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Supporting Information

Double duplex invasion of DNA induced by ultrafast photo-cross-linking for antigene method

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Experiments

General

Mass spectra were recorded on a Voyager PRO-SF, Applied Biosystems. HPLC was performed on a Chemcosorb 5-ODS-H column with JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. Reagents for the DNA sysnthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite , and CPG support were purchased form Glen research. Photoirradiation was performed by UV-LED (LUMEN DYNAMICS, Omni Cure LX405S)

Preparation of ^{CNV}K-modified and ^{CN}U-modified oligonucleotides

The phosphoramidite of ^{CNV}K and ^{CN}U was prepared following to previous reports[1,2]. The modified oligonucleotides containing ^{CNV}K were prepared, according to standard phosphoramidite chemistry using DNA sysnthesizer. Synthesized ODN were detached from the support by soaking in concentrated aqueous ammonia for 1 h at room temperature. They were deprotected by incubation with 28% ammonia for 4 h at 65°C and ammonia was removed by speedvac. The crude oligomer was purified by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% CH_3CN, linear gradient (30min) at a flow rate of 3.0 mL/min.

Photoirradiation

The ODN containing ^{CN}U (20 μM) and ODN containing ^{CNV}K (20 μM) in buffer solution (100 mM NaCl, 50 mM sodium cacodylate) was photoirradiated at 385 nm using UV-LED illuminator (LUMEN DYNAMICS, Omni Cure LX405S) at 4°C and then it was analyzed by UPLC

UPLC analysis

The samples were analyzed with UPLC system (Aquity, Waters) equipped with BEH Shield RP18 column (1.7μm, 2.1 × 50 nm, elution was with 0.05M ammnonium formate containing 1-10% CH_3CN, linear gradient (10min) at a flow rate of 0.2 mL/min, 60°C).

Denaturing PAGE analysis

The photoirradiated sample was diluted 10 times using formamide containing 8M urea.

The 1.5 uL diluted solution and 1 uL 10x loading dye were mixted and 2 uL solution was applied to 15%AA gel. Polyacrylamide gel electrophoresis (PAGE) was performed with 15% polyacrylamide containing 8 M urea. After the electrophoresis (150V-60min), the gel was imaged by Cy3 fluorescence (Light: Green 520 nm EPI, Filter 575DF20) by a luminescent image analyzer (LAS3000, Fujifilm). When the gel was imaged by SYBR gold, the gel was stained by SYBR gold for 30 min after electrophoresis. The fluorescent image was taken by a luminescent image analyzer.

Double duplex invasion (for long duplex DNA)

The solution of 100 nM duplex DNA and 10 μM probes in 50 mM cacodylate buffer (pH 7.4) containing 100 mM NaCl were incubated at 37°C for 1h, and then the solution was photoirradiated at 385 nm for 1 h.

Double duplex invasion (for genomic DNA) and Real-rime PCR

The solution of 0.01 ug/ul genomic DNA (Human Genomic DNA, Takara, Japan) and 50 nM probes in 10 mM Tris-HCl (pH 7.4) were incubated at 37°C for 1h, and then the solution was photoirradiated at 385 nm for 1h. The photoirradiated samples were subjected to Real-time PCR using automated real-time PCR system (Smart Cycler, Takara, Japan) with SYBR Premix Ex Taq II perfect real time (Takara, Japan) and 0.5 μM of forward and reverse primer. The target DNA was BRCA1 exon18. Inhibition efficiency was estimated form the change in C_T values.

T7 RNA polymerase transcription

We designed the forward primer (5'GCTAGTTAATACGACTCACTATAGGGAATG GAAAGCTTCTCAAAGTA-3') which containing T7 RNA promoter sequence and reverse primer (5'-ATGTTGGAGCTAGGTCCTTAC-3'). The PCR performed using human genomic DNA as template. The PCR product was purified by NucleoSpin Gel and PCR Clean-up. The purified linear template containing T7 RNA promoter region and BRCA1 exon 18 was used T7 RNA transcription as template strand. The 10 μg template DNA and 10 μM probes were incubated at 37°C for 1 h and photoirradiation at 385 nm for 1h (Total volume 20 μL). A transcription eleongation arrest assay (In vitro Transcription T7 Kit, Takara) was performed in 20 μl of transcription reaction mixture containing [1 mM NTP], 5 μg template, Rnase inhibitor, and T7 RNA polymerase at

42°C for 2h. After incubation, the sample was kept on ice or -20°C and the transcription mixture was analyzed on a 1% Agarose gel stained by EtBr.

