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Electronic Supplementary Information for Analyst

Label-free fluorometric detection of microRNA using isothermal rolling circle amplification generating tandem G-quadruplex

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Supplementary experimental methods

1. Ligation-mediated RCA (L-RCA) assay

The RCA assay employing the closed circular dumbbell padlock DNA was compared with the commonly used ligation-mediated RCA (L-RCA) assay using linear padlock DNA as a template in terms of signal-to-background ratio (S/B ratio). Both ends of the linear padlock probe DNA were designed to be hybridized with miRNA183 in order to generate a circular template by ligation for subsequent RCA. The ligation of the circular padlock probe DNA was performed in 10 μL of reaction mixture containing the padlock probe DNA and miRNA183 (125 nM each), 175 U of T4 DNA ligase, and 1 × ligation buffer (66 mM Tris-HCl, pH 7.6, 6.6 mM MgCl2, 10 mM DTT, and 0.1 mM ATP). The reaction mixture was heat-denatured at 95 °C for 3 min and cooled at 22 °C for 10 min prior to the T4 DNA ligase supplementation. The ligation reaction was then carried out at 22 °C for 1 h to generate the circular padlock template for the GQ-RCA. Next, 10 μL of ligation reaction mixture was supplemented to the RCA reaction mixture composed of 80 μg/mL BSA, 0.6 mM dNTP mixture, 15 μM ThT, 1 mM KCl, and 1 × buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 10 mM (NH4)2SO4, and 4 mM DTT) to make a total volume of 25 μL, which was then heat denatured at 95 °C for 3 min and cooled at 32 °C for 10 min. The RCA reaction was initiated by adding phi29 DNA polymerase (300 U) and incubated at 32 °C for 30 min, then terminated by heating at 65 °C for 10 min. The fluorescence intensity of the GQ-RCA reaction product was measured by scanning from 450 to 650 nm at an excitation wavelength of 425 nm by using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies., Santa Clara, CA, USA).

2. GQ-RCA for detection of miRNA with single nucleotide mutations or similar sequences

We prepared single nucleotide mutation (SNM_C and SNM_G)-containing miRNA183 variants and two different miRNA183 family miRNAs (miRNA96 and miRNA182) in this experiment (the sequences are listed in Table S1). The GQ-RCA was performed using 50 nM dumbbell padlock-183 with 50 nM of different miRNA sequences under the same conditions as described in experimental section of manuscript. We used GQ-RCA without miRNA under the same conditions as a negative control. The ThT fluorescence intensity of each GQ-RCA reaction product was measured by scanning from 450 to 650 nm with an excitation wavelength of 425 nm.

Supplementary data (tables and figures)

Table S1. Oligonucleotide sequences (5′ to 3′) used in this study.

Name	Sequence* (5′ to 3′)	Size* (nt)
miRNA 16	UAG CAG CAC GUA AAU AUU GGC G	22
miRNA 21	UAG CUU AUC AGA CUG AUG UUG A	22
miRNA 183	UAU GGC ACU GGU AGA AUU CAC U	22
miRNA 205	UCC UUC AUU CCA CCG GAG UCU G	22
miRNA 96	UUU GGC ACU AGC ACA UUU UUG CU	23
miRNA 182	UUU GGC AAU GGU AGA ACU CAC ACU	24
miRNA 183 SNM_C	UAU GGC ACU GGU AGA AUU GAC U	22
miRNA 183 SNM_G	UAU GGC ACU GGU ACA AUU CAC U	22
miRNA16 Dumbbell padlock (DP16)	p-CGA AAT TAT CCG TCC CAT CCC TAA CCC TAA CCC TAA CCC TAT TTC GGC AGT GTT TTT TTT TCG CCA ATA TTT ACT TTC TGC TTT CAC TGC	90
miRNA21 Dumbbell padlock (DP21)	p-CGA AAT CTA TTG CCC TAT TCC CTA ACC CTA ACC CTA ACC CTA TTT CGG CAG TGT CCT TTT CAA CAT CAG TCT GGT AAG CTA CCC TTT CAC TGC	93
miRNA183 Dumbbell padlock (DP183)	p-CGA AAT TAC CCT ACG CCT CCC TAA CCC TAA CCC TAA CCC TAT TTC GAT TTG GTC CTC AGT GAA TTC TAT GTG TGC CAT TTT TTC CCC AAA T	91
miRNA205 Dumbbell padlock (DP205)	p-CGA AAT TTA GGC CTT TAT TCC CTA ACC CTA ACC CTA ACC CTA TTT CGG CAG TGT CCC CAG ACT CCG GTG AAA TGA AGC CCA ACA CTG C	88
miRNA183 Specific-circular padlock	p-ACC AGT GCC ATA AAC GAC GAG CCC TAA CCC TAA CCC TAA CCC TCG CAA TAA GCT GTG AAT TCT	63

* The red-colored nucleotide indicates single nucleotide mutation (SNM) site; P, phosphorylation; nt, nucleotides

Table S2. Comparison of the assay number of labeling, number of enzymes, number of steps, duration time, and detection limit between fluorometric GQ-RCA and previously published miRNA detection methods.

