Supplementary Information

A lateral flow biosensor for detection of single nucleotide polymorphism by circular strand displacement reaction

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Experimental

Chemicals and reagents

The polymerase klenow fragment exo- and deoxynucleoside triphosphates (dNTPs) were purchased from New England Biolabs (New England, USA). Bovine serum albumin (BSA) and streptavidin (SA) were purchased from Sigma-Aldrich (Steinheim, Germany). Anti-digoxin was purchased from Beijing Biosynthesis Biotechnology (Beijing, China). Hairpin probe, reporter probe, primer and template DNA were synthesized by Shanghai Sangon Biological Engineering Technology (Shanghai, China). Nitrocellulose membrane was purchased from Shantou Ealon (Shantou, China). Fiberglass and absorbent paper were purchased from Shanghai Kinbio (Shanghai, China). All buffer solutions used in this study were prepared in our lab. Other chemicals were purchased from standard commercial sources and were of analytical grade purity.

Preparation of AuNPs and AuNP-SA conjugates

AuNPs with an average diameter of 25 nm were prepared using the citrate reduction method (Fang et al. 2010; Mao et al. 2009). Briefly, 4 mL of 1% trisodium citrate was added to 100 mL of a rapidly stirred and boiling $HAuCl_4$ solution (0.01%) in a 500-mL round bottom flask. After turning red, the solution was boiled for 10 additional min then cooled to room temperature with gentle stirring. The resulting AuNPs solution was stored at 4 °C and used for preparation of AuNP-SA conjugates. To prepare AuNP-SA conjugate, 10 μg (46 μL) SA and 4 μL 0.1 M K_2CO_3 were added to 1 mL AuNP solution and the mixture was shaken gently at room temperature for 1 h. BSA was added into the SA-coated AuNPs (1050 μL) to 1%. The solution was kept at room temperature for 1 h. Particles were centrifuged (12×10^3 rpm, 20 min) and rinsed three times with rinsing buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, 10% sucrose, and 0.1% NaN_3) to remove any unbound SA. The red pellet was re-suspended in 50 μL of rinsing buffer and then stored in a refrigerator at 4 °C until use.

Construction of lateral flow biosensor

Briefly, 30 μL of 1 mg/mL anti-digoxin and 30 μL of 100 μM 20-mer DNA probe (GGGCACTCTTGCCTACGCCA) were dispensed onto the nitrocellulose membrane (25 mm × 30 cm, capillary rate: 140 ± 40 s, thickness: 145 ± 20 μm) simultaneously, to form a test zone and a control zone with a lateral flow dispenser (Shanghai Kinbio, Shanghai, China). The membrane was then dried at room temperature for 12 h and stored at 4°C until use. Strips of fiberglass (16 mm in width) were used as sample pads after being soaked in sample pad buffer (1% Triton, 1%

BSA, 2% glucose, 50 mM boric acid, pH 8.0). The sample pads were dried and stored in low-humidity at room temperature. Absorbent pads were strips of thick absorbent paper of 17 mm in width. A strip of sample pad, nitrocellulose membrane and absorbent pad were attached along the long axis of an adhesive plate with an overlap of 2-3 mm according to the layout shown in Fig 1. The plate was then cut into 0.4 cm wide strips using a paper cutter (Programmed high speed cutter, Shanghai Kinbio, Shanghai, China).

Optimization of LFNAB Parameters.

In this assay, the sensor sensitivity depends on the concentration of AuNP-SA conjugate and the incubation time for the polymerase reaction. First different amounts of AuNP-SA conjugate were tested to determine the optimum concentration. 2 μL, 3 μL, 4 μL, 5 μL and 6 μL of AuNP-SA were used for the optimization test. Then different reaction time was tested to determine the best reaction time. As shown in Figure S1 A and B, 4ul AuNP-SA conjugate is the optimum concentration and 2h is the optimum reaction time.