1. Y. Yoshimura, K. Fujimoto, *Org. Lett.,* 2008, **9**, 2071.
2. J. C. Markley, P. Chirakul, D. Sologub, S. T. Sigurdsson, *Org. Lett.,* 2001, **11**, 2453.

Table S1. MALDI-TOF-MS analysis of synthesized ODN and photo-cross-linked duplex DNA

Entry	Sequence(5'-3')	Calcd. $[M+H]^+$	Found
ODN 1	TGCA^{CNV}KCCGT	2781.53	2781.09
ODN 2 (^{CN}U)	ACGGG^{CN}UGCA	2774.48	2773.89
ODN 1(K)▱ODN 2(^{CN}U)	TGCA^{CNV}KACGT ACG^{CN}UGTGCA	5555.01	5555.73

Table S2. Thermal denaturation temperature

Entry	T_m [°C]
ODN 1/ ODN 2(T)	29.8
ODN 1/ ODN 2(^{CN}U)	29.3

Table S3. LUMO energy of uracil base analogs.

Base	T-A	U-A	^{HM}U-A	^{F}U-A	^{TF}U-A	^{CN}U-A
LUMO [eV]	-0.04067	-0.04406	-0.04474	-0.05199	-0.06169	-0.06799

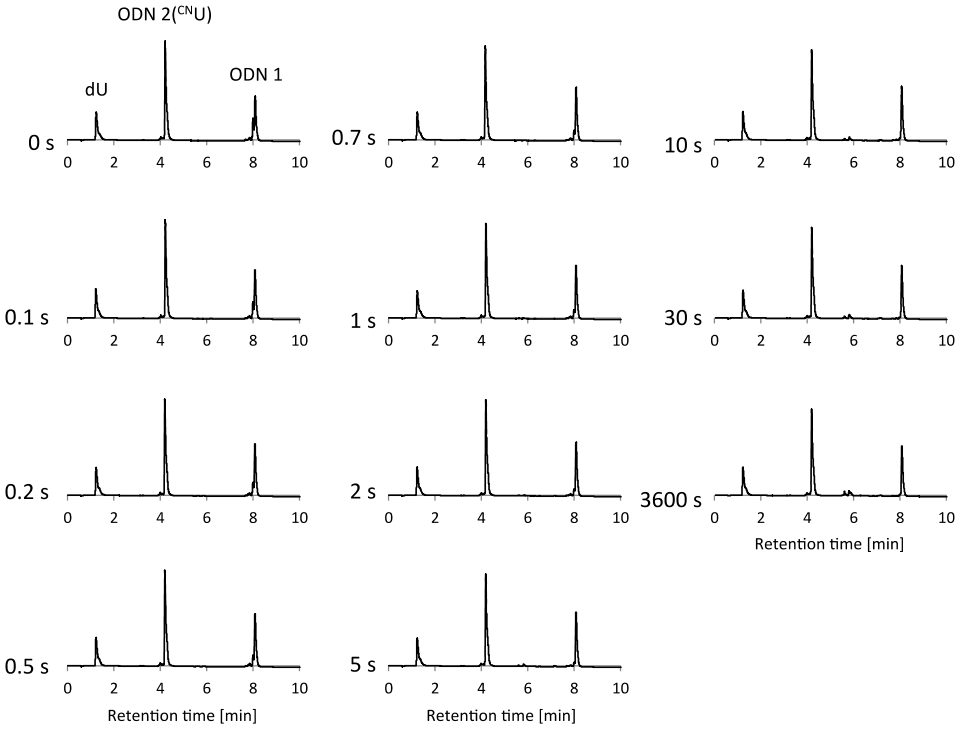

Fig. S1 UPLC analysis of photo-cross-linking reaction of ODN 1 and ODN 2(^{CN}U); The solutions of 10 μM ODN 1 and 10 μM ODN 2 (^{CN}U) in 50 mM sodium cacodylate buffer (pH 7.4) containing 100 mM NaCl were photoirradiated at 385 nm.