Target	Method	Label[a]	Enzyme[b]	Step	Time	LOD[c]	Application	Refs.
Let-7a	N-RCA/S-RCA-incorporated combined cascade amplification (rs-CCA)	-	3	3	5.5 h	5 pM	Total cellular RNA	[1]
miRNA122	Exo III-assisted DNA-AgNGs fluorescence quenching	-	1	3	2.5 h	84 pM	Human plasma	[2]
miRNA141	DSN and a perylene probe-based miRNA assay	-	2	1	3.5 h	5 pM	Human plasma	[3]
Let-7a	miRNA-triggered SDA	2	3	4	2.3 h	58 fM	-	[4]
Let-7b	branched rolling circle amplification (BRCA)	-	1	2	2.5 h	25 fM	Total cellular RNA	[5]
Let-7a	branched rolling-circle amplification (BRCA)	-	3	2	8 h	10 fM	Total cellular RNA	[6]
miRNA21	Isothermal amplification with GO	1	2	2	3 h	3 fM	??	[7]
miRNA21 miRNA-125b	label-free CHA based radiometric sensing method	-	0	2	1 h	3.1 pM	Cell lysate	[8]
miRNA21	GQ-based sensing system	-	1	1	0.5 h	4.5 nM	Human plasma	[9]
miRNA16, miRNA21, miRNA183, miRNA205	GQ-RCA	-	1	1	0.5 h	4.92 fM	Human plasma, total cellular RNA	This study

[a] Labels include quencher, fluorophore, and phosphate required for the assay

[b] The number of enzymes including polymerase, exonuclease, and nicking enzyme required for the assay

[c] The lowest quantity miRNA required to distinguish the sample and the blank

Abbreviations: DSN, duplex specific nuclease; SDA, strand displacement amplification; CHA, catalytic hairpin assembly

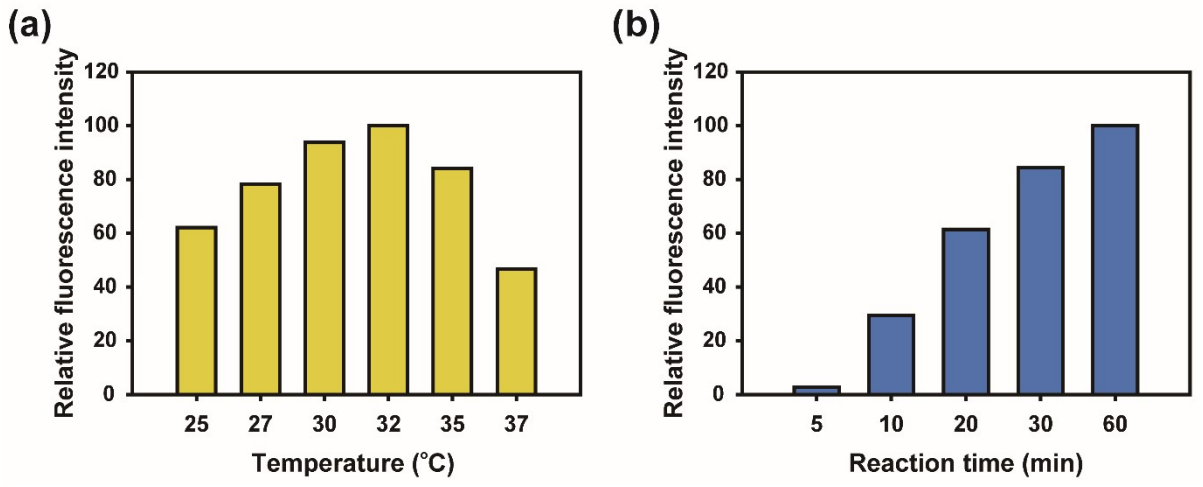

Fig. S1 The optimization of G-quadruplex generating RCA (GQ-RCA). (a) Relative fluorescence intensities of RCA products at different reaction temperatures (25 °C, 27 °C, 30 °C, 32 °C, 35 °C, and 37 °C) at 425 nm excitation and 488 nm emission wavelengths. (b) Relative fluorescence intensities of RCA products incubated at 32 °C for different reaction times (5 min, 10 min, 20 min, 30 min, and 60 min).

