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Supporting Information

MicroRNA detection using light-up aptamer amplification based on nuclease protection transcription

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Experimental

Materials and Reagents

S1 nuclease was purchased from Takara (Shiga, Japan). Streptavidin MagneSphere® paramagnetic particles (STA-MBs) were purchased from Promega (Madison, WI, USA). T7 RNA polymerase and nucleoside triphosphates were purchased from Takara. (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-1-(2,2,2-trifluoroethyl)-1H-imidazol-5(4H)-one (DFHBI-1T) was obtained from Tocris Bioscience (Bristol, United Kingdom). An RNase inhibitor was obtained from Solgent (Daejeon, Korea). Pfu DNA polymerase was purchased from Nanohelix (Daejeon, Korea), and the Wizard SV Gel and PCR Clean-up system were purchased from Promega. Tween 20 was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Phosphate-buffered saline (PBS) was obtained from Biosesang (Daejeon, Korea). Synthetic oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA, USA). Total RNA from the human liver was purchased from Takara. AFP-free human serum was purchased from Cone Bioproducts (Seguin, TX, USA).

Preparation of detection probe

The pUC57-Broccoli aptamer plasmids containing a T7 promoter, broccoli-derived aptamer, and terminator sequence were synthesized by Bionics (Seoul, Korea). PCR was performed using forward primer (F) and reverse primer (R) as detection probes (Table S1). PCR was performed according to the manufacturer's protocol as follows: 2 min of denaturation at 94 °C; 30 cycles at 95 °C for 20 sec, 54 °C for 40 sec, and 72 °C for 15 sec, followed by a final extension step at 72 °C for 5 min. PCR products were

analyzed via electrophoresis. After staining with GelRed, the gel blocks containing the detection probe sequence were excised under 365 nm UV illumination. The excised gel blocks were purified using a commercial PCR clean-up kit (Promega) according to the manufacturer's instructions.

Nuclease protection transcription for miRNA detection

For miRNA detection via the nuclease protection transcription assay, the detection probe (8 nM) was hybridized with the target miRNA in PBS (total volume, 10 μL) with RNase inhibitor (40 U/μL) for 30 min at 25 °C. The hybridized detection probe was added to the nuclease reaction mixture (total volume, 30 μL), S1 nuclease (20 U), and 1× S1 buffer. The nuclease reaction mixture was incubated at 25 °C for 10 min. Subsequently, 2 μM EDTA was added, followed by incubation for another 2 min to inactivate the S1 nuclease. The 20 μL of STA-MBs were added to the reaction mixture and incubated at 25 °C for 30 min. After washing with PBST (PBS at pH7.4 containing 0.1% Tween20), 40 μL of the transcription reaction mixture (2 μL of T7 RNA polymerase (50 U/μL), 3.2 μL of NTPs (each 2 mM), 4 μL of DTT (50 mM) and 1X RNA polymerase reaction buffer) was added and incubated for 2 h at 37 °C. The transcription mixture was then mixed with folding buffer (40 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, and 125 mM KCl) containing DFHBI-1T (1 μM) and incubated at 25 °C for 10 min. Fluorescence was measured using a VICTOR microplate reader (Model 2030-0050; PerkinElmer, Waltham, MA, USA). For the pretreatment of human serum, human serum was heated to 95 °C for 10 min to inactivate deoxyribonuclease. Subsequently, the serum sample was centrifuged at 13,500 rpm for 5 min at 4 °C, and

then, miR-122 was spiked into pretreated human serum (100%). The 2 μL of pretreated human serum spiked with miRNA was added to conduct the nuclease reaction at final 5% concentration.

RT-PCR

RT-qPCR was performed to quantify miR-122 expression levels. RT-PCR method was adopted the previous report by Hellens et al.[1] (Figure S5a) The M-MLV cDNA Synthesis kit was purchased from Enzynomics (Daejeon, Korea) for performing reverse transcription and TOPreal™ qPCR 2× Premix (SYBR Green with low ROX) was purchased from Enzynomics for signal analysis. Reverse transcription was performed in three steps: 10 min of primer hybridization at 25 °C, 60 min of reverse transcription at 42 °C, and 5 min of reverse transcriptase inactivation at 95 °C. PCR was performed as follows: 10 min of denaturation at 95 °C; 30 cycles at 95 °C for 10 sec, 54 °C for 45 sec, and 68 °C for 10 sec. RT-qPCR was performed using the MyGo Pro real-time PCR instrument (IT-IS Life Science Ltd., Dublin, Ireland) and MyGo Pro PCR Software for analysis. All primers used are listed in Table S1.

