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Supporting Information

A FRET-based DNA nano-tweezers technique for the imaging analysis of specific mRNA

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Experimental Section

Table S1. Synthetic DNA oligonucleotides used in this study. The target recognition sites on the oligonucleotide O1 and the targeted sites are written in red. The target recognition sites on the oligonucleotide O2 and the targeted sites are written in blue.

Synthetic DNA oligonucleotides for the FRET-based DNA-NTs	
Oligo Name	Sequence (5' to 3')
Hes-1 O1	**CTCAACTTTTATAATACAAA**TACATTTTACGCCTGGTGCC
Hes-1 O2	CCGACCGCAGGATCCTATAA**GGCGCAATCCAATAT**
Control O1	**CTTTTCGACAAGCGC**TACATTTTACGCCTGGTGCC
Control O2	CCGACCGCAGGATCCTATAA**TTAACCGATAAAGATGAA**
O3	Cy3-TTATAGGATCCTGCGGTCGGAGGCACCAGGCGTAAAATGTA-Cy5
Hes-1 DNA(A0)	**ATATTGGATTGCGCCTTTGTATTATAAAAGTTGAG**
Hes-1 DNA(A2)	**ATATTGGATTGCGCC**AA**TTTGTATTATAAAAGTTGAG**
Hes-1 DNA(A4)	**ATATTGGATTGCGCC**AAAA**TTTGTATTATAAAAGTTGAG**
Hes-1 DNA(A6)	**ATATTGGATTGCGCC**AAAAAA**TTTGTATTATAAAAGTTGAG**
Hes-1 DNA(A8)	**ATATTGGATTGCGCC**AAAAAAAA**TTTGTATTATAAAAGTTGAG**
Hes-1 DNA(A10)	**ATATTGGATTGCGCC**AAAAAAAAAA**TTTGTATTATAAAAGTTGAG**
Hes-1 4 base gap O1	**CATCTCAACTTTTATAATACA**TACATTTTACGCCTGGTGCC
Hes-1 4 base gap O2	CCGACCGCAGGATCCTATAA**CGCAATCCAATATGAA**
GLUT1 4 base gap O1	**CTCAGAGTTCGGTATTAGT**TACATTTTACGCCTGGTGCC
GLUT1 4 base gap O2	CCGACCGCAGGATCCTATAA**CCTTGGCCATAGGTCC**
PCR primers for the coloning of Hes-1 mRNA	
Oligo Name	Sequence (5' to 3')
Hes-1 Forward	TAGAATTCAGCTGGTGCTGATAACAGCGG
Hes-1 Reverse	ATGTCGACAAAAGCCTTCACTCTTTTATTATATTTTCTC

Construction of DNA-NTs

DNA-NTs were constructed by annealing three synthesized DNA oligonucleotides as shown in Fig. S1a. The sequences of these oligonucleotides were designed as in ref. 1 to form the tweezers part. Synthesized oligonucleotides O1, O2, and O3 (Table S1. IDT DNA technologies, 5 μM final) were mixed in a PBS solution and self-assembled with the annealing program as depicted in Fig. S1b. The product was directly used in further experiments. Each step of production was confirmed by 12% native PAGE analysis as depicted in Figure S1c for FRET-based DNA-NT.

