Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Photo-regulatable DNA isothermal amplification by template-mediated ligation

Bohao Cheng, Hiromu Kashida, Naohiko Shimada, Atsushi Maruyama* ,and Hiroyuki Asanuma *

Experimental Section

Materials

All conventional phosphoramidite monomers, CPG columns, and reagents for DNA synthesis were purchased from Glen Research. Other reagents for the synthesis of phosphoramidite monomers were purchased from Tokyo Chemical Industry, Wako, and Aldrich. All oligonucleotides except **Mod**, were purchased from Integrated DNA Technologies. T3, T4, and T7 ligase as well as reaction buffers were purchased from New England Lab (NEB).

Poly(*L*-lysine hydrobromide) (PLL-HBr, Mw = 7.5 × 10^3) and dextran (Dex, Mw = 8.0 × 10^3) were obtained from Sigma-Aldrich and Funakoshi Co., respectively. Poly(L-lysine)-*graft*-dextran (PLL-*g*-Dex) cationic comb-type copolymer was synthesized by a reductive amination reaction of dextran with PLL according to a previously published protocol.[1] The resulting copolymer was purified by ion exchange and dialysis, then obtained by freeze drying. The composition (10 wt% PLL and 90 wt% dextran) of the copolymer was confirmed by ^{1}H-NMR analysis.

Tem	5'-GTTACGCATCGCTGCTAGATCG-3'
SL1	5'-**/p/**CGATGCGTAAC**/TAMRA/-**3'
SL2	5'-CGATCTAGCAG
Mod	5'-CGA**X**TCT**X**AGC**X**AGCG**X**ATG**X**CGT**X**AAC-3'
Native-Mod	5'-CGATCTAGCAGCGATGCGTAAC-3'
Prod	5'-CGATCTAGCAGCGATGCGTAAC**/TAMRA/**-3'

X=2',6'-dimethylazobenzene moiety

p=phosphate group

Photo-irradiation

For *trans*-to-*cis* isomerization, UV light at 365 nm was applied using an OminiCure LX405s Spot Curing System with a UV LED head V2 019-00181R (Lumen Dynamics, 9500 mW/cm^2). For *cis*-to-*trans* isomerization, visible light at 455 nm was applied using a SLA-1000-2 two-channel Universal LED Driver with a BioLED Optical Head LCS-0455-03 (Mightex, 280 mW).

Synthesis of oligonucleotides

The modified oligonucleotide **Mod** tethering 2',6'-dimethylazobenzenes was synthesized on an automated DNA synthesizer (H-8-SE, Gene World) using phosphoramidite monomers containing 2',6'-dimethylazobenzene, which were synthesized as previously reported.[2, 3] After workup, the oligonucleotide was purified by reversed-phase HPLC and characterized using a MALDI-TOF mass spectrometer (Autoflex II, Bruker Daltonics). MALDI-TOF MS for

Mod: 9160 *m*/*z* (calculated for [**Mod**+H^+]: 9162).

General procedures for photo-regulatable amplification

All DNA strands (**Tem**, **SL1**, **SL2**, and **Mod**) were dissolved in 1 x ligase reaction buffer in microtubes to a final concentration as mentioned in captions, followed by the addition of PLL-*g*-Dex to a total volume of 50 μL. Throughout the experiments, the ratio of amino groups of the PLL-*g*-Des to phosphate groups of DNA (the N/P ratio) was kept 1.5. Then mixture was incubated at 37 ^oC for 10 min, before 10 min visible irradiation (465 nm light) was conducted. The ligase was added, and the reaction was initialized by irradiation with 365 nm light for 5 min. After UV irradiation, reactions were incubated in dark for 30 min and then irradiated with visible light for 5 min to finish cycle 1. Aliquots were taken after every cycle and mixed with loading buffer (80% formamide, 50 mM EDTA, 0.025% bromophenol blue, and 20% glycerol) to terminate the reaction. All the experiments were performed at 37 ^oC.

Photo-regulatable amplification in the absence of template, PLL-*g*-Dex, modulator or without light irradiation

DNA strands were dissolved in 1 x T3 ligase reaction buffer and treated for the indicated number of cycles as described in the general method. For the experiment without PLL-*g*-Dex, no polymer was added to the sample. For the experiment without light irradiation, the reaction was kept in dark after the addition of ligase and incubated for equivalent times as the other samples. For all four samples, amplification was performed 5 cycles.