Table S1. Sequence of oligonucleotides used in this study.

Name	Sequence (5' → 3')
Detection probe primers	
Detection probe-1_F	GTTGTAAAACGACGGCCAGTG
Detection probe-1_R	[Biotin]CAAACACCATTGTCACACTCCA[Spacer]CAAAAAACCCCT CAAGACCCG
Detection probe-2_F	[Biotin]CAAACACCATTGTCACACTCCA[Spacer]GTTGTAAAACGA CGGCCAGTG
Detection probe-2_R	CAAAAAACCCCTCAAGACCCG
RT-PCR primers	
Hairpin primer	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATG TGCGTCTCGCCTTCTTTCCAAACACC
Forward primer	AGGCTGTGGAGTGTGACAAT
Reverse primer	CGAGGAAGAAGACGGAAGAAT
miRNA sequence	
miR-122	UGGAGUGUGACAAUGGUGUUUG
miR-155	UAGCUUAUCAGACUGAUGUUGA
miR-21	UGAGGUAGUAGGUUGUAUAGUU
miR-141	UAACACUGUCUGGUAAAGAUGG
Let 7a	UUAAUGCUAAUCGUGAUAGGGGU
Mis-1	UGGAGUGUAACAAUGGUGUUUG
Mis-2	UGGAGUAUGACAAUGGAGUUUG
Mis-3	UGGAAUGUGAAAAUGGUGAUUG
Detection probe sequence	

Detector	CAAACACCATTGTCACACTCCA
Signal generator	GTTGTAAAACGACGGCCAGTGAATTCTAATACGACTCACTATAG **GGGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGA GTGTGGGCTCC***CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCT TGAGGGGTTTTTTG*

[Spacer]: six carbon glycol spacer that is capable of blocking extension by DNA polymerase. The mismatched sequence is underlined. T7 promoter sequence in signal generator is underlined. Broccoli aptamer sequence in signal generator is bolded. T7 terminator sequence in signal generator is italics.

Table S2. Determination of miR-122 in human serum (5%)

Spiked (pM)	Detected (pM)	Recovery (%)	RSD (%)
1250	1201	96	0.9
625	592	95	0.3
312.5	329	105	1.7

RSD, relative standard deviation (n=3)

Table S3. Comparison of different method for miRNA detection

Methods	Target	LOD	Linear range	Ref.
Target initiated strand displacement amplification using nicking enzyme and RNA aptamer amplification with transcription method	Let-7a	5.12 aM	10 aM-500 fM	2
Target-assisted ligation and RNA aptamer amplification with transcription method	miRNA-21/141	3 pM	5 pM-500 pM	3
Rolling circle amplification (RCA) and RNA aptamer amplification with transcription method	Let-7a	67.3 fM	100 fM-50 pM	4
Universal split spinach aptamer (USSA)	miRNA-99a	1.5 nM	0–100 nM	5
Target initiated amplification and RNA aptamer amplification with transcription method	miRNA-21/141	0.955 fM	1 fM-100 pM	6
Target initiated amplification and RNA aptamer amplification with transcription method	Let-7a	10 amol	10 amol–1 pmol	7
Duplex specific nuclease (DSN) assisted isothermal amplification detection using molecular beacon (MB)	Let-7a	3.8 pM	5 pM-500 pM	8
DSN-assisted target recycling signal amplification using RNA aptamer	miRNA 141	1.03 pM	0-10 μM	9
G-triplex based MB with DSN amplification	miRNA 141	0.25 pM	1 pM–5 nM	10
G-quadruplex based MB with DSN amplification	miRNA 141/ 21	1 pM	1 pM-5 nM	11
Catalyzed hairpin assembly (CHA)	miRNA-21	1.48 pM	4 pM-40 nM	12
Strand displacement amplification(SDA) and Rolling circle amplification (RCA)	let-7b	3.2 pM	10 pM-10 nM	13
Nuclease protection assay and transcription assisted RNA aptamer amplification	miRNA-122	178 fM	0.5 pM–5 nM	This work

