Supporting information

L-DNA-tagged fluorescence *in situ* hybridization for highly sensitive imaging of RNAs in single cells

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Experimental Section

Synthesis of chimera oligonucleotides. Oligonucleotides were synthesized with a phosphoramidite method (H6 DNA/RNA synthesizer). Besides standard phosphoramidites, L-DNA phosphoramidites were purchased from Glen Research Inc. The synthesized oligonucleotides were cleaved from the support with 28% aqueous ammonia and deprotected at 55°C for 4 h. The products were purified and confirmed by PAGE.

PCR. Polymerase chain reaction (PCR) reactions were carried out in a mixture containing 1×PCR buffer, 50 ng of the template DNA (pUC19), 200 nM of forward and reverse primers, 2 units of KOD Dash (Toyobo), and 100 μM of dNTPs. For labeling with fluorescent dyes, 60 μM of Fluorescein-12-dUTP (Thermo Fisher Scientific) and 40 μM of dTTP was used instead of the 100 μM dTTP. PCR was performed in LifeECO ver2.0 (Bioer Technology). After an initial denaturation at 94°C for 2 min, 30 cycles of PCR were carried out with denaturation at 94°C for 30 sec, annealing at 53°C for 2 sec and extension at 74°C for 30 sec. LT-PCR products were purified with agarose gel purification or Gel/PCR Extraction Kit (FastGene).

Fluorescence quantification of single fluorescein-labeled oligodeoxynucleotide probe. Streptavidin Mag Sepharose (GE Healthcare) was washed with tris buffered saline (TBS). 50 μL of 100 nM Biotin-labeled miRNA (Biotin-let-7a) was added and incubated at room temperature for an hour with mixing. Supernatant was removed and beads were washed with TBS 3 times. 50 μL of X nM (X = 2, 4, 6) a single fluorescein-labeled probe (FAM-probe(let-7a)) was added and incubated at room temperature in dark for 2 h with mixing. Supernatant was removed and beads were washed with TBS three times. 200 μL of TBS was added to resuspend the beads. The fluorescent intensity was measured with plate reader (Biotek Cytation 5) at $\lambda_{ex}/\lambda_{em}$: 484/525 nm. The excitation and emission band passes were set at 10 nm.

Fluorescence quantification of fluorescently-labeled LT-PCR products. Streptavidin Mag Sepharose was washed with tris buffered saline. 50 μL of 100 nM Biotin-let-7a was added and incubated at room temperature for 1 h with mixing. Supernatant was removed and beads were washed with TBS three times. 50 μL of 100 nM L-D chimera probe (L-probe(let-7a)) was added and incubated at room temperature for 1 h with mixing. Supernatant was removed and beads were washed with TBS three times. 50 μL of X nM (X = 2, 4, 6) LT-PCR products were added and incubated at room temperature in dark for 2 h with mixing. Supernatant was removed and beads were washed with TBS three times. 200 μL of TBS was added to resuspend the beads. The fluorescent intensity was measured with plate reader (Biotek Cytation 5) at $\lambda_{ex}/\lambda_{em}$: 484/525 nm. The excitation and emission band passes were set at 10 nm.

Cell culture. The human liver carcinoma cell line HepG2 cells and Human lung adenocarcinoma epithelial cell line A549 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing heat-inactivated fetal bovine serum (10%), penicillin (50 mg/mL), and streptomycin (50 mg/mL) under a humidified atmosphere (5% CO_2) at 37°C. Reagents for culturing were purchased from Gibco. For the following imaging experiments, cells were cultured in glass-based dishes (Matsunami).

LT-FISH. HepG2 and A549 cells were fixed in 4% (w/v) formaldehyde at room temperature for 20 min. After fixation, slides were washed with DEPC-treated PBS (DEPC-PBS) and dehydrated through three-time washing with 70% ethanol for 3 min each time. This was followed by two brief wash with DEPC-PBS. L-D chimera probe was diluted and applied at 200 nM in hybridization buffer (4×SSC, 0.5mM EDTA, 10% dextran sulfate, 25% deionized formamide in DEPC-H_2O) and incubated at 37°C for 1.5 h. After the hybridization of L-D chimera probe, excess probe was washed with DEPC-PBS for four times, and 10 nM 606-bp PCR product was hybridized in dark at 45°C for 2 h. The samples were washed with PBS three times, and then mounted onto glass slides with DAPI (Abcam) to stain the nucleus.

Confocal microscopy imaging. Confocal microscopy imaging was conducted using Observer Z1 microscope (Carl Zeiss, Jena, Germany). For nucleus imaging, DAPI was excited at the wavelength of 405 nm. The emissions were collected from λ = 420 nm to λ = 480 nm. Each probe was excited at the wavelength of 488 nm. All the emissions were collected at longer than λ = 515 nm. The acquired images were processed with ZEN 2009.