Table S4. The sequence of probe ODN and long duplex DNA

Entry	Sequence(5′–3′)	Calcd. for $[M+H]^+$	Found	T_m[°C]
Probe#1(T)	CTCTCGGCAGA^{CNV}KTCATTGGTC	6478.13	6477.45	59.8
Probe#1(^{CN}T)	C^{CN}UCTCGGCAGA^{CNV}KTCATTGGTC	6489.10	6489.62	59.2
Probe#2(T)	ATCTGCCGAGA^{CNV}KAGAGGATTA	6584.18	6584.23	59.5
Probe#2(^{CN}T)	A^{CN}UCTGCCGAGA^{CNV}KAGAGGATTA	6595.15	6595.33	59.1

Entry	Sequence(5′–3′)	T_m[°C]
Templ1	Cy3–AGGCATTGTTCTCTGACCAATGAATCTGCCGAGAGAGAGGATTAACCCGCATTGGAACGAAAAG	75.6
Templ2	Cy3–GTTCCAATGCGGGTTAATCCTCTCTCTCGGCAGATTCATTGGTCAGAGAACA	

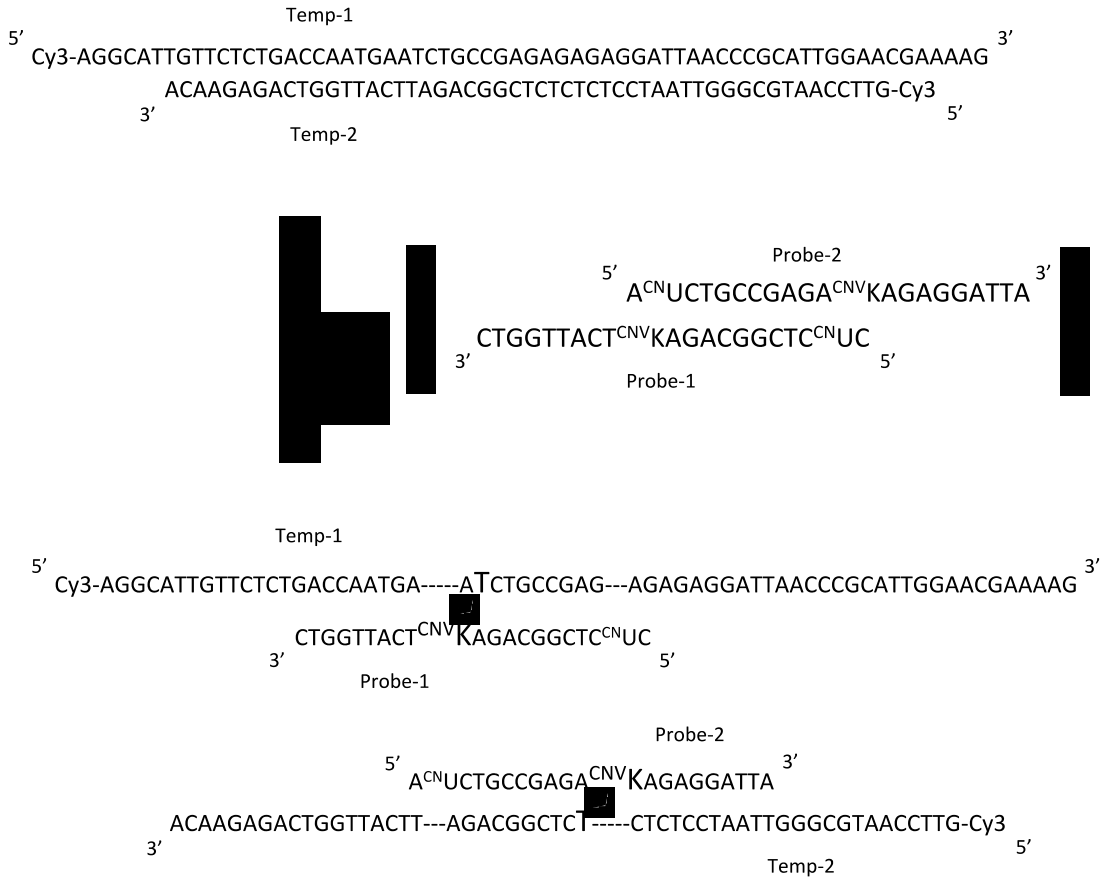

Fig. S2 Scheme of double duplex invasion using photo-reactive Probe-1 and Probe-2.