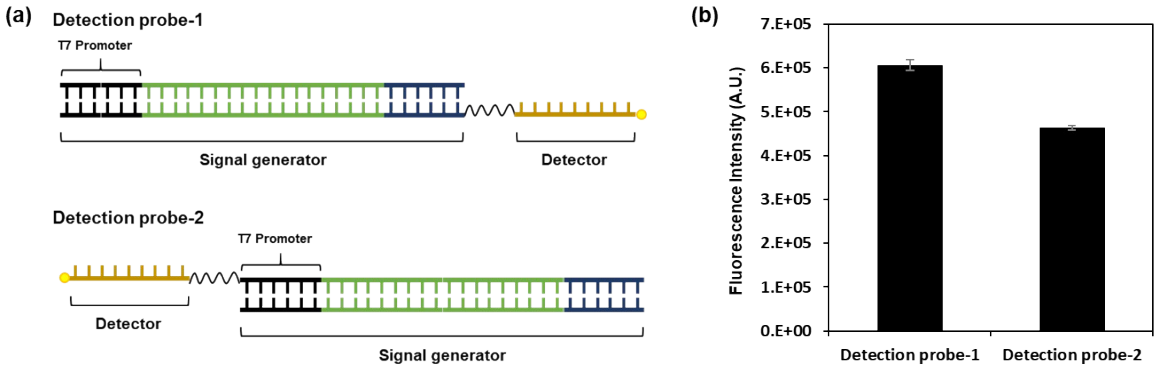

Fig. S1. The effect of biotin position in the detection probe on the immobilization efficiency. (a) Design of detection probe. The biotinylated detection probes were prepared: Detection probe-1, biotin at the 5' end near the promoter region of detection probe; Detection probe-2, biotin at the 5' end near the terminator region of detection probe. The biotinylated detection probes were immobilized on the streptavidin coated magnetic bead (STA-MB), and transcription efficiency was determined by the fluorescence signal from the generated Broccoli aptamer. (b) The fluorescence intensity of Broccoli aptamer from detection probes with different position of biotin. Error bars indicate the standard deviations of three independent measurements.

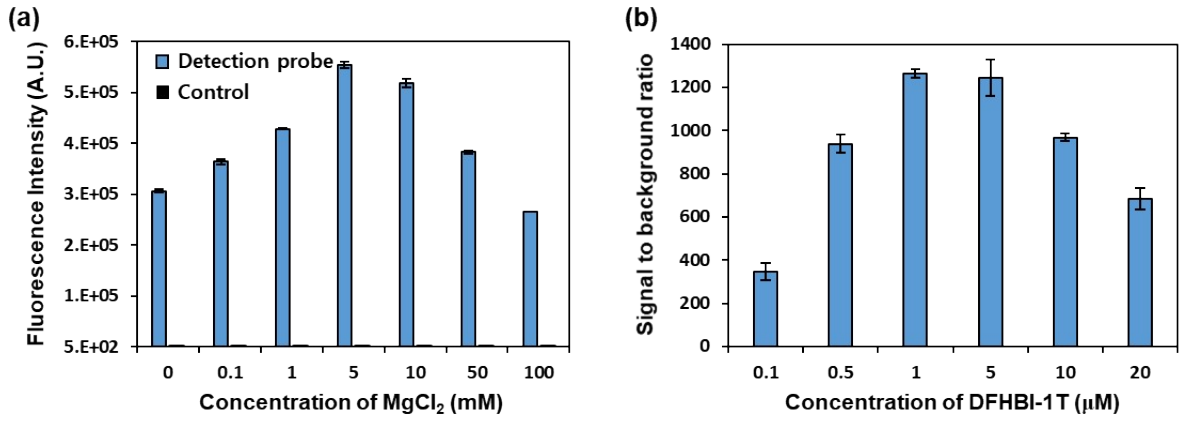

Fig. S2. Optimization of Broccoli aptamer folding conditions. (a) Optimization of the concentration of $MgCl_2$. The concentration of DFHBI-1T in fold buffer was 1 μM. (b) Optimization of the concentration of DFHBI-1T. The concentration of $MgCl_2$ in fold buffer was 5 mM. For *in vitro* transcription, 8 nM of detection probe was incubated with transcription mixture at 37 °C for 2 h. The control (background) is the fluorescence signal obtained in the absence of detection probe. Error bars indicate the standard deviations of three independent measurements.

