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Electronic supporting information for

Preparation and affinity-based purification of caged linear DNA for light-controlled gene expression in mammalian cells

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Synthesis of Bio-Bhc-diazo

Synthetic procedures and spectroscopic characterization of NHS-Bhc-hydrazone and Bio-Bhc-diazo have been reported in the literature[1)]. Briefly, to a stirred solution of NHS-Bhc-hydrazone (71.2 mg, 120 μmol) and EZ-Link Pentylamine-Biotin (Piers, 44.0 mg, 134 μmol) in DMF (0.5 mL) was added Et_3N (18.5 μL, 132 μmol). The stirring was continued at ambient temperature for 13 h. The solvents and the volatile materials were evaporated under vacuum to yield crude products.

To a stirred suspension of the crude products in MeOH (2 mL) was added Et_3N (18.5 μL, 132 μmol). After 5 h, the resultant precipitate was collected by filtration, washed with a small amount of methanol and dried under vacuum to yield 72.4 mg (111 μmol, 93% yield) of Bio-Bhc-diazo.
^{1}H NMR (DMSO-d_6) δ 1.25–1.65 (12H, m), 2.04 (2H, t, J = 7.0 Hz), 2.57 (1H, d, J = 12 Hz), 2.81 (1H, dd, J = 12 & 5.0 Hz), 3.00 (2H, m), 3.07–3.14 (2H, m), 4.12 (2H, m), 4.28 (1H, m), 4.73 (2H, s), 5.81 (1H, s), 6.37 (1H, s), 6.44 (1H, s), 6.68 (1H, s), 7.02 (1H, s), 7.74 (1H, t, J = 5.5 Hz), 8.00 (1H, t, J = 5.5 Hz), 8.08 (1H, s)

PCR amplification of linRL-SV40 from pRL-SV40

The linRL-SV40 was amplified from the pRL-SV40 (Promega Corp.) using primers linRLSV40f (5'-TATGGAAAAACGCCAGCAAC-3') and linRLSV40r (5'-AAAGTGCCACCTGGATCCTT-3'). The primers were synthetic oligo DNAs purchased from FASMAC (Japan). Polymerase chain reaction (PCR) was carried out using KOD-Plus-Ver. 2 (Toyobo Co. Ltd., Japan) including KOD-Plus- (1 U/μL), 10 × PCR Buffer for KOD-Plus-Ver.2, 25 mM $MgSO_4$ and 2 mM dNTPs. Each PCR reaction mix (100 μL) contained 10 μL of the 10 × PCR buffer, 10 μL of 2 mM dNTPs, 6 μL of 25 mM $MgSO_4$, 3 μL of each primer (10 μM in TE buffer), 20 ng of the template pRL-SV40 (40 ng/ μL), 2 U of the KOD-Plus- (1 U/μL), and 65.5 μL of

MilliQ water to fill. The PCR conditions were 1 cycle of 2 min at 94°C, followed by 40 cycles of denaturation (10 s at 98°C), annealing (30 s at 58°C), and extension (2 min at 68°C) using a thermal cycler (iCycler; Bio-Rad Laboratories, Inc., USA). Five sets of the PCR products (total of 500 μL volume) were combined and subjected to membrane purification tubes (MinElute PCR purification Kit; Qiagen Inc., USA) to remove the primers and the template plasmid DNA. The PCR products were quantified by measuring the absorbance at 260 nm using a spectrophotometer (V630BIO; Jasco, Japan). A typical concentration of the linRL-SV40 was 1200 ng/μL (20 μL).

Synthesis and affinity-based purification of Bio-Bhc modified pRL-SV40

A plasmid DNA pRL-SV40 (1.5 μL, 1043 ng/μL in TE buffer) was reacted with 9 μL of Bio-Bhc-diazo (6.7 mM in DMSO) at 37°C for 1 hr in a dark place. The reaction mixture was subjected to gel permeation chromatography (Micro Bio-Spin P 30 Tris RNase Free; Bio-Rad) to remove excess Bio-Bhc-diazo and its decomposed materials. The eluted fraction (15 μL) was added to a suspension of streptavidin magnetic beads (New England Bio Labs) in a wash/binding buffer (250 μL) in a 1.5-mL tube and stood at room temperature for 15 min. The 1.5-mL tubes were set into a magnetic rack (MagnaRack; Invitrogen Corp., USA). The supernatant was discarded to a 1.5-mL tube (fraction B). The beads were washed three times with a wash/binding buffer (250 μL each). To the beads was added 25 μL of an elution buffer, 20 mM biotin in 50 mM Tris-HCl buffer (pH 8). The suspension was heated at 70°C for 5 min and cooled on an ice for 1 min. The supernatant was collected in another 1.5-mL tube (fraction C).