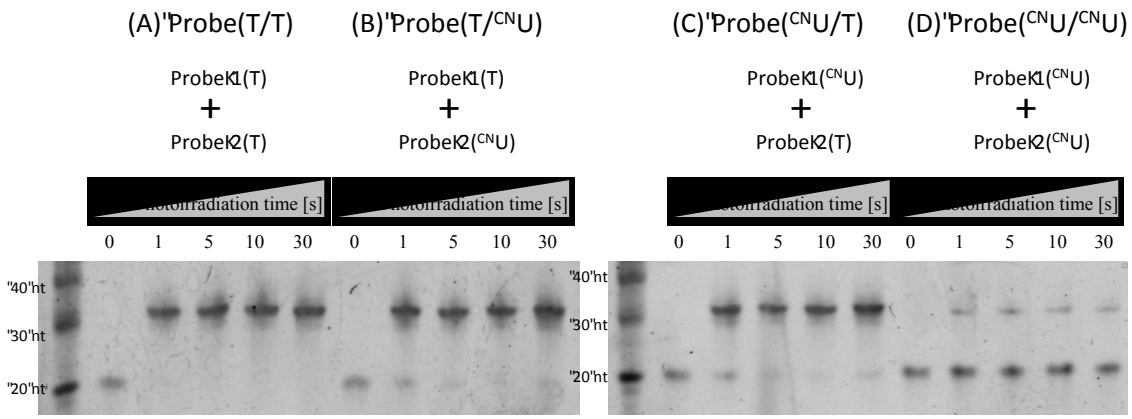

Fig. S3 Denaturing PAGE analysis of photo-cross-linking between probe 1 and 2.

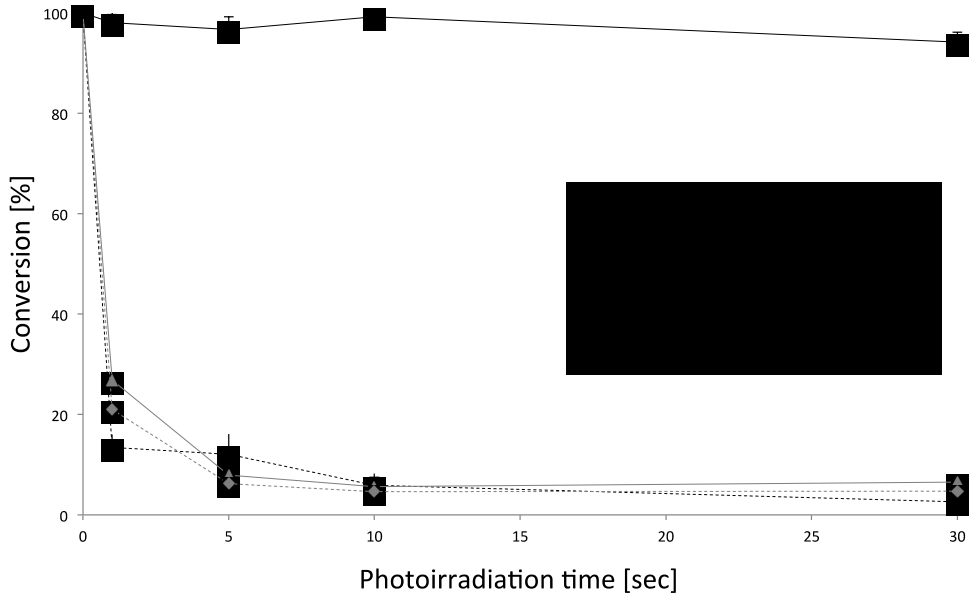

Fig. S4 Time course of photo-cross-linking ratio between probe 1 and 2.