Table S1: DNA sequences used in this work

Names	Sequences (5'-3')
L-FW	*GGCAGATTGTGTGGACAGGTAATGGTTGTC*TTCCCAGTCACGACGTTGTA
D-FW	GGCAGATTGTGTGGACAGGTAATGGTTGTCTTCCCAGTCACGACGTTGTA
FW	TTCCCAGTCACGACGTTGTA
RV-1	ACAATTTCACACAGGAA
RV-2	CAATACGCAAACCGCCTCTC
RV-3	GGGGAAACGCCTGGTAT
Biotin-let-7a	Biotin-UGAGGUAGUAGGUUGUAUAGUU
FAM-probe(let-7a)	FAM-ACTCCATCATCCAACATATCAA
L-probe(let-7a)	*GACAACCATTACCTGTCCACACAATCTGCCTTT*ACTCCATCATCCAACATATCAA
L-probe(survivin)	*GACAACCATTACCTGTCCACACAATCTGCCTTT*TAGAGATGCGGTGGTC
L-probe(miR-155)	*GACAACCATTACCTGTCCACACAATCTGCCTTT*ACCCCTATCACGATTAGCATTAA
L-probe(miR-21)	*GACAACCATTACCTGTCCACACAATCTGCCTTT*TCAACATCAGTCTGATAAGCTA
D-probe(survivin)	GACAACCATTACCTGTCCACACAATCTGCCTTTTAGAGATGCGGTGGTC
D-probe(miR-155)	GACAACCATTACCTGTCCACACAATCTGCCTTTACCCCTATCACGATTAGCATTAA
D-probe(miR-21)	GACAACCATTACCTGTCCACACAATCTGCCTTTTCAACATCAGTCTGATAAGCTA
FAM-probe(survivin)	FAM-TAGAGATGCGGTGGTC

Underlined italic: L-DNA, others: D-DNA. (Oligonucleotides except for ones including L-DNA were purchased from Hokkaido System Science Co., Ltd.)

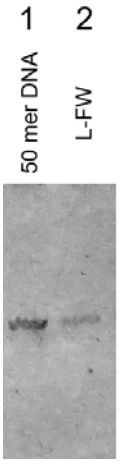

Fig. S1 Synthesis and characterization of L-DNA tagged PCR primer. A purchased 50 mer DNA (Hokkaido System Science Co., Ltd.) (lane 1) and purified L-FW (lane 2) were loaded onto 15% polyacrylamide gel containing 7M urea. Gel bands were stained with SYBR Gold.

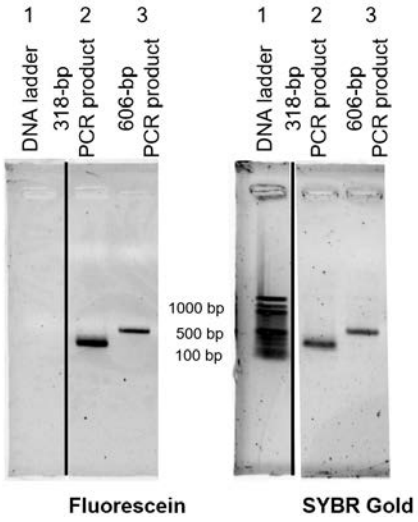

Fig. S2 318 and 606 bp fluorescein-labeled PCR products tethering an L-DNA tag. DNA ladder (lane 1), 318 bp PCR product by a L-FW and RV2 primer set (lane 2). 606 bp PCR product obtained by a L-FW and RV3 primer set (lane 3) were loaded onto 1% Agarose gel. Band images were obtained through direct detection of fluorescein or after SYBR Gold staining.

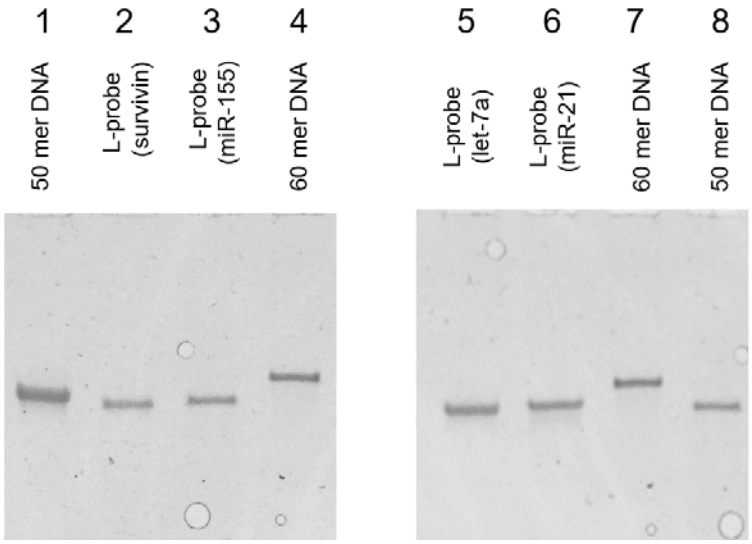

Fig. S3 Synthesis and characterization of chimera probes. 50 mer DNA (lane 1), 49 mer chimera DNA (L-probe(survivin)) (lane 2), 56 mer chimera DNA (L-probe(miR-155)) (lane 3), 60 mer DNA (lane 4), 55 mer chimera DNA (L-probe(let-7a)) (lane 5) 55 mer chimera DNA (L-probe(miR-21)) (lane 6) 50 mer DNA (lane 7), and 60 mer DNA (lane 8) were loaded onto 15% polyacrylamide gel containing 7M urea. Gel bands were stained with SYBR Gold.