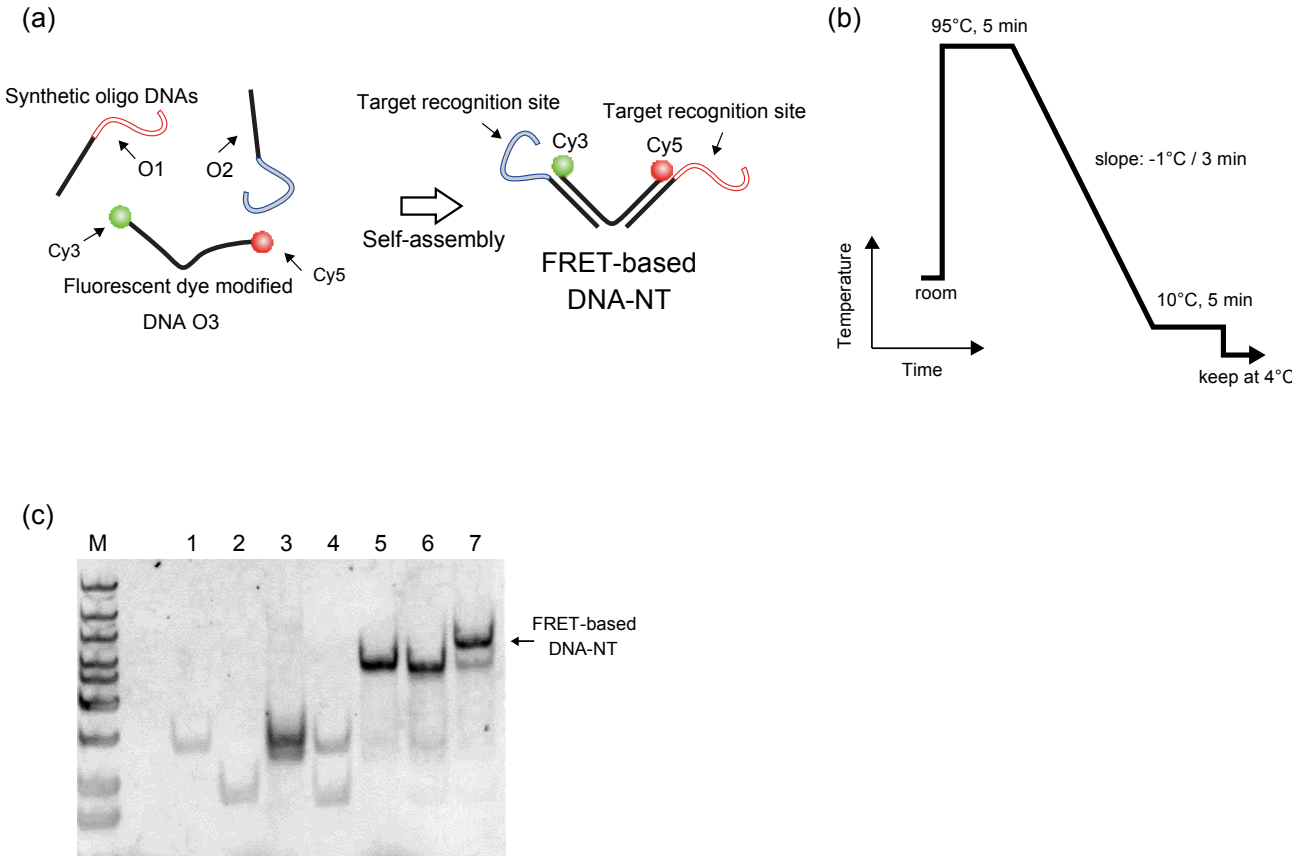

Fig. S1. The construction of FRET-based DNA-NT. (a) The basic strategy for construction. Three synthesized oligonucleotides were self-assembled to form DNA-NT by annealing. (b) The annealing program for DNA-NT formation via self-assembly. (c) Gel Star-stained native PAGE gel image to confirm FRET-based DNA-NT formation. M, DNA ladder (10–300 bp; Wako, Osaka, Japan); Lane 1, O1; Lane 2, O2; Lane 3, O3; Lane 4, O1+O2;Lane 5, O1+O3; Lane 6, O2+O3; and Lane 7, O1+O2+O3.

Production of the target model mRNA, Hes-1

Hes-1 was cloned from mouse ES cells (EB3[2,3]; Riken, Japan). Total RNA was extracted using an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. A cDNA library was then constructed using a cDNA Synthesis Kit (Takara, Shiga, Japan) according to the manufacturer's protocol. This library was subjected to PCR amplification using the primers Hes-1 Forward and Hes-1 Reverse (Table S1), using KOD-Plus-Neo polymerase (Toyobo, Osaka, Japan). The amplified gene was cloned into *Eco*RI and *Sal*I restriction sites of the pTNT vector (Promega) after restriction enzyme digestions. The transcripts of Hes-1 mRNA were produced with the vector using RiboMAX Large Scale RNA Production Systems (Promega) according to the manufacturer's instructions. The purity of the RNA product was confirmed by denaturing gel electrophoresis (Fig. S2).

