Supplementary Material (ESI) for The Analyst

SUPPLEMENTARY INFORMATION

Homogeneous and Label-free Bioluminescence Detection of Single Nucleotide Polymorphism with Rolling Circle Amplification

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Experimental Details

Materials

D-luciferin, luciferase, adenosine 5'-phosphosulfate (APS), apyarse, polyvinylpyrrolidone (PVP), bovine serum albumin (BSA)，and ATP sulfurylase were purchased from Sigma Co. Ltd. Ampligase Thermostable DNA Ligase and Phi29 DNA Polymerase were purchased from Epicentre Technologies (Madison, WI). All the synthetic DNA and deoxynucleotides (dNTPs) used in this study were obtained from TaKaRa Biotechnology Co. Ltd. (Dalian, China). The DNA sequences used for RCA were listed as follows: Padlock probe，5'-Phosphate-GTC TCT CCC AGG ACA GGC TTT CAT TTA CAG TTT ACG GTT TAG CAT TTC ATT ACT TTC ATT TAC TTT ACG ATT TCG GCT CTT CCT CTG TGC GCC A-3'; Mutant DNA: 5'-GCG TGT TTG TGC CTG TCC TGG GAG AGA CTG GCG CAC AGA GGA AGA G-3' (The underlined base is the mutant site); Wild DNA: 5'-GCG TGT TTG TGC CTG TCC TGG GAG AGA CCG GCG CAC AGA GGA AGA G-3'; Second primer: 5'-CAG TTT ACG GTT TAG CAT TTC-3'. The mutant DNA and wild DNA are fragments of human p53 gene (exon8). All the oligonucleotides

were PAGE purified. All solutions were prepared in deionized and sterilized water. Other reagents were of analytical reagent grade and used as purchased without further purification.

Ligation Reaction and RCA

For ligation reactions, 1.5 U of Ampligase was added to 20 μL reaction mixture containing 20 mM Tris-HCl (pH 8.3), 25 mM KCl, 10 mM $MgCl_2$, 0.5 mM nicotinamide adenine dinucleotide (NAD), 0.01% Triton X-100, 10 nM padlock probe and appropriate amount of target DNA in one tube. After an initial incubation at 95℃ for 3 minutes to denature the target DNA and padlock probe, ligation reactions were performed at 46℃ for 30 minutes. Subsequently, 20 μL reaction mixture containing 80 mM Tris-HCl (pH7.5), 100 mM KCl, 20 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 8 mM dithiothreitol (DTT), 250 μM each dNTP, and 20 U phi29 DNA polymerase was transferred into the ligation products. RCA reactions were performed at 30℃ for 3 hour and terminated by incubation at 65℃ for 10 minutes. BRCA reactions were conducted by adding 100 nM the second primer to the RCA mixture and following the RCA procedures listed above.

Preparation of Luciferin-luciferase Reaction Solution

The luciferin-luciferase reaction solution was prepared containing 50 mM glycylglycine (pH7.6), 1 mM EDTA, 10 mM magnesium acetate, 0.4 mM D-luciferin and 3 mg/mL luciferase, which was stored at 4 ℃.

PPi Conversion and Bioluminometric Assay

Prior to conversion of PPi, 0.08 U apyrase was used to degrade the excess dNTPs at room temperature for 10 minutes after RCA reaction and heat-inactivated at 95℃ for 3 minutes. After the reaction was cooled to room temperature, the PPi was converted to ATP in 200 μL volume, containing 0.1 M Tris-HCl (pH7.6), 2 mM EDTA, 10 mM $MgCl_2$, 0.1% BSA, 0.4 mg/mL PVP, 1mM DTT, 15 μM APS and 0.04 U ATP sulfurylase. Afterward, an aliquot of 50 μL above reaction products was transferred to a 40×14 mm quartz tube, then 200 μL of luciferin-luciferase reaction solution was injected, and the bioluminescence signal was measured with the BPCL luminescence analyzer (Institute of Biophysics Chinese Academy of Sciences, Beijing, China).

Allele Frequencies Determination

Mutant DNA and wild DNA were mixed at different ratios ranging from 0 to 100% and used as DNA samples. The total amount of the mutant and wild DNA was controlled at 500 pM. Then, the bioluminescent signal of the artificial DNA sample was obtained by following the ligation, BRCA, PPi conversion and luciferin bioluminescence reaction as described above.

Agarose gel electrophoresis experiment

The rolling circle amplification of mutant and wild DNA were verified and discriminated by agarose gel electrophoresis. As shown in Figure S1, due to the RCA products were too large to enter agarose gels, a long product appears in lane 2 (mutant DNA) but not in lane 1 (wild DNA).

