Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Highly Sensitive, Selective, and Rapid Detection of miRNA-21 using an RCA/G-Quadruplex/QnDESA Probing System

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1. General Information

Natural oligonucleotides having the sequences listed in **Table S1** and diethyl pyrocarbonate deionized water (DEPC-DW) were purchased from Bioneer (South Korea). phi-29 DNA polymerase and SplintR Ligase were purchased from New England Biolabs (United States). The dNTP mixture (dATP, dTTP, dCTP, and dGTP) was purchased from Enzynomics (South Korea). Nuclease-free water was purchased from Invitrogen by Life Technologies (USA). 40% Acrylamide/Bis solution was purchased from BIO-RAD (USA). Urea (extra pure) bought from DAEJUNG Chemicals (South Korea). QnDESA fluorophore was synthesized in the Synthetic Biology and Medicinal Chemistry Laboratory, Jeonbuk National University. Human serum (H4522) was purchased from Sigma Aldrich (USA origin).

Absorbance spectra were recorded using a Cary 100 UV–Vis spectrophotometer (Agilent Technologies, USA). Fluorescence spectra were recorded at room temperature using a quartz cuvette (path length: 1 cm) and a FluoroMate FS-2 fluorescence spectrophotometer (Scinco, Seoul, South Korea). The PAGE apparatus was purchased from CBS Scientific (California, USA). The agarose electrophoresis device was purchased from Mupid-2plus (ADVANCE) (Kobe, Japan).

2. Experimental Procedure for Enzymatic Reaction

The RCA reaction mixture (total volume: 20 µL) contained the 22AG padlock template and miRNA-21 (100 pmol/µL, 2 µL each), 10X BSA (2 µL), SplintR Ligase (25,000 U/mL, 1 µL), phi-29 DNA polymerase (10 U/µL, 1 µL), SplintR Ligase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM DTT; pH 7.5 at 25 °C; 2 µL), phi-29 DNA polymerase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 4 mM DTT; pH 7.5 at 25°C; 2 µL), and a dNTP mixture (dATP, dTTP, dCTP, and dGTP; 2 mM, 8 µL). This single-tube reaction mixture was slightly vortexed and spin down, then incubated at 37 °C for 1 h.

3. Table S1: Oligonucleotides used in this study

Name	Sequence (5´→3´)
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-21 mismatch 1	UAG CUU AUC ACA CUG AUG UUG A
miRNA-21 mismatch 2	UAG CUU AUC ACA UUG AUG UUG A
miRNA-21 mismatch 3	UAG CUU AUG ACA UUG AUG UUG A
miRNA 25b	UCC CUG AGA CCC UAA CUU GUG A
miRNA 24-3P	UGG CUC AGU UCA GCA GGA ACA G
miRNA 146-a	CCU CUG AAA UUC AGU UCU UCA G
22AG G-Quadruplex	AGG GTT AGG GTT AGG GTT AGG G
22AG Padlock Template	5´ [Phosphate] - CTG ATA AGC TAA TGA CGG ATT GAT GAC ACC CTA ACC CTA ACC CTA ACC CTG ATA GTC TAC ATG TTA ACG ATC AAC ATC AGT

4. Detection of the amplified tandem 22AG G-quadruplex

At the completion of the RCA reaction, 100 mM KCl, 100 mM Tris-HCl, 1 µM QnDESA, and distilled water were added to adjust the volume to 1 mL. The fluorescence data were analyzed using Origin software. Gel electrophoresis was performed using either 2% agarose gel (AGE) or 20% polyacrylamide gel (PAGE). In the case of agarose gel, 1X TBE buffer (50 mL) was melted with agarose powder (2 g) and then loaded at 50 V for 50 min. For confirmation of the circular-DNA and for testing of the sensitivity, 20% non-denaturing PAGE was used. For gel preparation, 40% acrylamide/Bis solution (15 mL), 10X TBE buffer (3 mL), and 20% ammonium persulfate solution (APS; dissolved in water; 200 µL) were mixed in single tube filled with H_2O to give a total volume of 30 mL and finally added 20 µL of TEMED to make the gel. In case of 20% denaturing PAGE, 15g of Urea(16.65 M) was added into the solution.

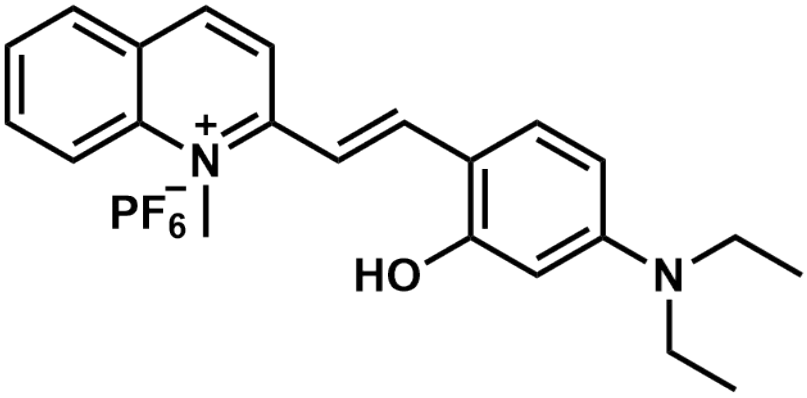

Figure S1: Structure of the fluorophore QnDESA. For the synthesis of QnDESA, see ref. 34.