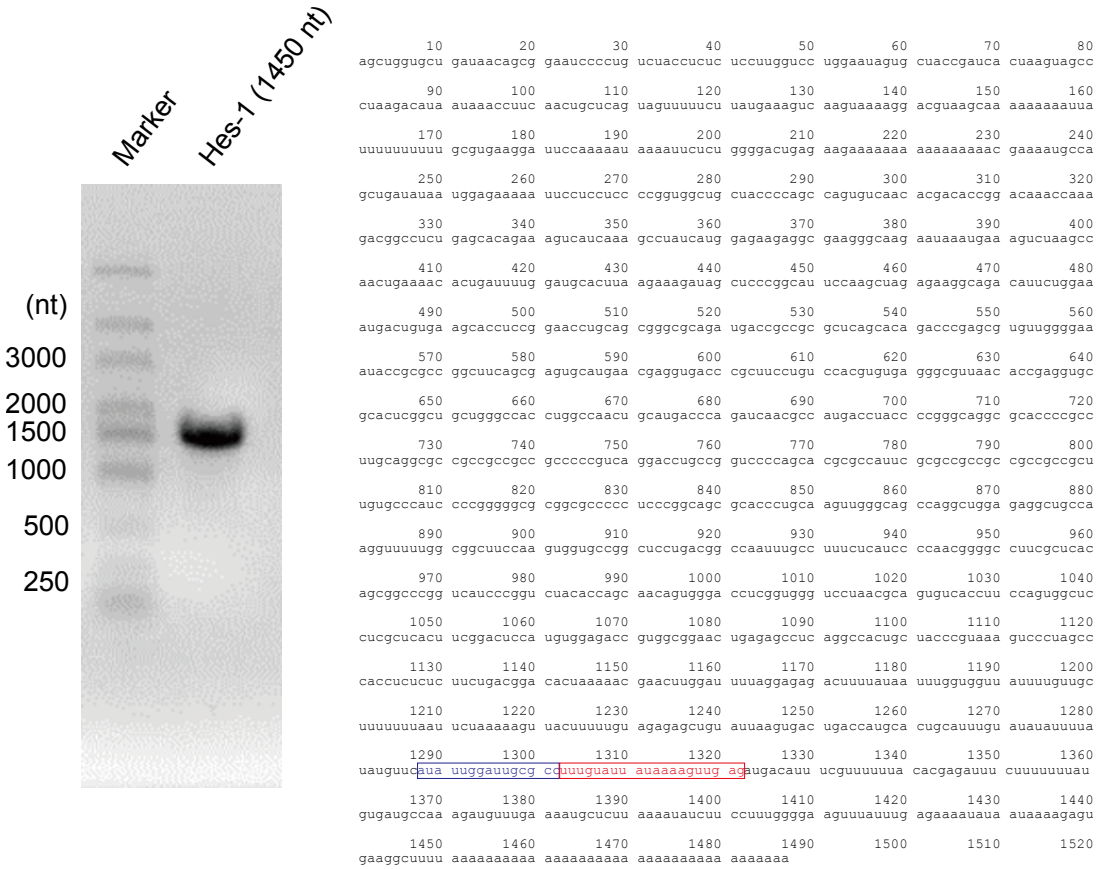

Fig. S2. Denaturing gel electrophoresis of Hes-1 mRNA target models. *In vitro* transcripts of Hes-1 mRNA (1450 nt) and its sequence. The blue part is recognized by O2 and the red part is targeted by O1 of the FRET-based DNA-NT.

Electrophoresis Analysis

FRET-based DNA-NT (500 nM final) was mixed with the same concentration of *in vitro* transcripts of Hes-1 mRNA in a PBS solution for 1 h at room temperature. A 2% native agarose gel electrophoresis with 1.2 μL of each sample was carried out to confirm the target-recognition ability of DNA-NT. Before the Gel Star staining, the fluorescence (Cy3) image was taken with a Typhoon 9210 (Molecular Dynamics) using a 532 nm excitation and a 580BP30 filter for the emission image. The Gel Star-stained image was then obtained by BioDoc-IT™ System (UVP) equipped with the filter set for ethidium bromide staining.

Fluorescence measurement

FRET-based DNA-NT (500 nM final) was mixed with each concentration of *in vitro* transcripts of Hes-1 mRNA in a PBS solution for 1 h at room temperature. Fluorescence was then measured with a multiplate reader, SpectraMax M5 (Molecular Devices) in a 96-well format with 100 μL of the mixture. FRET efficiency was calculated as fluorescence intensity at 662 nm/fluorescence intensity at 565 nm. Background fluorescence intensity was subtracted from each fluorescence intensity prior to use in the FRET calculations. The lower detection limit of 3.29 standard deviations from the average of the blank value (0.0643 ± 0.00144, at 0 vs. DNA-NT) was 0.035 (vs. DNA-NT) as calculated from the linear approximation (Fig. S3). This value is corresponding to 17.6 nM of Hes-1 mRNA in this experimental condition.