Template-dependency analyses

In these experiments, the template strand (**Tem**, **Mis11**, **Mis7/16**, **Mis11/16**, or **PolyT**) was dissolved in 1 x T3 ligase reaction buffer, and reactions were performed as described in the general method. For all four samples, amplification was performed 5 cycles.

Photo-stability of ligase

Before ligation, T3 ligase equivalent (16 U/μL) together with PLL-*g*-Dex (N/P=1.5) was pre-irradiated with UV and visible light (each 30 min) or incubated in dark (60 min). Then the DNA mixture (**Tem**, **SL1**, and **SL2**) was added (volume up to 50 μL) to start the ligation, after 30 min reaction in dark, the reaction was terminated with loading buffer.

Gel electrophoresis quantification of yield

Samples mixed with loading buffer were subjected to electrophoresis on a 20% polyacrylamide gel containing 8 M urea at 750 V for 2.5 h. Images were analyzed with an

FLA-9500 scanner (GE Healthcare) by monitoring the fluorescence of TAMRA attached to the substrate **SL1**. Quantitative analyses were conducted using Image Quant TL (GE Healthcare). Color intensity of the areas including substrate bands (I_s), product bands (I_p), and background (I_b) were quantified. Degree of Amplification value was calculated by the following formula:

$$\text{Degree of Amplification} = (I_p - I_b)/(I_s + I_p - 2 \times I_b) / 0.02$$

Solution containing 5 μM **SL1** and 5 μM **Prod** was subjected to the lane 1 of gel as the marker.

Melting temperature measurements

The melting curves were obtained with a JASCO model V-560 spectrometer and a JASCO model FP-6500 spectrometer equipped with programmable temperature controllers; 10mm x 2mm quartz cells were used. T_m value was determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm (in UV/Vis case) as a function of temperature. The temperature ramp was 1.0 ^oC/min. Before **Tem/*trans*-Mod** measurements, **Modulator** was incubated at 90^oC for 10 min to isomerize as much azobenzene derivatives into *trans*-form. Before **Tem/*cis*-Mod** measurements, **Modulator** was irradiated by UV light for 10 min at 60^oC. Conditions: 1 μM DNA, 100mM NaCl, 10 mM phosphate buffer (pH=7.0).

Reference

A. Maruyama, H. Watanabe, A. Ferdous, M. Katoh, T. Ishihara and T. Akaike, *Bioconjugate Chem.*, 1998, **9**, 292-299.

Y. Hara, T. Fujii, H. Kashida, K. Sekiguchi, X. G. Liang, K. Niwa, T. Takase, Y. Yoshida and H. Asanuma, *Angew Chem Int Edit*, 2010, **49**, 5502-5506.