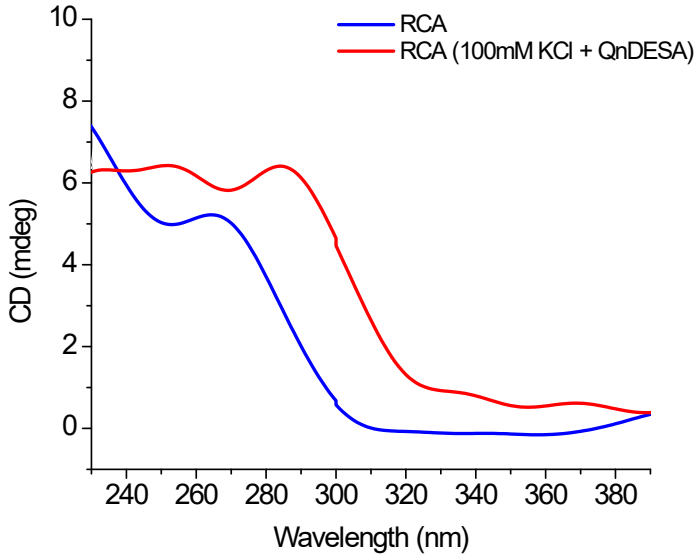

Figure S2: CD spectra of the 22AG RCA product in the range 240–380 nm. Blue: RCA product only; red: RCA product with stable G-quadruplex in the presence of K^+ ions and QnDESA.

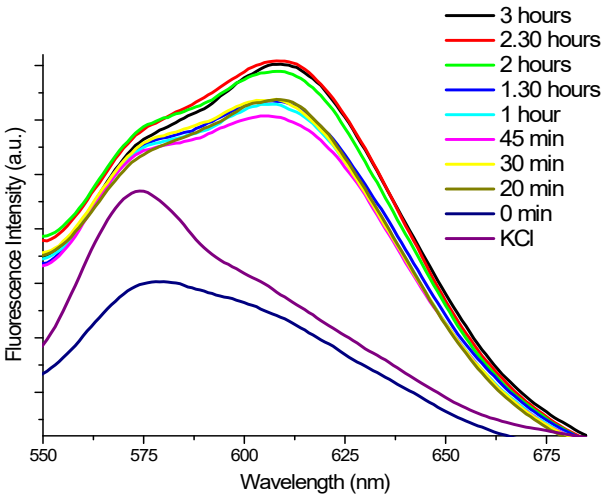

Figure S3: Sensitivity of the time-dependent fluorescence spectra from 0 min to 3 h. Fluorescence spectra were recorded using the optimized concentration of QnDESA (1 µM). All samples were excited at 525 nm.

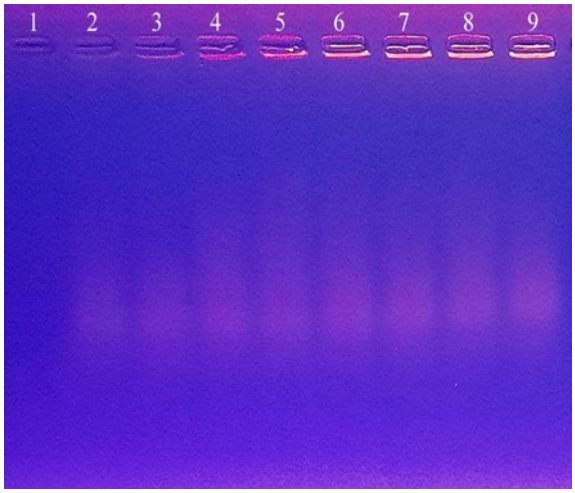

Figure S4: RCA band detected through agarose gel electrophoresis from 0 min to 3 h. Lane 1: RCA 0 min; lane 2: RCA 20 min; lane 3: RCA 30 min; lane 4: RCA 45 min; lane 5: RCA 1 h; lane 6: RCA 1:30 h; lane 7: RCA 2 h; lane 8: RCA 2:30 h; lane 9: RCA 3 h. Considering both the RCA band intensity and the fluorescence intensity, 1 h was the optimized period for detection.

