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

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A lateral flow biosensor for detection of single nucleotide polymorphism based on circular strand displacement reaction (CSDPR) has been developed. Taking advantage of high fidelity of T4 DNA ligase, signal amplification by CSDPR, and the optical properties of gold nanoparticles, this assay has reached a detection limit of 0.01 fM.

Single nucleotide polymorphisms (SNPs) are single base DNA mutations among individuals. Many health problems are caused by SNPs, such as genetic diseases, cancers, drug resistance of infectious agents, and disease susceptibility. They are also the key to personalized medicine. Therefore, a convenient and accurate SNP detection method for clinical diagnosis and research is demanded.

Recently, many techniques have been developed to meet the need for SNP detection. Such techniques include SNP microarray,[1,2] polymerase chain reaction (PCR),[3] fluorescence resonance energy transfer[4,5] and electrochemical detection.[6] These techniques have been widely applied for SNP detection and were shown to be effective. But these techniques require expensive instruments and highly trained personnel, and are time-consuming.

As a new method for nuclei acid detection, circular strand displacement reaction (CSDPR) has attracted extensive interest.[7] It is simple and convenient. Compared to traditional PCR, CSDPR can be carried out at a constant temperature, thus requiring no special instruments. Also, ligation reaction performed by DNA ligase can be used to detect SNP with high specificity.[8,9] Ligase can covalently join two adjacent probes annealed on the SNP site with high fidelity. Recently, lateral flow biosensors (LFBs) have attracted a great deal of research interest for they offer a promising approach to realizing point of care nucleic acid detection. In the past two years, we have developed LFBs for the detection of nucleic acids,[10] proteins,[11] cancer cells[12] and copper ions,[13] which are time-saving and do not need intensive labor.

Herein, we developed an LFB for specific, sensitive and visual detection for SNP analysis. This method takes advantage of high fidelity of T4 DNA ligase, excellent signal amplification by CSDPR, and the special optical properties of gold nanoparticles (AuNPs), providing an alternative to complex instrument-dependent methods used for SNP detection. As shown in Fig. 1, this SNP detection system consists of a biotin modified hairpin DNA probe (PO_4-TGGCGTAGG-CAAGAGTGCCCTGCCTACGCCATTCC-$(T)_{13}$-biotin), a short primer (GGAATGGCG), a digoxin-labeled reporter DNA probe (digoxin-TTTTTTTTTCTTGTGGTAGTTG-GAGCTGG), T4 DNA ligase, Klenow polymerase *exo*-, dNTPs and buffer solution. In the presence of synthetic wild-type target DNA (TTTTGGGCACTCTTGCCTACGC-CACCAGCTCCAACTACCACAAGCCCC, part of K-ras oncogene sequence, the underlined cytosine is the SNP site in codon 12), the hairpin probe is opened. After the hairpin probe and the digoxin-labeled reporter DNA probe anneal to the synthetic wild-type target DNA, T4 DNA ligase covalently joins the two adjacent probes that flank the mutation site by 5′-PO_4 of the hairpin probe and 3′-OH of the reporter probe. Then the short primer anneals to the open hairpin probe and starts a polymerization reaction with polymerase and dNTPs. The reaction product, a biotinylated digoxin-modified DNA sequence, is synthesized while the target is displaced, which triggers a new round of polymerization reaction. This repeating process generates a large number of biotinylated digoxin-modified DNA sequences. The reaction product is mixed with streptavidin–Au nanoparticles (AuNP-SA) before being loaded to the LFB. The biotinylated digoxin-modified DNA sequence joins to AuNP-SA and is then captured by anti-digoxin on the test zone of the nitrocellulose membrane. The DNA probe (GGGCACTCTTGCCTACGCCA) on the control zone can open the hairpin conjugated with AuNP-SA and thus capture the superfluous AuNP-SA-hairpin. The red line at the control zone indicates that the biosensor works properly, and the red line at the test zone indicates the presence of wild-type target nucleic acid. Qualitative analysis is performed by observing the color change caused by aggregation of AuNPs on the test zone, and semi-quantitative detection can be