H. Nishioka, X. G. Liang, H. Kashida and H. Asanuma, *Chem. Commun.*, 2007, 4354-4356.

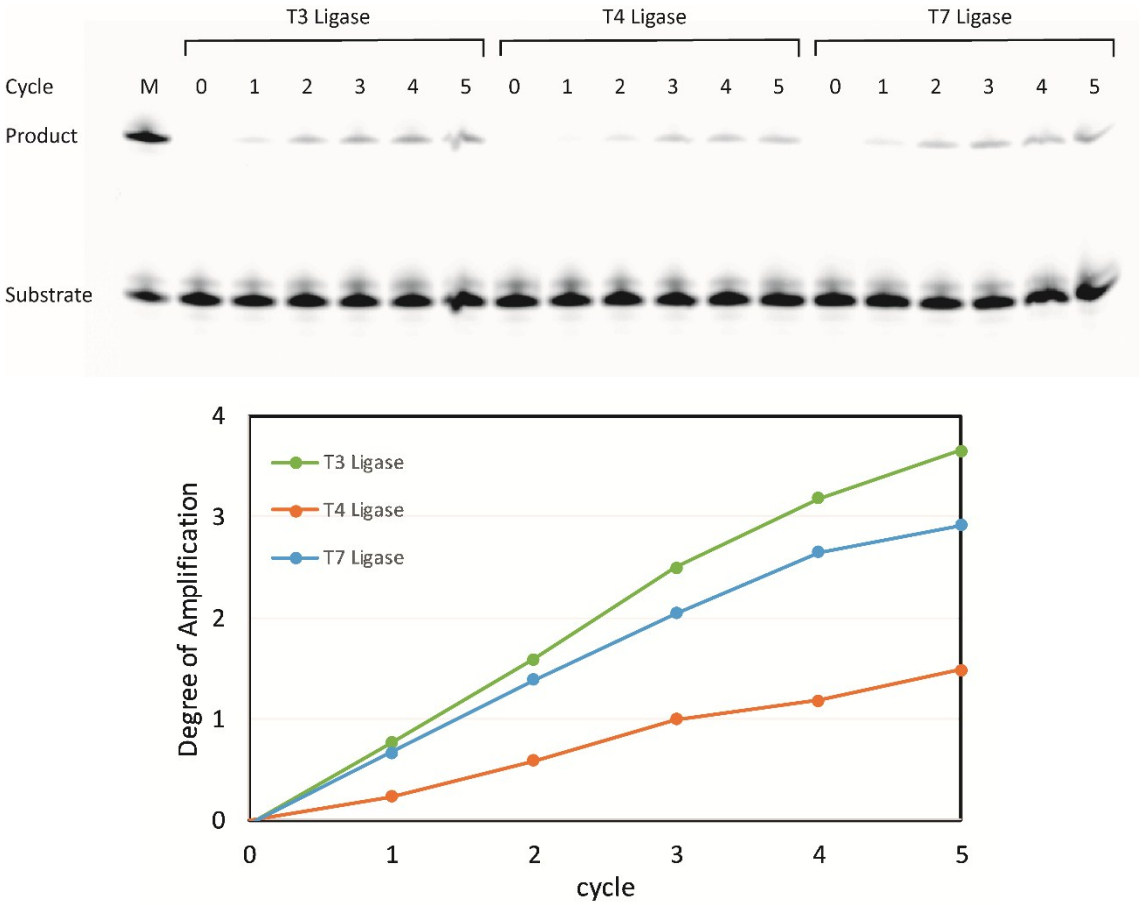

Figure S1. PAGE image (upper) and calculated yields of photo-regulatable amplifications with T3, T4, and T7 ligases. Lane M indicates the maker lane. Degree of amplification is calculated from PAGE (see Experimental Section). Conditions: 200 nM Template, 10 μM each Substrate, 2 μM Modulator, PLL-*g*-Dex at N/P = 1.5, 16 U/μL ligase, performed in 1 x T3, T4 or T7 reaction buffer at 37 ^{o}C.

Figure S2. PAGE image of enzymatic ligation catalyzed with or without irradiation of T3 ligase: lane M, marker; lane 1, with irradiated T3 ligase and lane 2, with non-irradiated T3 ligase. Conditions: 5 μM Template, 5 μM each Substrate, PLL-*g*-Dex at N/P = 1.5, 16 U/μL T3 ligase in T3 reaction buffer at 37 ^{o}C. Reaction time is 2 hrs.

Figure S3. PAGE analyses of reactions with and without template, PLL-g-Dex, modulator, azobenzene-incorporation and light irradiation: lane M, marker; lane 1, amplification with all factors; lanes 2-6, amplifications in the lack of respective factor as shown in table. Conditions: 200 nM Template, 10 μM each Substrate, 2 μM Modulator/Native-Mod, PLL-*g*-Dex at N/P = 1.5, 16 U/μL T3 ligase in 1 x T3 reaction buffer at 37 °C. For all samples, 5 cycles of amplification were conducted.

