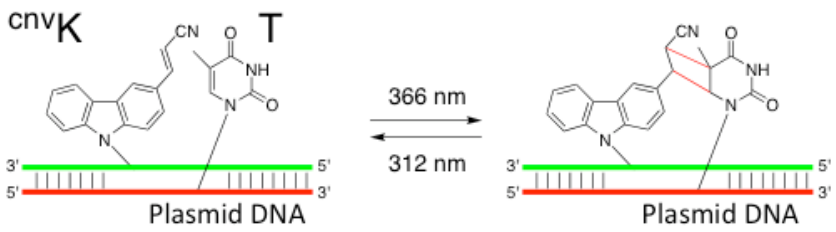

Figure S1. Photocrosslinking reaction on plasmid DNA using photoresponsive ODNs containing ^{CNV}K.

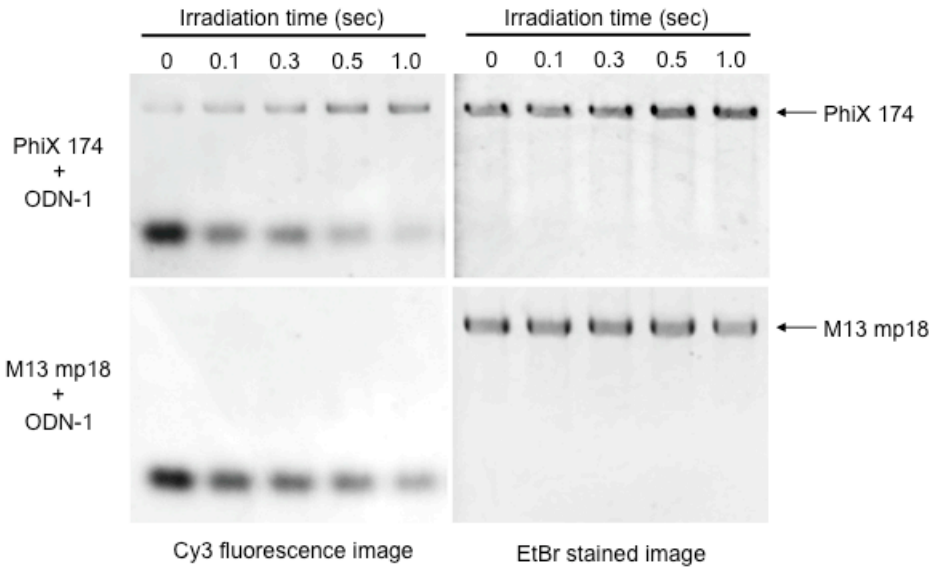

Figure S2. Agarose gel electrophoresis of the fluorescent labeled PhiX 174 with ODN-1. After the 366 nm irradiation, 0.7% agarose gel electrophoresis was performed and imaged with Cy3 fluorescence and ethidium bromide staining. Conditions for photoreaction: [ODN-1] = [PhiX 174 or M13 mp18] = 50 nM in 50 mM Na-cacodylate buffer (pH 7.0) containing 150 mM NaCl, 50ºC.

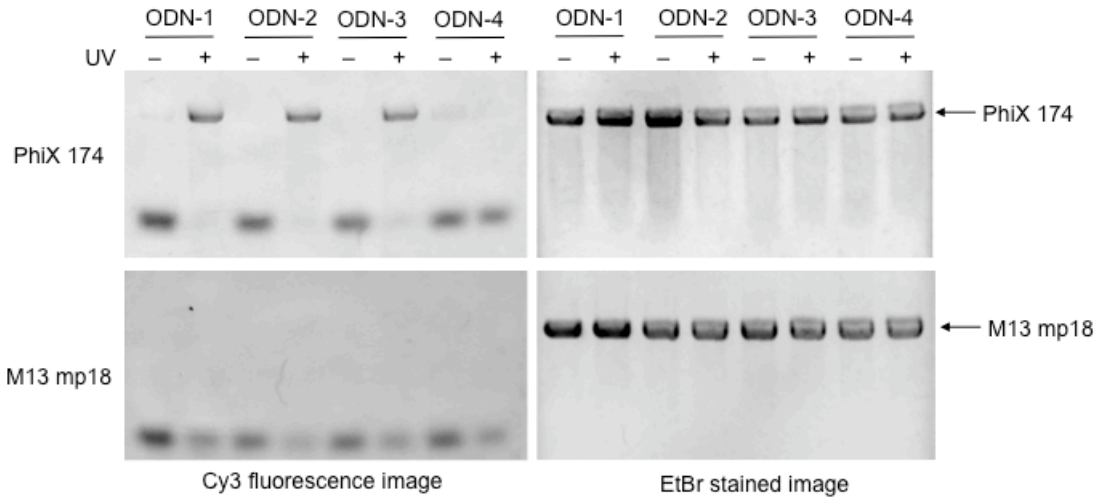

Figure S3. Agarose gel electrophoresis of the fluorescent labeled PhiX 174 with ODN-1, -2, -3 and -4. After the 366 nm irradiation, 0.7% agarose gel electrophoresis was performed and imaged with Cy3 fluorescence and ethidium bromide staining. Conditions for photoreaction: [ODN] = [PhiX 174 or M13 mp18] = 50 nM in 50 mM Na-cacodylate buffer (pH 7.0) containing 150 mM NaCl, 50ºC.