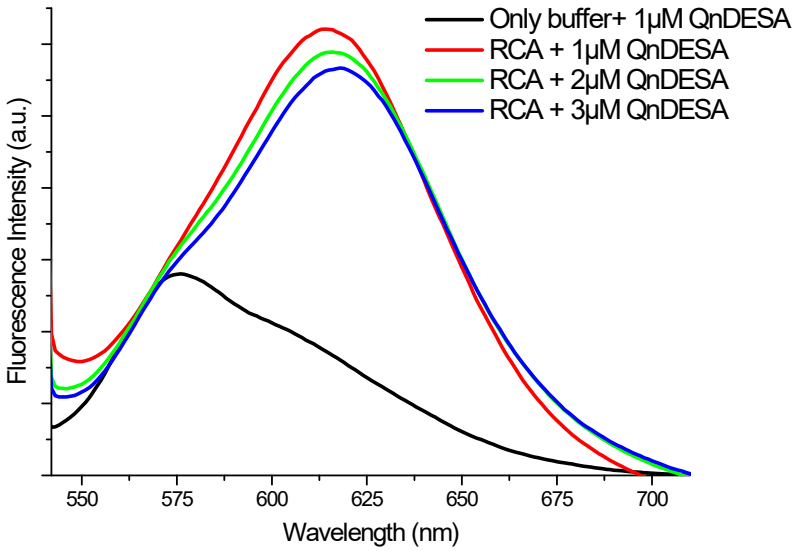

Figure S5: Fluorescence spectra of the RCA product in the presence of various concentrations of QnDESA. Here, increasing the QnDESA concentration led to continuous red-shifting. All samples were excited at 525 nm.

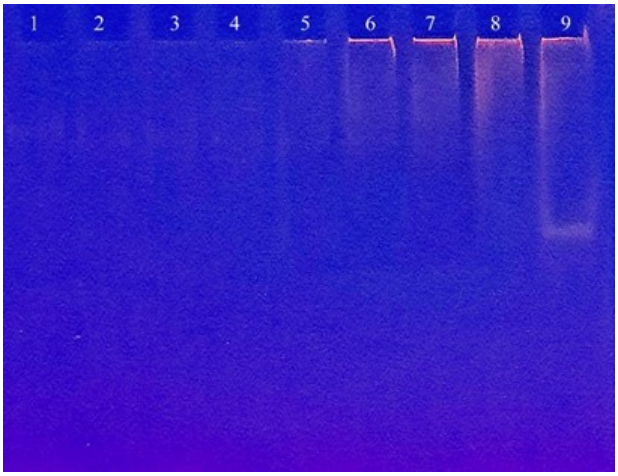

Figure S6: 20% Non-denaturing PAGE testing of the RCA sensitivity in the presence of various concentrations of miRNA-21. As gel sensitivity is less than fluorescence that's why clear band shows lane 6-9. Lane 1, 1 aM; lane 2, 10 aM; lane 3, 100 aM; lane 4, 1 fM; lane 5, 10 fM; lane 6, 100 fM; lane 7, 1 pM; lane 8, 10 pM; lane 9, 100 pM.

Figure S7: 2% Agarose gel electrophoresis for testing of selectivity. Lane 1, miR-21 (**positive target**); lane 2, miR-21-mismatch 1; lane 3, miR-21-mismatch 2; lane 4, miR-21-mismatch 3; lane 5, miR-25b; lane 6, miR-146-a; lane 7, miR-24-3P; lane 8, no target/ no miR-21.

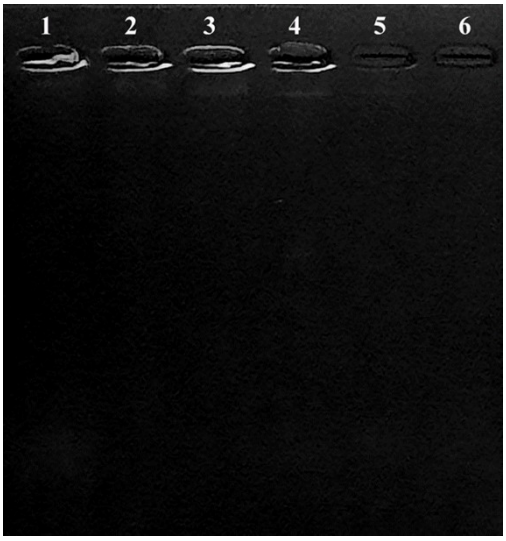

Figure S8: 2% Agarose gel electrophoresis for testing of RCA sensitivity in the presence of various concentrations of miRNA-21 in spiked human serum sample. As agarose gel sensitivity is less than fluorescence that's why band shows lane 1-4. Lane 1, 100 pM; lane 2, 10 pM; lane 3, 1 pM; lane 4, 100 fM; lane 5, 10 fM; lane 6, 1 fM.

Figure S9: 2% Agarose gel electrophoresis for testing of selectivity of miRNA mixture. Lane M, 25/100 bp ladder; Lane 1, **negative target** (miRNA 25b, miRNA 24-3P, miRNA 146a); lane 2, **positive target** (miRNA 21, miRNA 25b, miRNA 24-3P, miRNA 146a).