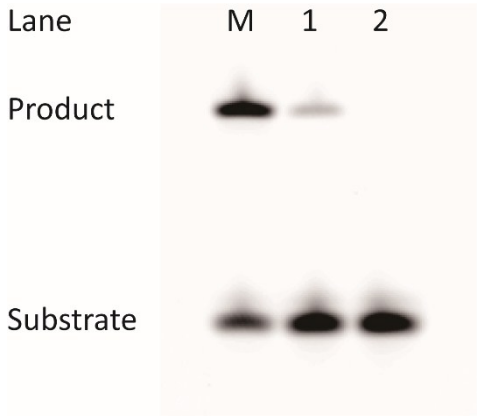

Figure S4. PAGE of reactions with PLL-*g*-Dex or PLL: lane M, marker; lane 1, with PLL-*g*-Dex and lane 2, with PLL. Conditions: 200 nM Template, 10 μM each Substrate, 2 μM Modulator, PLL-*g*-Dex at N/P = 1.5, 16 U/μL T3 ligase in 1 x T3 reaction buffer at 37 ^{o}C. For both samples, 5 cycles of amplification were conducted.

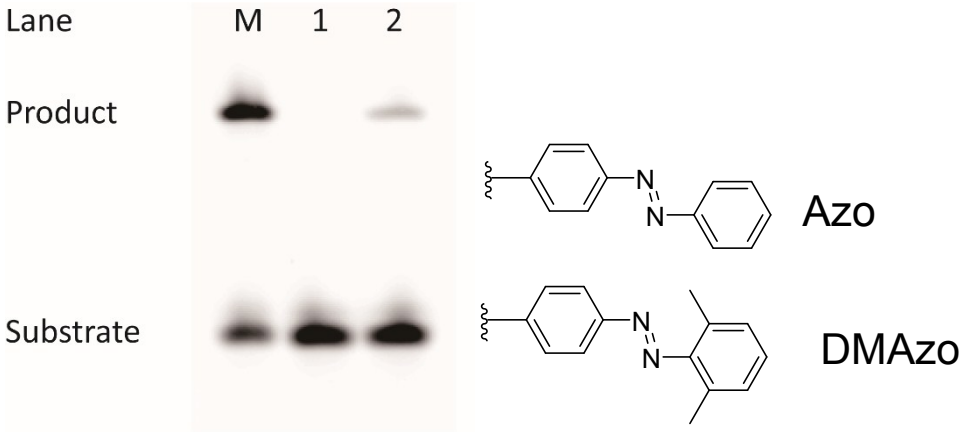

Figure S5. Photo-regulatable DNA amplification with azobenzene tethered modulator: lane M, marker; lane 1, with Azo-tethered modulator and lane 2, with DMAzo-tethered modulator. Conditions: 200 nM Template, 10 μM each Substrate, 2 μM Modulator, PLL-*g*-Dex at N/P = 1.5, 16 U/μL T3 ligase in 1 x T3 reaction buffer at 37 ^{o}C. For both samples, 5 cycles of amplification were conducted. The chemical structures of azobenzene (Azo) and 2',6'-dimethylazobenzene are shown.

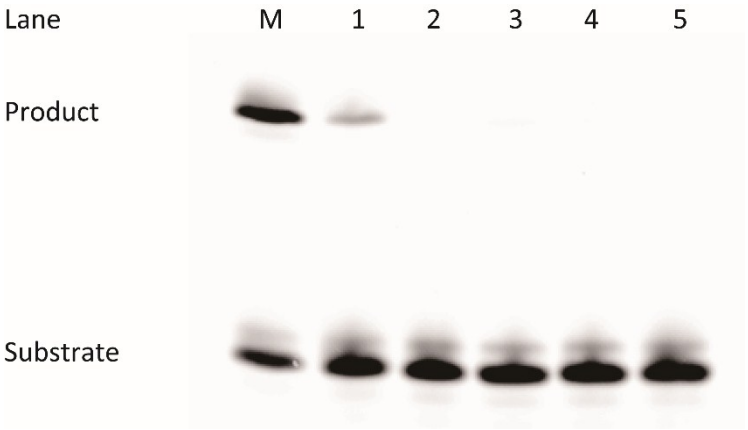

Figure S6. PAGE analyses of amplification reactions in the presence of indicated templates lane M, marker; lane 1, amplification with full-matched Template; lanes 2-5, amplifications with Mis11, Mis7/16, Mis11/16 or PolyT template. Conditions: 200 nM each template, 10 μM each Substrate, 2 μM Modulator, PLL-*g*-Dex at N/P = 1.5, 16 U/μL T3 ligase in 1 x T3 reaction buffer at 37 ^{o}C. For all samples, 5 cycles of amplification were conducted.

