# **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	a oligonucleotides for the FRET-based DNA-NTs
Oligo Name	Sequence (5' to 3')
Hes-1 01	CTCAACTTTTATAATACAAATACATTTTACGCCTGGTGCC
Hes-1 02	CCGACCGCAGGATCCTATAAGGCGCAATCCAATAT
Control O1	CTTTTCGACAAGCGC TACATTTTACGCCTGGTGCC
Control O2	CCGACCGCAGGATCCTATAA <b>TTAACCGATAAAGATGAA</b>
03	Cy3-TTATAGGATCCTGCGGTCGGAGGCACCAGGCGTAAAATGTA-Cy5
Hes-1 DNA(A0)	ATATTGGATTGCGCCTTTGTATTATAAAAGTTGAG
Hes-1 DNA(A2)	ATATTGGATTGCGCCAATTTGTATTATAAAAGTTGAG
Hes-1 DNA(A4)	ATATTGGATTGCGCCAAAATTTGTATTATAAAAGTTGAG
Hes-1 DNA(A6)	ATATTGGATTGCGCCAAAAAATTTGTATTATAAAAGTTGAG
Hes-1 DNA(A8)	ATATTGGATTGCGCCAAAAAAAATTTGTATTATAAAAGTTGAG
Hes-1 DNA(A10)	<b>ATATTGGATTGCGCC</b> AAAAAAAAAA <b>TTTGTATTATAAAAGTTGAG</b>
Hes-1 4 base gap Ol	CATCTCAACTTTTATAATACATACATTTTACGCCTGGTGCC
Hes-1 4 base gap O2	CCGACCGCAGGATCCTATAACGCAATCCAATATGAA
GLUT1 4 base gap 01	<b>CTCAGAGTTCGGTATTAGT</b> TACATTTTACGCCTGGTGCC
GLUT1 4 base gap O2	CCGACCGCAGGATCCTATAACCTTGGCCATAGGTCC
PCR p	rimers 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  $\mu$ M 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<sup>2,3</sup>; 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<sup>TM</sup> 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  $\mu$ L 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<sup>4,5</sup> (Riken) cultured on Nunc<sup>™</sup> Lab-Tek<sup>™</sup> 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 =  $(f) - [(b) / (a)] \times (e) - [(c) / (d)] \times (g)$  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  $\mu$ M of DNA-NT and 12.6 ng/ $\mu$ L 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  $\mu$ M of CellTracker<sup>TM</sup> 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.

10 20 30 40 50 60 70 80 90 100 uacacececag aaccaaugge ggegguceua uaaaaaggea geueeggeg eueueececea agageagagg euugeuugua gagugaegau eugageuaeg 110 120 130 140 150 160 170 180 190 200 gggucuuaag ugcgucaggg cguggagguc uggcgggaga cgcauaguua cagcgcgucc guucuccguc ucgcagccgg cacagcuaga gcuucgagcg 210 220 230 240 250 260 270 280 290 300 cagegeggee auggaueeea geageaagaa ggugaeggge egeeueaugu uggeuguggg aggageagug eueggaueae ugeaguuegg euauaaeaeu 310 320 330 340 350 360 370 380 390 400 ggugucauca acgecececa gaagguuauu gaggaguucu acaaucaaac auggaaccac egeuaeggag ageceaueee auceaecaea eucaecaege 410 420 430 440 450 460 470 480 490 500 uuugguucu cuceguggee aucuucucug uegggggeau gauugguuce uucucugueg geeucuuugu uaauegeuuu ggeaggegga acueeaugeu 510 520 530 540 550 560 570 580 590 600 gaugaugaac cuguuggccu uuguggcugc ugugcuuaug ggcuucucca aacugggcaa guccuuugag augcugaucc ugggccgcuu caucaucggu 610 620 630 640 650 660 670 680 690 700 guguacugog gocugacuac uggouuugug occauguaug ugggagaggu gucaccuaca goucuaogug gagoccuagg cacacugoac cagougggaa 710 720 730 740 750 760 770 780 790 800 ucgucguugg cauccuuauu gcccaggugu uuggcuuaga cuccaucaug ggcaaugcag acuuguggcc ucugcugcuc agugucaucu ucaucccagc 810 820 830 840 850 860 870 880 890 900 