Detection of nucleic acid by lateral flow biosensor

To detect SNP using the LFB, 1 μL of synthetic wild-type target (TTTTGGGCACTCTTGCCTACGCCACCAGCTCCAACTACCACAAGCCCC), mutant DNA (TTTTGGGCACTCTTGCCTACGCCAACAGCTCCAACTACCACAAGCCCC), or extracted human genome DNA was added to 25 μL reaction solution [1.2 μM hairpin probe (PO4-TGGCGTAGGCAAGAGTGCCCTGCCTACGCCATTCC-(T)13-Thiol), 12 nM primer (GGAATGGCG), 9.6 nM reporter probe (TTTTTTTTTCTTGTGGTAGTTGGAGCTGG-Digoxin), 10 U T4 DNA ligase, 5 U polymerase Klenow fragment exo-, 50 μM dNTPs, and 1X T4 DNA ligase reaction buffer (40 mM Tris–HCl pH7.8, 10 mM DTT, 10 mM MgCl2,)] was incubated at 25°C for 2 h and then 25 μL product was loaded onto the sample pad along with 4 μL AuNPs-SA and 50 μL 4X SSC. The biosensor was scanned using a hand-held strip reader (Shanghai Kinbio, Shanghai, China) 30 min later.

Statistical analysis

SPSS 10.0 software (SPSS Inc., IL, US) was used for statistical analysis. The results were expressed as means ± SD from three independent experiments. Individual comparisons were made by Student's t-test for paired data and p-values less than 0.05 were considered to be significant.

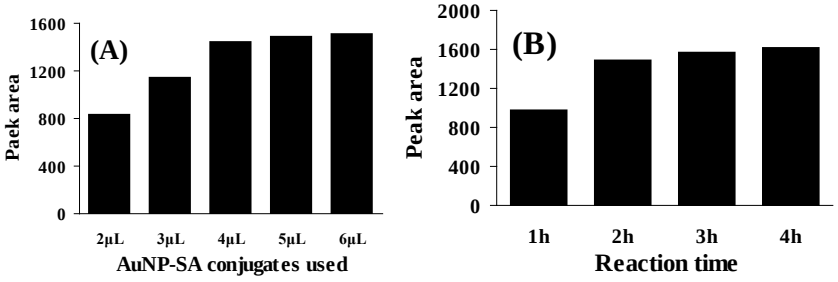

Fig. S1 (A) Optimization test to determine the optimum AuNP-SA concentration used in this assay; (B) Optimization test to determine the best reaction time.

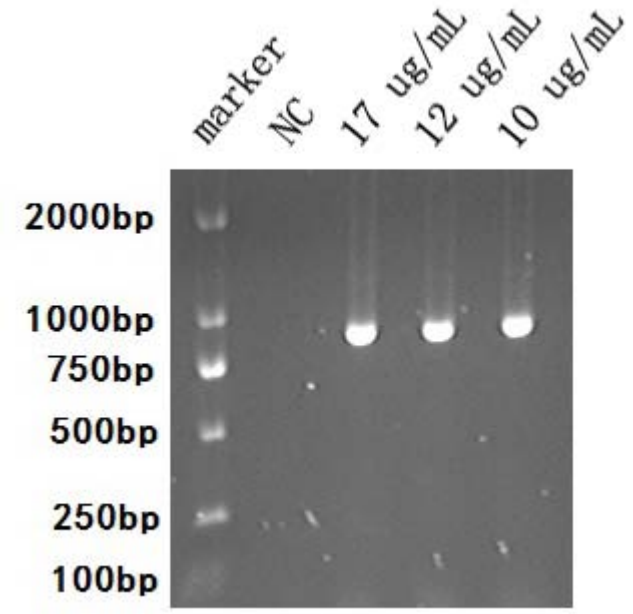

Fig. S2 Photo image of gel electrophoresis of human genomic nucleic acid PCR product.

References

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