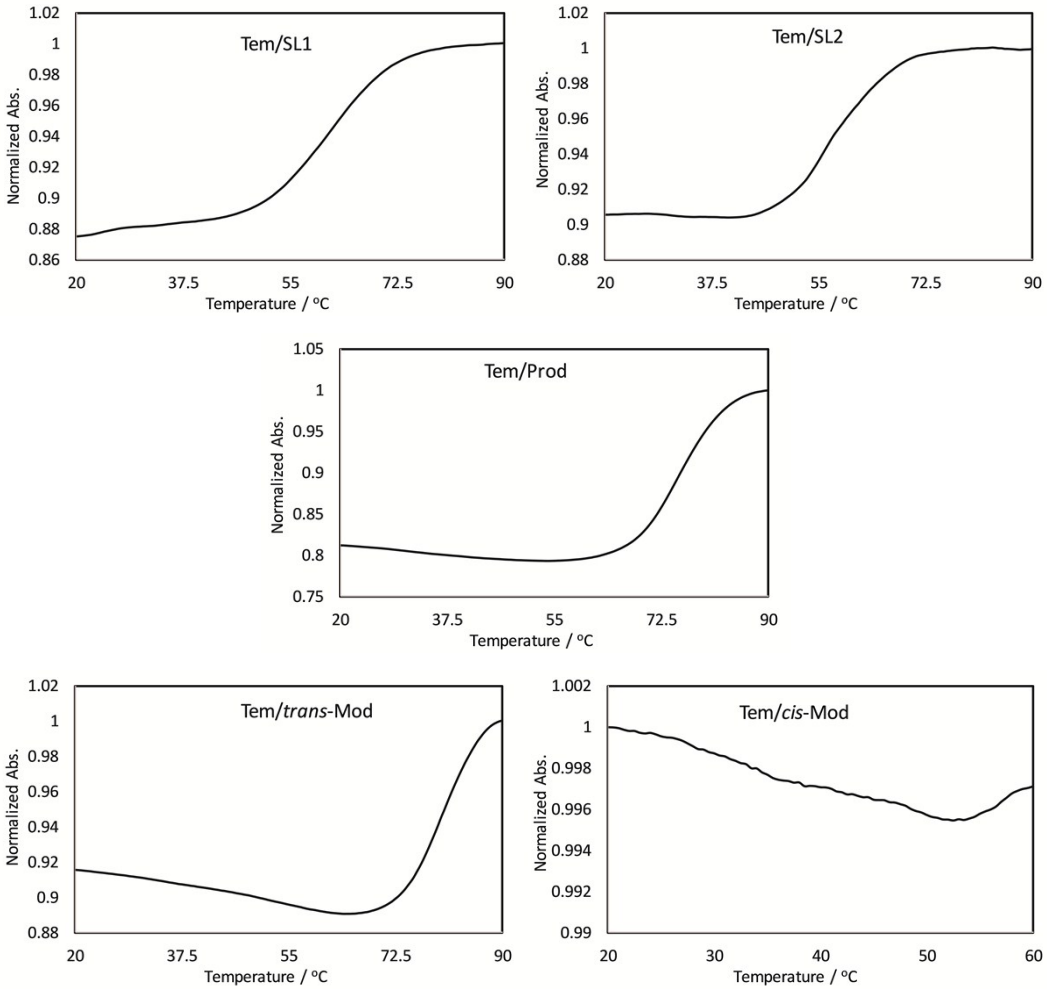

Figure S7. Normalized melting curves of all duplexes in photo-amplification system. Conditions: 1 μM Template with 1 μM SL1/SL2/Product/*trans*-Modulator/*cis*-Modulator, PLL-*g*-Dex at N/P = 1.5 in phosphate buffer (10 mM phosphate group, 100 mM NaCl, *p*H=7.00).

Table S1. Melting temperatures (T_ms) of duplexes.

Duplex	T_m/°C	
	trans-	*cis*-
Tem/SL1	58.5	
Tem/SL2	56.0	
Tem/Prod	75.0	
Tem/Mod	79.5	n.d.