ccugcuacag uguauccugu ugcccuucug ccccgagage cccccgcuuce ugcucaucaa ucguaacgag gagaaccggg ccaagagugu gcugaagaag 910 920 930 940 950 960 970 980 990 1000 cuucgaggga cageegaugu gaeeegaga cugeaggaga ugaaagaaga gggueggeag augaugeggg agaagaaggu caceaucuug gageuguuce 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100 geucaecege cuaecegeag eccauceuca uegeuguggu geugeageug uecageage ugueggguau caaugeugug uucuaeuaeu caaegageau 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200 cuucgagaag gcaggugugc agcagccugu guacgccacc aucggcuccg guaucgucaa cacggccuuc acuguggugu cgcuguuugu uguagagcga 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 gcuggacgac ggacccugca ccucauuggc cuggcuggca uggcaggcug ugcugugcuc augaccaucg cccuggccuu gcuggaacgg cugccuugga 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 uguccuaucu gagcaucgug gccaucuuug gcuuuguggc cuucuuugaa guaggcccug guccuauuce augguucauu guggccgage uguucagca 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 ggggccccgu ccugcugcua uugcuguggc uggcuucce aacuggaccu caaacuucau ugugggcaug ugcuuccagu auguggagca acugugcggc 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600 cccuacgucu ucaucaucuu cacggugcuc cucgugcucu ucuucaucuu caccuacuuc aaagucccug agaccaaagg ccgaaccuuc gaugagaucg 1610 1620 1630 1640 1650 1660 1670 1680 1690 1700 cuuceggeuu ceggeagggg ggugeeagee aaagugaeaa gaeaecegag gageueuuee aeceueuggg ggeggaeuee caagugugag gageeceaea 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 cccagcccgg ccugcucccu gcagcccaag gaucucucug gagcacagge agcuagauga gaccucuucc gaaccgacag aucucgggea agccgggecu 1810 1820 1830 1840 1850 1860 1870 1880 1990 1900 gggcgccuuu ccucagccag cagugaaguc caggaggaua uucaggacuu ugauggcucc agaauuuuua augaaagcaa gacugcugcu cagaucuauu 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 садаиаадса дсаддишина иааишинини аинасидани индинанини ининининан садосасиси ссиансисса сасиднадис инсассинда 2010 2020 2030 2040 2050 2060 2070 2080 2090 2100 uuggeeeagu geeugaggu ggggaeeaeg eeeugaeag eeuugeeu ucuuugeeaa geuaaueugu aggg<del>ggae cuauggeeaa gg</del>aead<mark>aeua</mark> 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 [auaccgaacu cugaguagg aggcuuuacc gcuggaggcg guagcugcca cccacuuccg caggccugga ccucggcacc auaggggucc ggacuccauu 2210 2220 2230 2240 2250 2260 2270 2280 2290 2300 uuaggauucg eceauuccug ucucuuccua eceaaceacu caauuaaucu uuccuugccu gagaceaguu ggaageacug gagugeaggg aggagaggga 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400 agggccaggc ugggcugcca gguucuaguc uccugugcac ugagggccac acaaacacca ugagaaggac cucggaggcu gagaacuuaa cugcugaaga 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 cacggacacu ccugcccugc uguguauaga uggaagauau uuauauauuu uuugguuguc aauauuaaau acagacacua aguuauagua uaucuggaca 2510 2520 2530 2540 2550 2560 2570 2580 aacccacuug uaaauacacc aacaaacucc uguaacuuua ccuaagcaga uauaaauggc ugguuuuuag aaa 2590 2600

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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