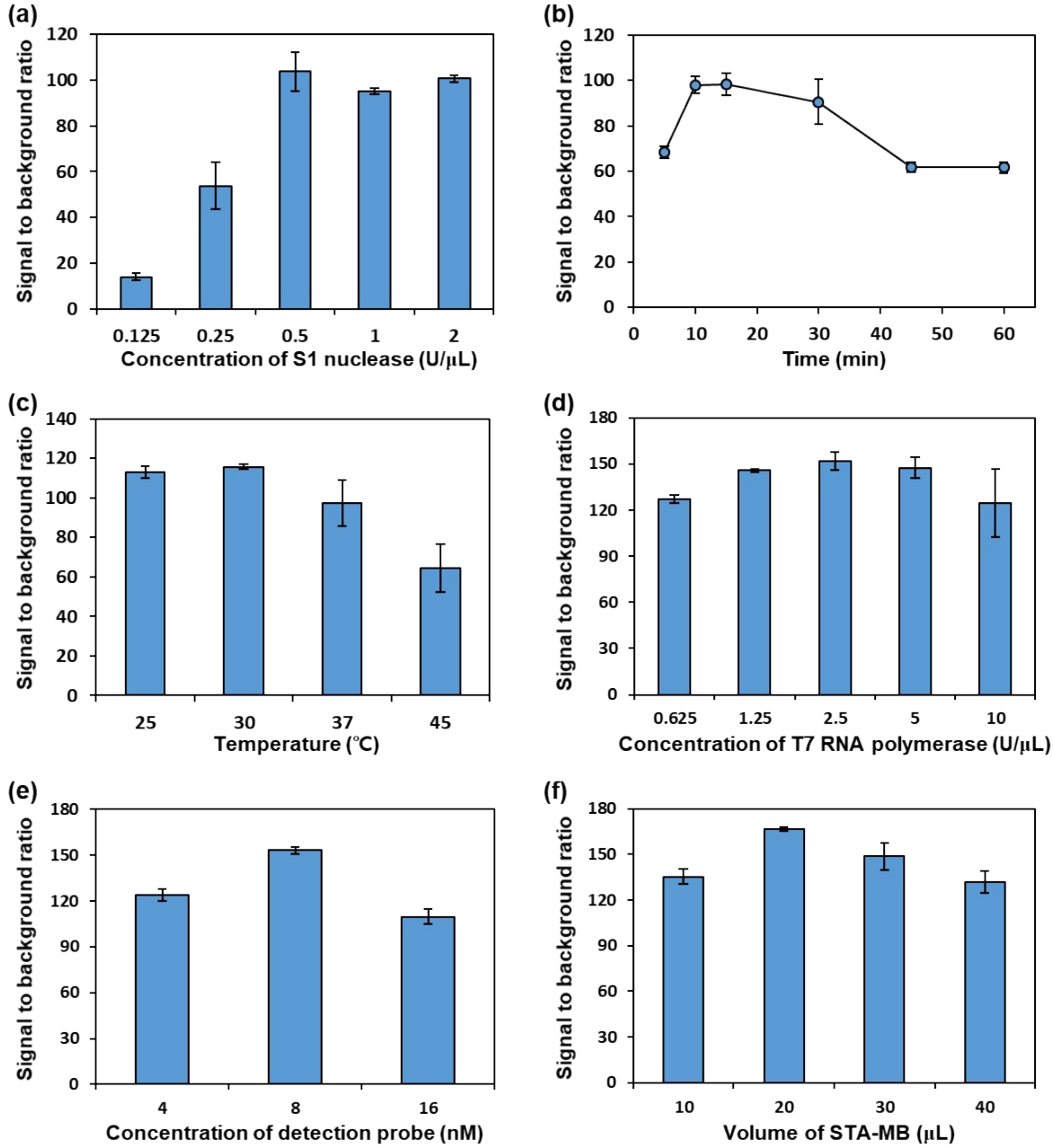

Fig. S3. Optimization of experimental condition. (a) Effect of the concentration of S1 nuclease. The reaction time and temperature of S1 nuclease were 15 min and 25 °C. The concentration of detection probe and T7 RNA polymerase were 8 nM and 1.25 U/μL, respectively. The volume of STA-MBs was 10 μL. (b) Optimization of S1 nuclease reaction time. The concentration of S1 nuclease was 0.5 U/μL. (c) Optimization of S1 nuclease reaction temperature. The concentration and reaction time of S1 nuclease were 0.5 U/μL and 10 min. (d) Optimization of the concentration of T7 RNA polymerase. The