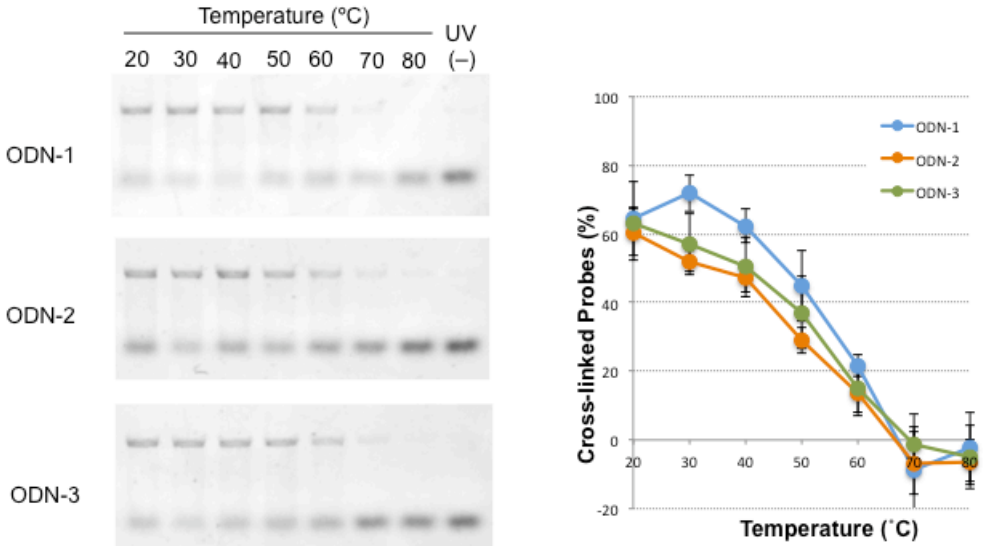

Figure S4. Temperature dependence of the photolabeling reaction of PhiX 174 with ODN-1, -2 and -3. Conditions for photoreaction: [ODN] = [PhiX 174] = 50 nM in 50 mM Na-cacodylate buffer (pH 7.0) containing 150 mM NaCl, 50ºC, 366 nm irradiation for 3 sec.

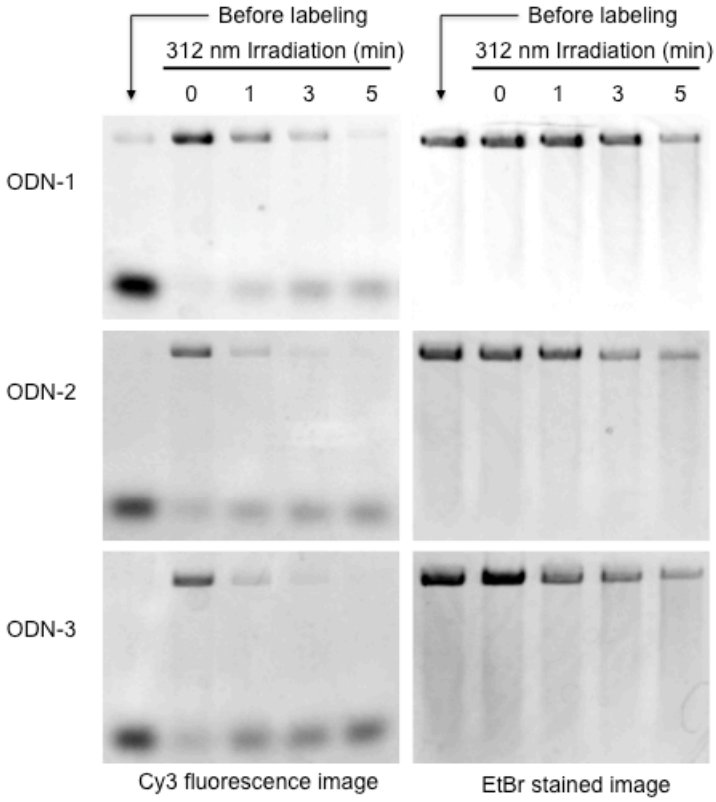

Figure S5. Agarose gel electrophoresis of the photoinduced de-labeling product of Cy3-labeled PhiX 174. Conditions for photoreaction: [ODN] = [PhiX 174 ssDNA] = 50 nM in 50 mM Na-cacodylate buffer (pH 7.0) containing 150 mM NaCl, 50ºC.