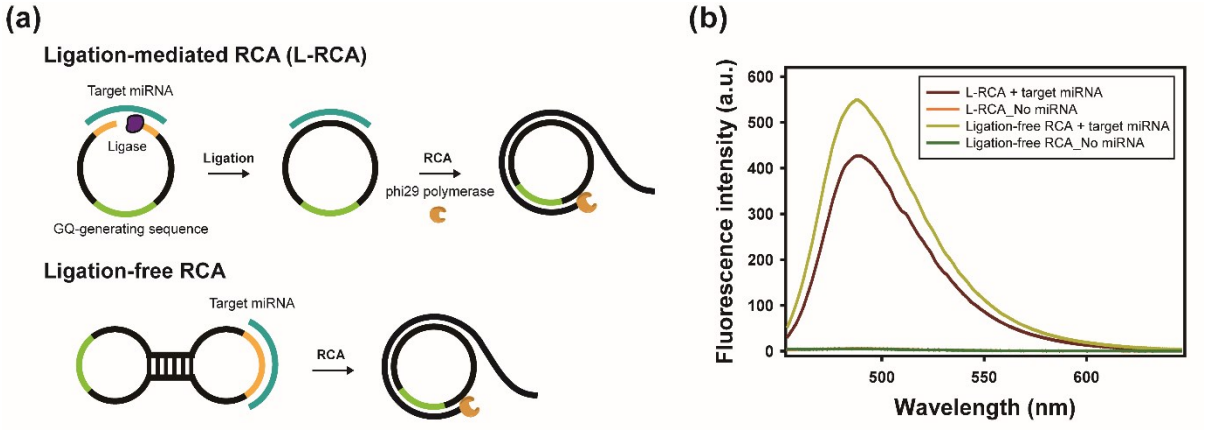

Fig. S2 Comparison of RCA efficiency between the ligation-mediated RCA (L-RCA) and the ligation-free RCA. (a) Schematic illustration of the ligation-mediated and ligation-free RCA methods. (b) RCA reaction conducted at 32 °C for 30 min using the same amount of template DNA and miRNA. Fluorescence emission spectra (450 nm < λ_{em} < 650 nm at λ_{ex} = 425 nm) were obtained from each type of GQ-RCA reaction product. S/B ratio was calculated to be 80.5 and 91.5 in the case of the ligation-mediated and ligation-free RCA methods, respectively.

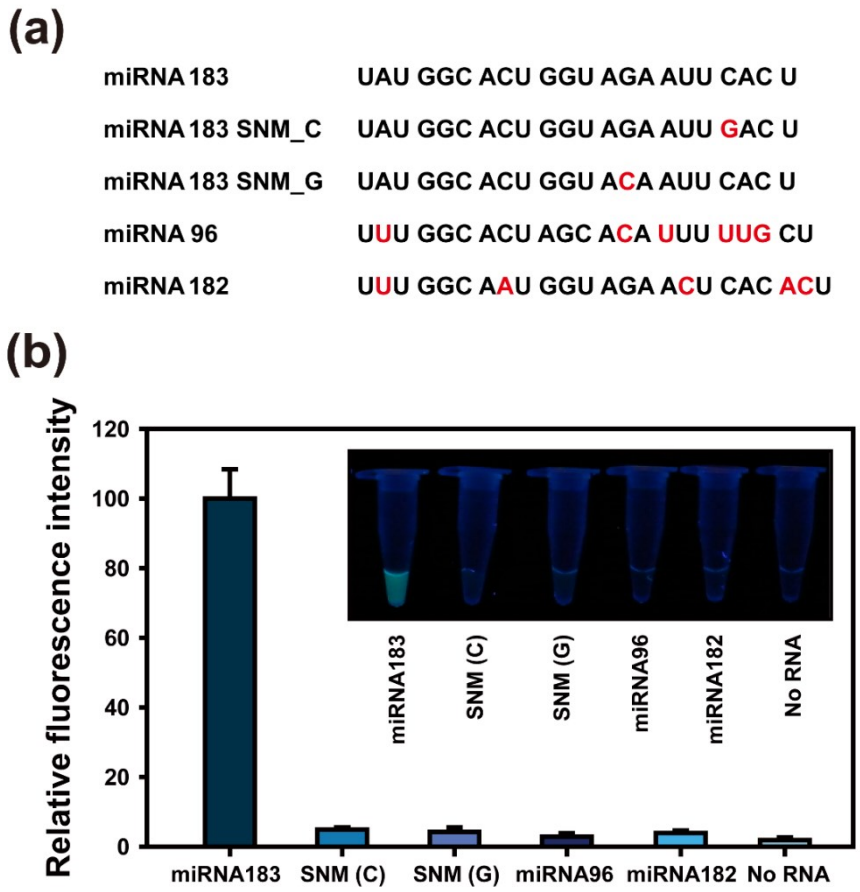

Fig. S3 Sequence specificity of the miRNA detection system using GQ-RCA. (a) Sequences of wild-type miRNA183, single nucleotide mutation (SNM)-containing mutant miRNA183, and miRNA183 family (miRNA96 and miRNA182). Different nucleotides from the wild-type miRNA183 sequence are highlighted in red. (b) The inset images represent the ThT fluorescence of the RCA products under UV light. Enhanced bright blue fluorescence was observed only in the case of the wild-type miRNA183. The bar graphs represent ThT fluorescence intensities, quantified for each miRNA relative to the target value of 100. The data are presented as the average and error denoting standard deviation (SD) of three experiments.

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