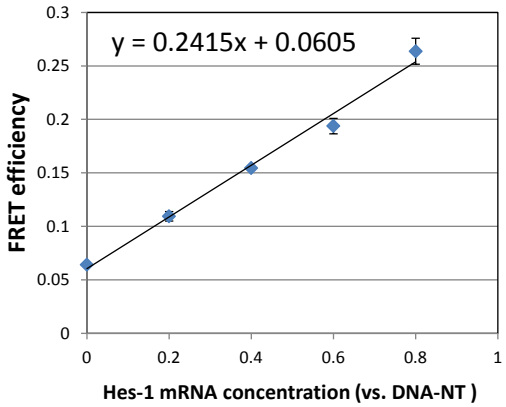

Fig. S3. The linear approximation for the calculation of lower detection limit.

Detection of pre-fixed mRNA produced by a living cell

Mouse hepatocarcinoma Hepa 1-6 cells[4,5] (Riken) cultured on Nunc™ Lab-Tek™ Chamber Slide Systems (Thermo Scientific) were first washed with PBS buffer and fixed with 3.7% formaldehyde for 10 min. The slides were then washed twice with PBS buffer, followed by a 70% ethanol treatment for 2 h at 4 °C to permeabilize the cellular membrane. The DNA-NT (0.6 μM final) in Hybridization buffer [10% dextran sulfate (Sigma–Aldrich), 0.02% RNase free BSA (Promega), 2 mM vanadyl ribonucleoside complex (New England Biolabs), 10% formamide, 1.25 U ribonucleic acid, transfer from *Escherichia coli* (Sigma–Aldrich), 2× SSC Buffer (Sigma–Aldrich)] was added to the cells and left overnight at 37 °C, after which they were washed with 10% formamide. The cells were further washed with 10% formamide, 15% formamide, and 20% formamide, for 30 min at 37 °C each. Finally, the cells were washed with 40% formamide for 30 min at 37 °C twice, and with SSC buffer three times. Anti-fade solution (prolong gold, Invitrogen) was added to the sample, and fluorescent images were then taken by an Olympus FV1000 confocal microscope. The Cy3 image was taken with a 559-nm laser and a variable barrier filter set at 570–625 nm (Cy3 excitation and Cy3 emission). The Cy5 image was obtained with a 635-nm laser and a variable barrier filter set at 655–755 nm (Cy5 excitation and Cy5 emission). The FRET image was obtained utilizing Olympus FV10-ASW software as pFRET images. Briefly, 4 images with 2 samples were obtained first as a control image. Two images, “Cy3 excitation and Cy3 emission” and “Cy3 excitation and Cy5 emission” termed as (a) and (b) respectively, were obtained with a cellular sample stained with the DNA-NT modified with only Cy3. Two images, “Cy3 excitation and Cy5 emission” and “Cy5 excitation and Cy5 emission” termed as (c) and (d) respectively, were also obtained from the other sample stained with the DNA-NT modified with only Cy5. Then, 3 images, “Cy3 excitation and Cy3 emission”, “Cy3 excitation and Cy5 emission” and “Cy5 excitation and Cy5 emission” termed as (e), (f), and (g), respectively, were obtained with the sample of interest which was stained with the DNA-NT modified with both Cy3 and Cy5. Finally, a FRET image was calculated as pFRET = (f) – [(b) / (a)] × (e) - [(c) / (d)] × (g) utilizing the software.