The obtained fractions were analyzed using 1% agarose gel electrophoresis. An aliquot (10 μL) of the fractions was mixed with 2 μL of Fermentas 6 × DNA Loading Dye (Thermo Scientific) and loaded into each lane on the gel. The gel was electrophoresed (TAE buffer, 100 V, 30 min) and stained using fluorescent dye (SYBR Gold nucleic acid stain; Molecular Probes Inc.) to visualize the pRL-SV40 (Fig. 2a in the main text). Then the same gels were blotted onto nylon membranes (Hybond N+; Amersham Biosciences Corp.). The biotin-labeled DNAs were detected by incubation with an avidin-HRP conjugate (Pierce Chemiluminescent Nucleic Acid Detection Module; Thermo Scientific). Luminescence was detected using a gel documentation system (LAS-1000 Plus; FujiFilm, Japan) (Fig. 2b). Luminescence intensity of the bands corresponding to the pRL-SV40 was quantified using Image J software[2)].

Synthesis and affinity-based purification of Bio-Bhc caged linRL-SV40

A linear dsDNA linRL-SV40 (6 μL, 500 ng/μL in TE buffer) was reacted with 54 μL of Bio-Bhc-diazo (6.7 mM in DMSO) at 37°C for 3 hr in a dark place. The reaction mixture was subjected to the MinElute PCR purification Kit to remove the excess Bio-Bhc-diazo, its decomposed materials, and DMSO. The eluted fraction (10 μL) including the modified and unmodified dsDNAs

was added to streptavidin magnetic beads (New England Bio Labs) and was let to stand at room temperature for 15 min. The supernatant (approximately 10 μL) was discarded to a 1.5-mL tube (fraction B). The beads were washed three times with a wash/binding buffer (250 μL each). Unlike the modified plasmids, the captured linear dsDNA derivatives could not be released efficiently from the beads with the biotin elution buffer. To the beads was added 25 μL of a mixture of 10 mM EDTA (pH 8) and formamide (1:19 in volume). The suspension was heated at 65°C for 2 min and cooled on ice for 1 min. The supernatant was collected in a 1.5-mL tube (fraction C). The elution process was repeated once. The supernatants were combined, subjected to the MinElute PCR purification Kit to remove DMF and used in electrophoretic analysis or the transfection study.

To optimize the preparation, linRL-SV40 was reacted with Bio-Bhc-diazo for the indicated reaction times. The supernatants and eluted fractions of the magnetic beads treatment were analyzed using agarose gel electrophoresis (1% agarose, TAE buffer, 100 V, 30 min) The dsDNAs were visualized using SYBR Gold staining (Fig. S1). The intensities of the fluorescent bands were quantified using ImageJ software. The results shown in Fig. S1 demonstrated that the relative amount of unmodified linRL-SV40 left in the reaction mixtures was, respectively, 82% for 3 hr, 15% for 5 hr, 5% for 7 hr, and 1.5% for 20 hr. The amount of Bio-Bhc caged linRL-SV40 obtained from the eluted fractions was smaller than expected when the reaction time exceeds 5 hr. For example, the relative amount of the caged linRL-SV40 was 54% for the 5-hr reaction and 22% for the 20-hr reaction.