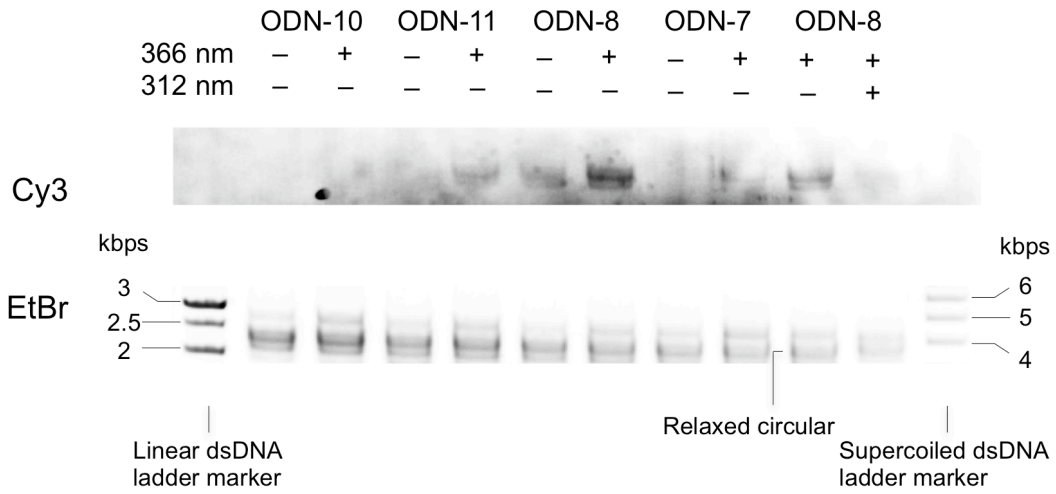

Figure S6. Photoinduced fluorescence labeling of double-stranded plasmid DNA, pUC18, after Topoisomerase I treatment. a) 0.7% Agarose gel electrophoresis of photo-labeling/delabeling of double-stranded plasmid DNA, pUC18, with ODN-7, -8, -10, and 11. Conditions for photoreaction: [plasmid DNA] = 50 nM, [ODN] = 5 μM in 50 mM sodium cacodylate buffer (pH 7.0) containing 100 mM NaCl. 366 nm irradiation (3 min, r.t.) was performed after the boiling (100ºC, 5 min) and rapid cooling (0ºC, 2 min) of the reaction mixtures. 312 nm irradiation (3 min) was performed at 50ºC. ODN-10 : Cy3-CTTCTA^{CNV}KTGTAGC, ODN-11; Cy3-TTCTTCTA^{CNV}KTGTAGCCG.

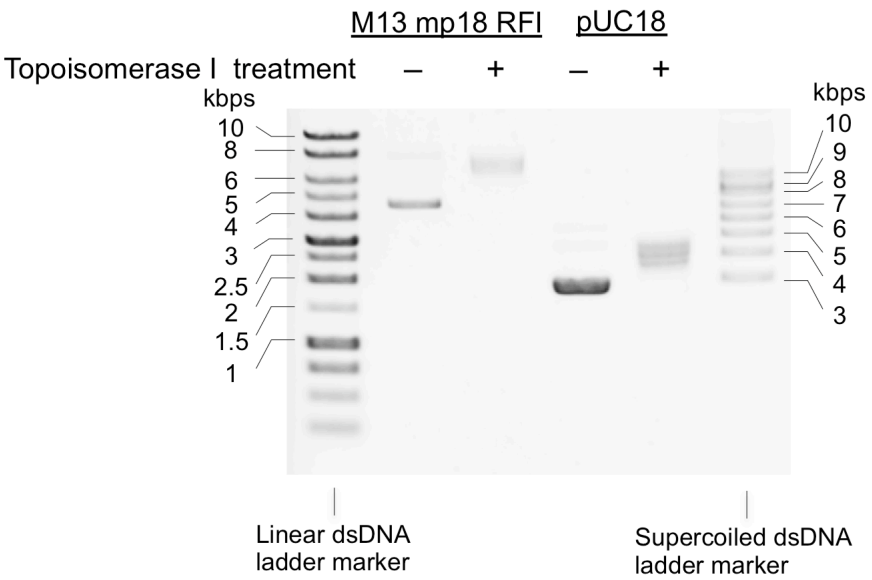

Figure S7.Agarose gel electrophoresis of double-stranded plasmid DNAs after Topoisomerase I treatment.