

Supporting Information

Lateral flow biosensor for detection of nucleic acid with high sensitivity and selectivity

Puchang Lie,^a Jie Liu,^a Zhiyuan Fang,^a Boying Dun,^a Lingwen Zeng^{*a}

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). Biotinylated streptavidin-modified hairpin probe 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-DNA 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 H₂AuCl₄ 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-DNA conjugates. To prepare AuNP-DNA conjugate, 100 μL of 5'-biotinated 3'-thiolated DNA hairpin probe (100 μM) (probe 1, Biotin-5'-TCTTGGACACA ACTAACGCCATGGCTAGACTGTGTCCAAGA-3'-thiol) was added to 500 μL of 4-fold concentrated AuNP solution and the mixture was shaken gently at 4°C for 24 h. The DNA-coated AuNPs (500 μL) were subjected to "aging" by adding 55 μL 100 mM phosphate buffer (pH 7.0) with 1% sodium dodecyl sulfate and 1.5 M NaCl. The solution was kept at 4°C for 12 h. Particles were centrifuged (12×10³ rpm, 20 min) and rinsed three times with rinsing buffer (20 mM Na₃PO₄, 5% BSA, 0.25% Tween-20, 10% sucrose, and 0.1% NaN₃) to remove any unbound DNA. The red pellet was re-suspended in 500 μL of rinsing buffer and then stored in a refrigerator at 4 °C until use.

Construction of lateral flow biosensor

Briefly, 30 μL of 100 μM 15-mer DNA probe (probe 4 GAAAGATAGAAAGAT) and 30 μL of 1 mg/mL streptavidin (SA) 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-DNA conjugate and the incubation time for the polymerase reaction. First we investigated the amounts of AuNP-DNA conjugate used for this assay and determine the optimum concentration. The amounts of AuNP-DNA of 1 μ l, 2 μ l, 3 μ l and 4 μ l were used for optimization test. Then different reaction time is tested to determine the best reaction time. As shown in Figure S1 A and B, 2 μ l AuNP-DNA conjugate is the optimum concentration and 30 min is the optimum reaction time.

Detection of nucleic acid by lateral flow biosensor

To detect nucleic acid using the lateral flow biosensor, 2 μ L of hairpin probe (probe 1) conjugated AuNPs was mixed with different amounts of synthetic target DNA (target DNA, GTCTAGCCATGGCGTTAGTTGTGTCTTT), non-target DNA with random sequences (random sequence 1, GGTAGAAGGGAGGGCTAGTTGTGTCTTT, random sequence 2, GTCAGTCCCCTTGTTCTGTGTCTTT, random sequence 3, AGTGTGGATTTCGCACTCCTTGTGTCTTT), extracted human genome DNA, hepatitis C virus (HCV) RNA, swine DNA, or tuberculosis (TB) DNA in 20 μ L reaction solution [Tris-HCl (50 mM, pH 8.0), 50nM tag-primer (probe 2), 3 U polymerase Klenow fragment exo-, 50 μ M dNTPs, 6% DMSO, 0.1% BSA, 1 mM DTT, 5 mM MgCl₂, and 1X enzyme reaction buffer]. The mixture was incubated at 42 °C for 30 min and then 20 μ L ISDPR product was loaded onto the sample pad along with 20 μ L 4X SSC. The biosensor was scanned using a hand-held “strip reader” (Shanghai Kinbio, Shanghai, China) 10 min later.

Statistical analysis

SPSS 10.0 software (SPSS Inc., IL, US) was used for statistical analysis. The results were expressed as means \pm 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.

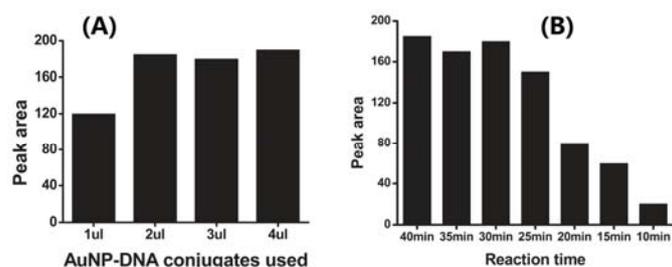


Fig. S1 (A) Optimization test to determine the optimum AuNP-DNA concentration used in this assay; (B) Optimization test to determine the best reaction time.

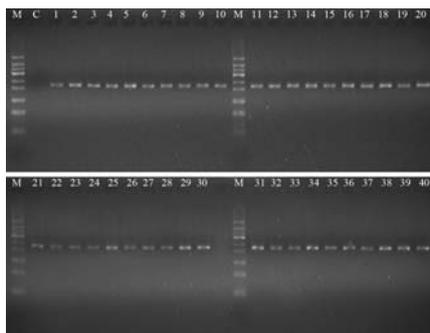


Fig. S2 Photo image of gel electrophoresis of human genome nucleic acid PCR product.

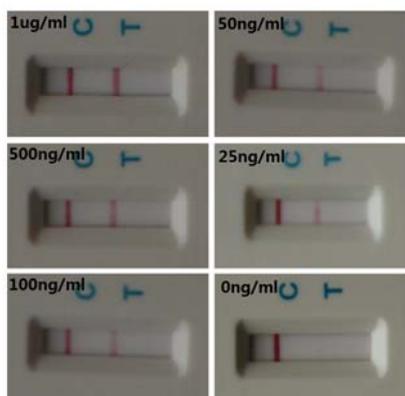


Fig. S3 Typical photo images of LFB in the presence of different concentrations of human nucleic acid. The concentrations are from top to bottom and left column to right column 1 $\mu\text{g/ml}$, 500 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 0 ng/ml (negative control).

References

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- 2 X. Mao, Y.Q. Ma, A.G. Zhang, L.R. Zhang, L.W. Zeng, G.D. Liu, *Anal. Chem.*, 2009, **81**, 1660-1668.