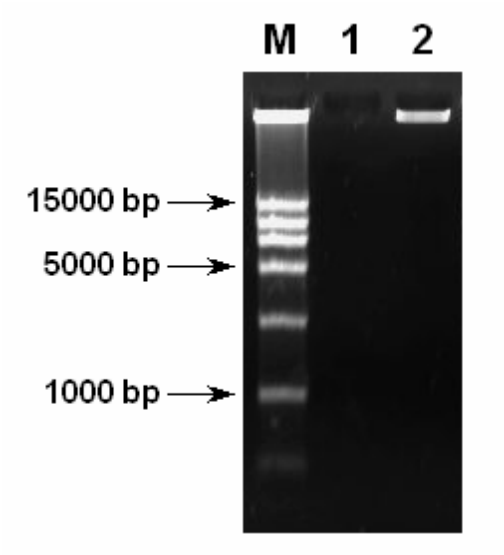

Figure S1. Agarose gel electrophoresis (1.0%) of RCA products stained with SYBR Green I dye. The reaction mixture contained 1 nM padlock probe, 0.5 nM wild DNA (lane 1) and 0.5 nM mutant DNA (lane 2). The marker was indicated by M. The RCA reaction time was 3 hours.

Optimization of the amounts of the APS

APS, an analog of ATP, also reacts with luciferin to emit a small luminescent signal. The amout of APS in solution is one of the dominant factors that determine the sensitivity and the dynamic range of the assay. To ensure a certain quantitative PPi detection, the amout of APS has been optimized. Figure S2 shows the preferable 15 μM APS can be used in the present experiment,

where the bioluminescent intensity of PPi produced by RCA reaction reaches its maximum value and the background signal produced by APS keeps a low value.

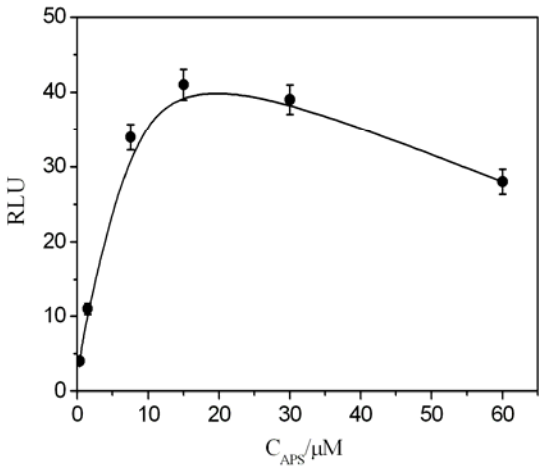

Figure S2. Effect of the concentration of APS on the bioluminescent intensities. Experimental conditions: 100 pM mutant DNA primed amplification with 500 pM padlock probe at 30 ℃ for 3 hours. PPi was converted to ATP with ATP sulfurylase and different concentrations of APS. The relative bioluminescent unit (RLU) was the net intensities produced by mutant DNA, where the background signal had been substracted for each value. Error bars were estimated from three replicate measurements.

Optimization of the RCA reaction time

The optimal amplification time was studied as shown in Figure S3, the bioluminescent intensities of mutant DNA arising from RCA increasingly increased within 3 hours. After 3 hours, the bioluminescent signals of mutant DNA reached its maximum and kept stable. However, the wild DNA caused by the limited RCA-free extension only generated the minimal PPi resulting in its bioluminescent intensities enhanced little. The most bioluminescent discrimination between the mutant and wild DNA was obtained in the time of 3 hour, which was selected as optimum for the experiments.

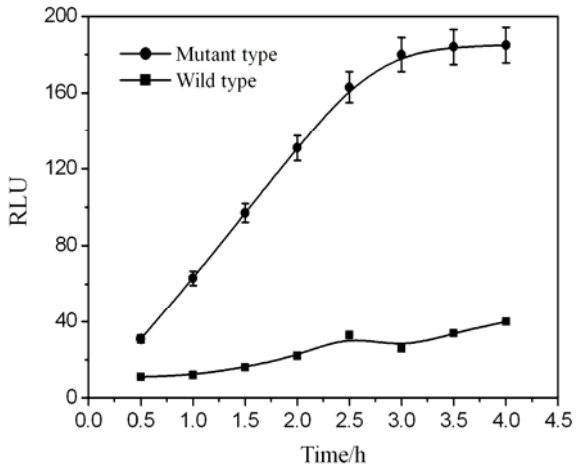

Figure S3. Effect of the amplification time of mutant DNA and wild DNA. Experimental conditions: 250 pM mutant and 250 pM wild DNA primed amplification with 500 pM padlock probe at 30 ℃ for different times. Sequential bioluminometric assay was carried out as described in the Experimental Section. Error bars were estimated from three replicate measurements.