Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Label/quencher-free detection of single-nucleotide changes in DNA using isothermal amplification and G-quadruplexes

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Experimental methods

DNA oligonucleotides and reagents

DNA oligonucleotides (padlock probes, wild type DNA, target mutant DNA, and RCA primers) were synthesized and purified by the High Affinity Purification (HAP) method and polyacrylamide gel electrophoresis (Bionics, Seoul, Republic of Korea). The sequences of DNA oligonucleotides are described in Table S1. Among these oligonucleotides, ligation templates (PL1 and PL2) were phosphorylated at 5' terminal for ligation. *Taq* DNA ligase and phi29 DNA polymerase, purified from recombinant sources, were purchased from New England BioLabs (Ipswich, MA, USA). Reagents used for RCA (dNTP mix and BSA) and fluorescence detection (Thioflavin T) were purchased from TaKaRa Korea Biomedical Inc. (Seoul, Republic of Korea) and Sigma-Aldrich Korea (Yongin, Republic of Korea), respectively.

Ligation of padlock probe and rolling circle amplification (RCA)

Ligation of padlock probes was performed in 10 μL of reaction mixture, which was composed of 1× *Taq* DNA ligase reaction buffer (20 mM Tris-HCl, 25 mM KOAc, 10 mM $Mg(OAc)_2$, 1 mM NAD^+, 10 mM DTT, and 0.1% Triton X-100, pH 7.5), 0.4 U of *Taq* DNA ligase (40 U/μL), 100 nM of padlock probe (PL1), and 100 nM of target DNA oligonucleotide (WT or T790M). Reaction mixtures were heated at 95°C for 3 minutes and incubated at 65°C for 60 minutes. After ligation, 5 μL of ligation product was added to 15 μL of RCA reaction mixture, which contained 1×phi29 DNA polymerase reaction buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, and 4 mM DTT, pH 7.5), 10 U phi29 DNA polymerase, 100 nM RCA primer, 100 μg/mL BSA, and 0.5 mM dNTP mixture (dATP, dTTP, dCTP, and dGTP). Rolling circle amplification of DNA was performed by incubation at 30°C for 1 h, and the reaction was quenched by incubation at 65°C for 10 minutes. Agarose gel (1.0%) electrophoresis was performed, and amplified DNA species in the agarose gel were identified by ethidium bromide staining under UV illumination.

Intercalation of thioflavin T and fluorescence detection

For fluorescence detection of the RCA product, 5 μL RCA product was added to 45 μL of fluorescence detection mixture, which contained 15 μM of thioflavin T. The mixture was incubated at room temperature for 30 min. For fluorescence measurements, the incubated

mixture was transferred to a quartz cuvette (Hellma Analytics, Singapore), and the fluorescence was scanned using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The excitation wavelength was 425 nm, and emission spectra were scanned from 450 nm to 650 nm.

Target Sensitivity and Selectivity

The target sensitivity assay was performed in 10 µL of reaction mixture, which contained 1×*Taq* DNA ligase reaction buffer, padlock probe (100 nM), 0.4 U of *Taq* DNA ligase, and various concentrations (100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, and 0.1 nM) of target DNA oligonucleotides (either wild type or mutant DNA). Ligation of padlock probes (PL1), rolling circle amplification, and fluorescence detection using thioflavin T were performed as described above. The target selectivity assay was performed using 100 nM of a target DNA oligonucleotide mixture containing both wild type and mutant DNA species (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100% of mutant DNA). Ligation of PL1, rolling circle amplification, and thioflavin T-coupled fluorescence detection was performed as described above.

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Table S1. Sequences of DNA oligonucleotides.

Name	Sequence
Padlock (PL1)	5'- pTGA TGA GCT GCA CGG AAA **CCC TAA CCC TAA CCC TAA CCC TAA** AAA *GGG CTG CCA GAT ACT CTT CGC* AAA TTT AGG GCA TGA GCT GCA -3'
Padlock (PL2)	5'- pTGA TGA GCT GCA CGG TTA GTA CTA TGG TGG CCA AAC GAC *GGG CTG CCA GAT ACT CTT CGC* GAG AAA AAT TTT AGG GCA TGA GCT GCA -3'
Wild	5'- CCG TGC AGC TCA TCA **C**GC AGC TCA TGC CCT -3'
Mutant	5'- CCG TGC AGC TCA TCA **T**GC AGC TCA TGC CCT -3'
RCA Primer	5'- GCG AAG AGT ATC TGG CAG CCC -3'

(Bold-faced nucleotides in PL1: G-quadruplex-forming site. Italicized nucleotides in PL1: RCA Primer-binding site. Bold-faced nucleotides in WT and Mutant: Single-nucleotide mismatch (SNP) site)

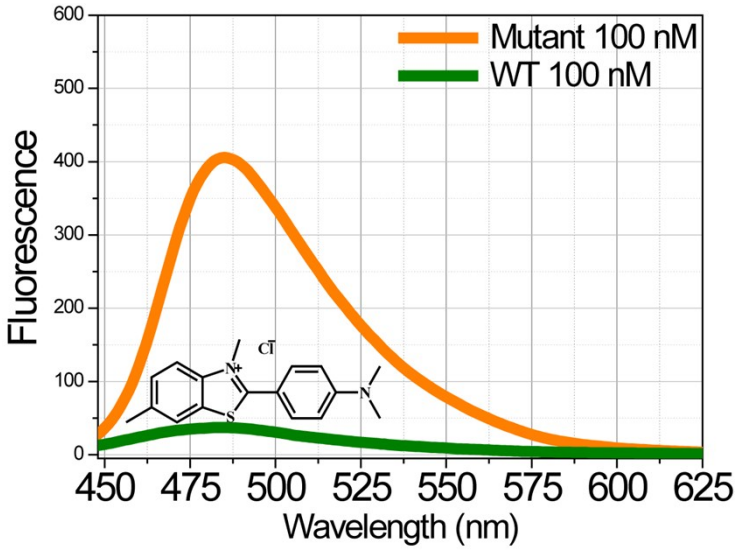

Fig. S1. Fluorescence emission spectra (λ_{ex} = 425 nm) of thioflavin T in presence (mutant) or absence (WT) of RCA product (RCAP) using PL2 (without C-rich sequence).