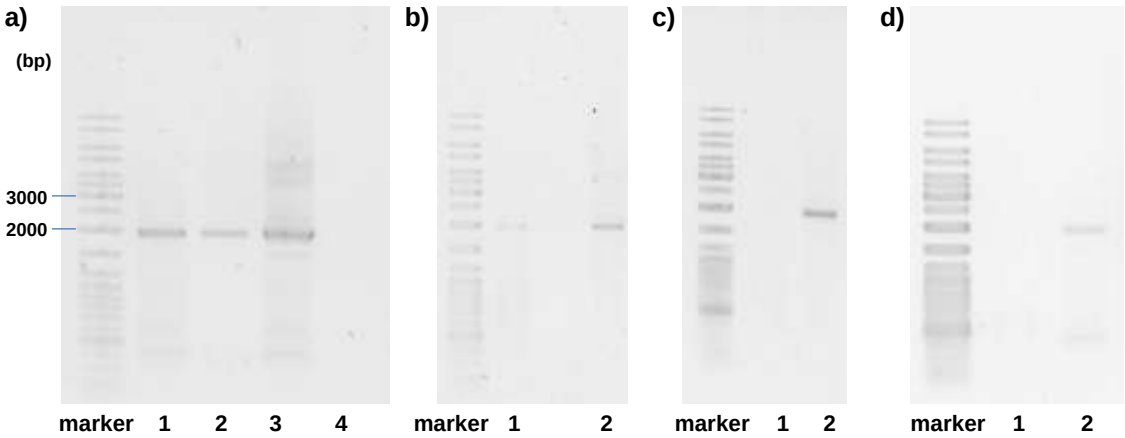

Fig. S1 Relative amount of the unmodified linRL-SV40. Results of the agarose gel electrophoresis stained by SYBR gold. A linear dsDNA linRL-SV40 was reacted with Bio-Bhc-diazo for 3 hr (a), 5 hr (b), 7 hr (c), and 20 hr (d). Lane 1: supernatants of the streptavidin magnetic bead treatment. Lane 2: eluted fractions. For comparison, linRL-SV40 (not caged) was subjected to the magnetic beads. Lane 3: supernatant. Lane 4: eluted fraction. The fluorescence intensity of the band in lane 3 was set as 100%.

Additional experiments were performed to ascertain whether non-specific capture of linRL-SV40 on the streptavidin magnetic bead is negligible or not. The linRL-SV40 (10 μL, 500 ng/μL in TE buffer) was subjected to the magnetic bead treatment as described above. The supernatants and the eluted fractions after the biotin treatment or the EDTA/formamide treatment were applied to an agarose gel electrophoresis (1% agarose, TAE buffer, 100 V, 30 min). The dsDNAs were visualized by SYBR Gold staining (Fig. S2). Nearly quantitative recovery of the linRL-SV40 was observed in the supernatants (lanes 1–9 in Fig. S2).

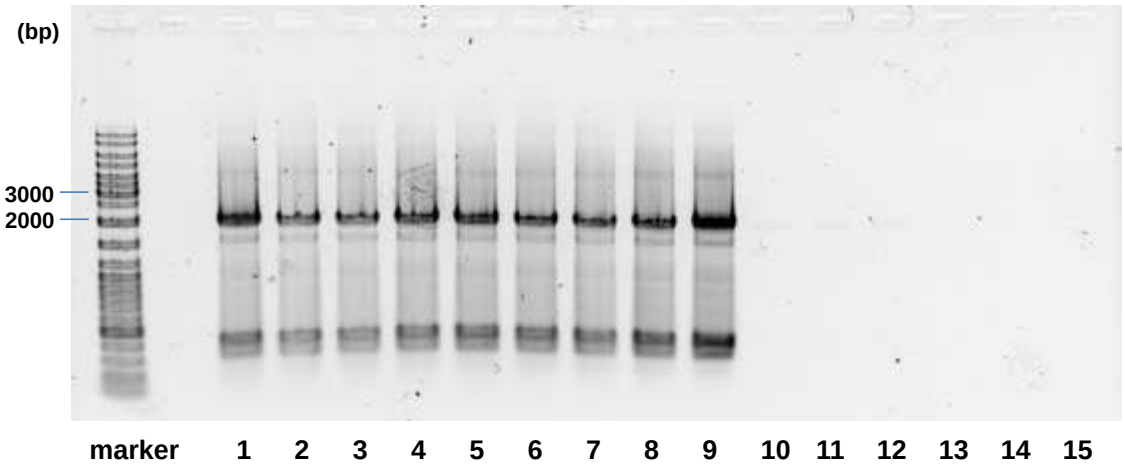

Fig. S2 Agarose gel electrophoresis of linRL-SV40 after the streptavidin magnetic bead treatment. The linear dsDNA linRL-SV40 was added to the magnetic beads and stood at room temperature for 15 min. Lanes 1–3: linRL-SV40 (500 ng/μL in TE buffer). Lanes 4–9: the supernatants of the bead treatment. Lanes 10–12: the eluted fractions with 10 mM biotin treatment. Lanes 13–15: the eluted fractions with the formamide/EDTA treatment.