concentration, reaction time and temperature of S1 nuclease were 0.5 U/μL, 10 min and 25 °C. (e) Optimization of the detection probe. The concentration, reaction time and temperature of S1 nuclease were 0.5 U/μL, 10 min and 25 °C. The concentration of T7 RNA polymerase was 2.5 U/μL. (f) Effect of volume of STA-MB. The concentration, reaction time and temperature of S1 nuclease were 0.5 U/μL, 10 min and 25 °C. The concentration of detection probe and T7 RNA polymerase were 8 nM and 2.5 U/μL, respectively. 5 nM of miR-122 was analysed for condition optimization. The background is signal from a control reaction without miRNA. The fluorescence intensity represents the average of three individual measurements, and error bars indicate standard deviation.

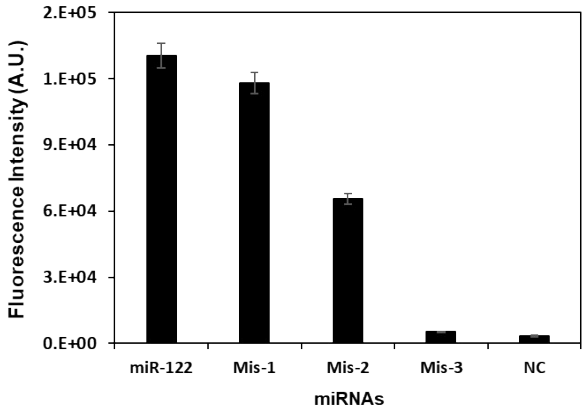

Fig. S4. Specificity of the nuclease protection transcription assay for miR-122. The concentration of miR-122 and three mismatched sequence Mis-1 (single-base mismatched), Mis-2 (two-base mismatched) and Mis-3 (three-base mismatched) were 5 nM. Data represent the average of three individual measurements, and error bars indicate standard deviation. NC, negative control.

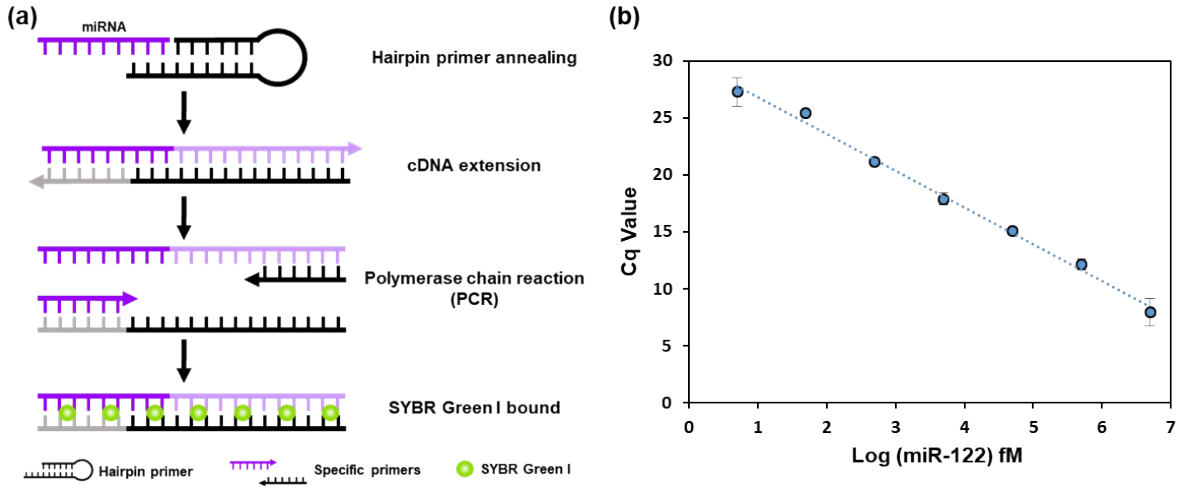

Fig. S5. Quantitative miR-122 detection using RT-qPCR method. (a) Schematic of the stem-loop RT-PCR reaction, showing the participating components in the reaction. (b) Calibration curves with different miR-122 concentrations (5 fM – 5 nM) obtained by conventional RT-q-PCR. The regression equation was y= -3.2332x + 30.08 (R^2 = 0.9948) in the linear range. The data are presented as the average of three independent experiments, and error bars indicate standard deviation.

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