Detection of target mRNA inside a living cell after fixation

EB3 cells cultured on a glass bottom dish were washed with PBS followed by incubation with 1 μM of DNA-NT and 12.6 ng/μL of SLO (Sigma–Aldrich) in serum-free medium for 10 min at 37 °C. After washing with serum-free medium three times, the cells were incubated in medium with serum (10% FBS) for 30 min at 37 °C. The cells were further incubated with 10 μM of CellTracker™ Blue CMF2HC (4-Chloromethyl-6,8-Difluoro-7-Hydroxycoumarin) (Life Technologies) in serum-free medium for 30 min at 37 °C, followed by incubation in medium with serum for 30 min at 37 °C. The cells were then washed three times with PBS and fixed with 3.7% formaldehyde for 15 min. After being washed with PBS, anti-fade solution was added to the cells. The fluorescent images were then taken by an Olympus FV1000 confocal microscope. The Cy3 image was taken with a 559-nm laser and a variable barrier filter set as 570–625 nm. The Cy5 image was obtained with a 635-nm laser and a variable barrier filter set as 655–755 nm. The FRET image was obtained using Olympus FV10-ASW software as described above.

DNA-NT for GLUT1 mRNA detection

The sequences of mouse GLUT1 mRNA and GLUT1 DNA-NT are shown in Fig. S4 and Fig. S5, respectively.

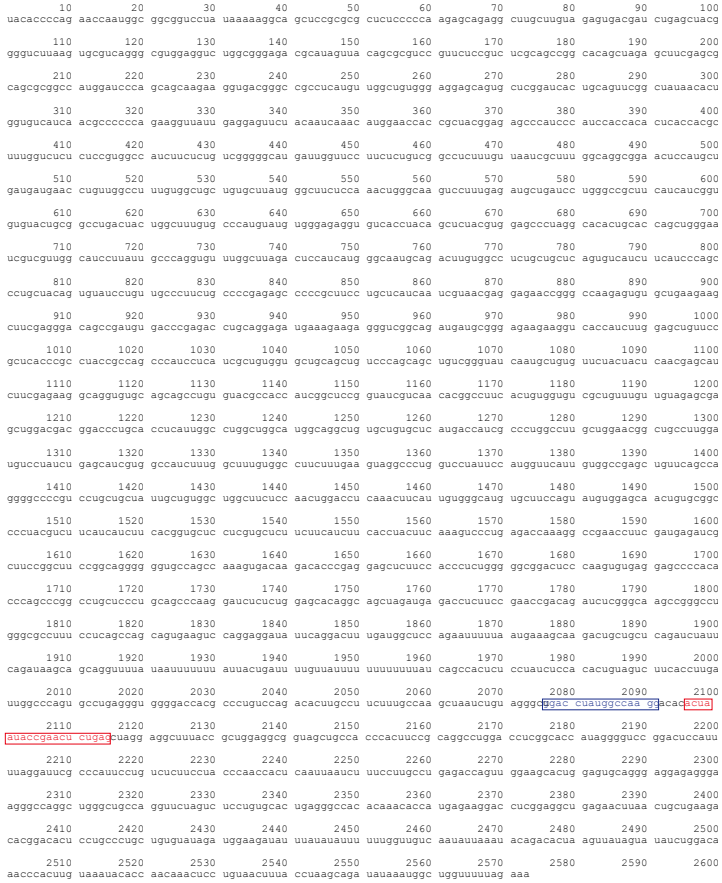

Fig. S4. The sequence of mouse GLUT1 mRNA. The blue part is recognized by O2 and the red part is targeted by O1 of the FRET based DNA-NT.

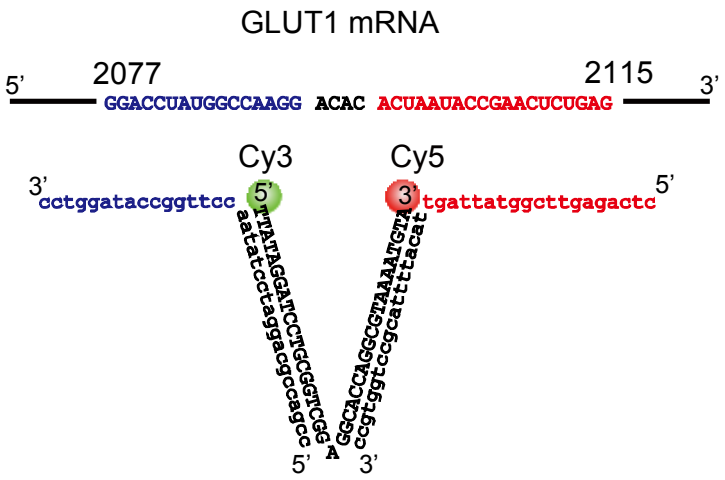

Fig. S5. The sequence of GLUT1 DNA-NT.

Reference

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