Table S5 MALDI-TOF-MS analysis of probe ODN

Entry	Sequence	Calcd.#[M+H]$^+$	Found	T_m#[°C]#
ProbeL3	GTAGCTGTTAGA^{CNV}KGGCTGGCT	6598.14	6597.55	58.3
ProbeL3(^{CN}U)	G^{CN}UAGCTGTTAGA^{CNV}KGGCTGGCT	6609.11	6608.03	57.8
ProbeL4	TTCTAACAGCTA^{CNV}KCCTTCCAT	6381.12	6380.65	57.1
ProbeL4(^{CN}U)	T^{CN}UCTAACAGCTA^{CNV}KCCTTCCAT	6392.09	6390.69	57.2

Table S6. The sequence of primer set

Entry	Sequence(5’-3’)
Forward primer	AATGGAAAGCTTCTCAAAGTA
Reverse primer	ATGTTGGAGCTAGGTCCTTAC

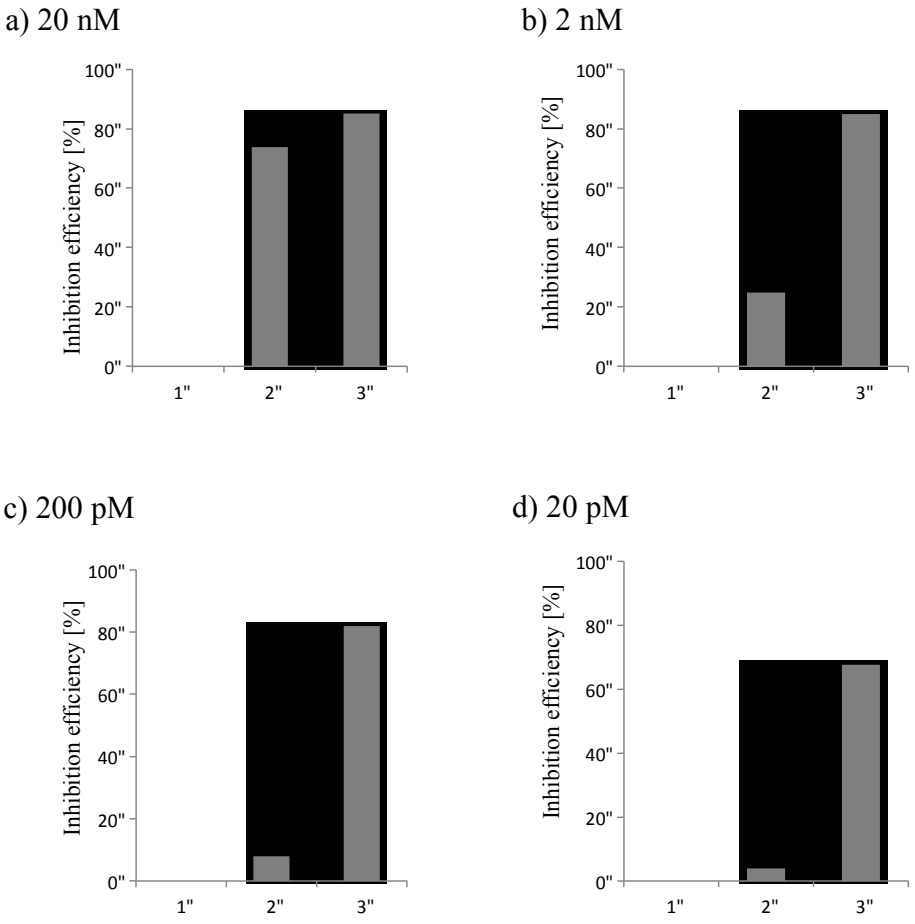

Fig. S5 Inhibition efficiency of the DNA amplification of human genomic DNA dependent in the probe concentration. The solution of 0.01 ug/ul genomic DNA and probes(20 nM, 2 nM, 200 pM, 20 pM) in 10 mM Tris-HCl (pH 7.4) were incubated at 37°C for 1h, and then the solution was photoirradiated at 385 nm for 1h. Inhibition efficiency was calculated using the C_T value of the amplification curve of the real-time PCR. Lane 1: Without probe, Lane 2: With probe and non-photoirradiation: Lane 3: With probe and photoirradiation for 60 min.