In vitro photolysis of Bio-Bhc caged linRL-SV40

The purified samples of Bio-Bhc caged linRL-SV40 in TE buffer were transferred into thin walled 0.2-mL PCR tubes and irradiated at 350 nm using four RPR 350-nm lamps (2 mW/cm^2). The products were analyzed using 1% agarose gel electrophoresis, as described above (Fig. 3).

Cell culture and transfection study

HeLa cells were cultured at 37°C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM, GIBCO, USA) supplemented with 20% fetal bovine serum (FBS). Cells were trypsinized, resuspended in DMEM and transferred into 24-well plates (Tissue Culture Plate, 353047; BD Falcon, USA) at a density of 50 000 cells per well in a volume of 500 μL. After 24 hr of growing, the media were changed to 500 μL/well of a reduced serum media Opti-MEM (GIBCO,

USA). A plasmid DNA coding for a firefly luciferase (pGL3; Promega Corp.) and a linear dsDNA coding for a renilla luciferase (linRL-SV40 or Bio-Bhc-caged linRL-SV40) were mixed with a lipofection reagent (Lipofectamine 2000; Invitrogen Corp., USA). Transfection was performed following the manufacturer's standard protocol. A typical condition is as follows. For each transfection (for three wells), a mixture of 300 ng of the Bio-Bhc-caged linRL-SV40 and 300 ng of the pGL3 in Opti-MEM (total volume of 150 μL) was prepared in a 1.5-mL tube. In a separate tube, 2.4 μL of Lipofectamine 2000 was mixed with 147.6 μL of Opti-MEM and incubated for 5 min at room temperature. The Lipofectamine 2000 solution was combined with the diluted oligo DNAs and incubated for 20 min at room temperature. The Lipofectamine 2000-oligo DNAs complex (100 μL per well) was added to the cells in the 24-well plate. The cells were incubated in the presence of the lipofection complex at 37°C in a 5% CO_2 atmosphere for 3 hr. For the UV treated cells, the media containing lipofection complex were replaced with 500 μL of Opri-MEM (no phenol red). The cells were irradiated through the bottom of the plate at 350 nm for 5 min using four RPR 350-nm lamps (2 mW/cm^2). The media were changed to 500 μL of DMEM (10% FBS). The cells were cultured at 37°C for an additional 24 hr and lysed. The amount of Rluc expression was quantified using a dual luciferase reporter assay system (PicaGene Dual Luciferase Assay Kit, Toyo Ink Co. Ltd., Japan) by a luminometer (TD-20/20; Turner BioSystems, USA). The procedure was performed following the manufacturer's standard protocol.

The amount of the DNA used for the transfection study was set to 100 ng for one well because the relative luminescent intensity of Rluc was linearly proportional to the amount of the linRL-SV40 in the range from 0 ng to 200 ng of the DNA (Fig. S3).

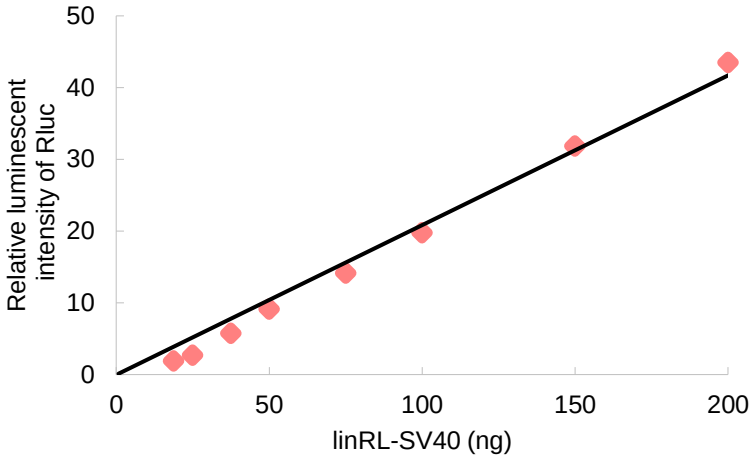

Fig. S3 Relative luminescent intensity of Rluc.

References

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(2) M. D. Abramoff, P. J. Magalhaes and S. J. Ram, *Biophotonics International*, **2004**, *11*, 36.