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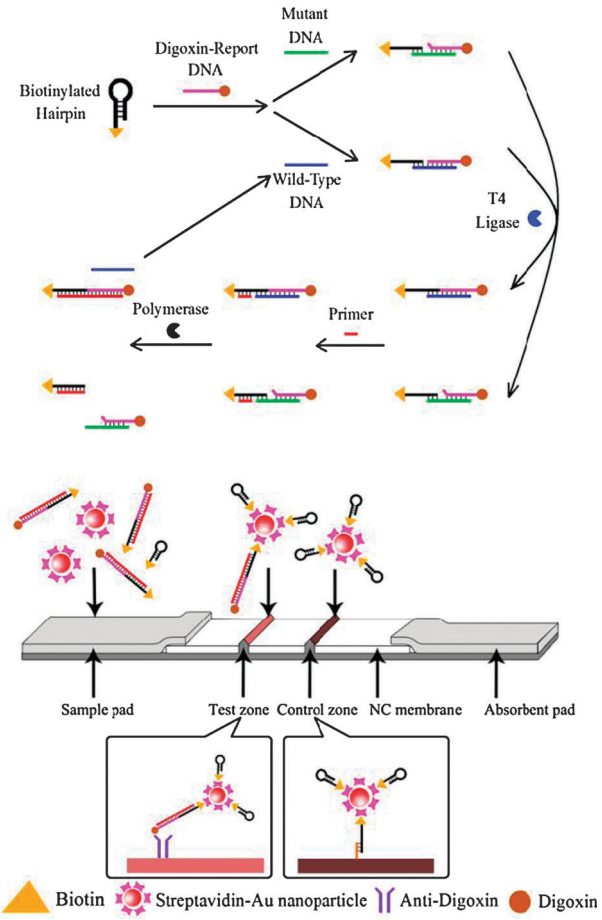

Fig. 1 Schematic illustration of the production of joined DNA based on CSDPR and ligase reaction, and the structure of lateral flow biosensor.

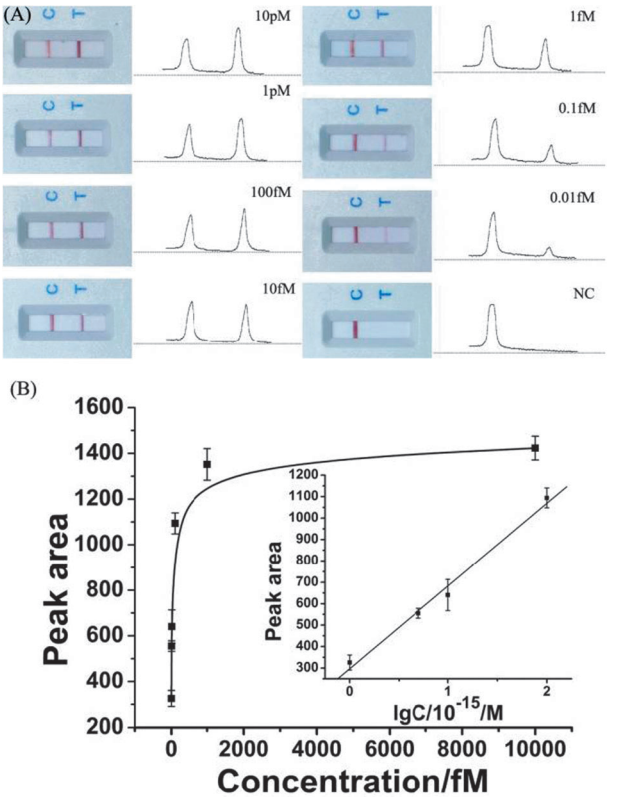

Fig. 2 (A) Typical photo images and corresponding intensities of a LFB in the presence of different concentrations of synthetic target DNA and negative control. (B) Calibration curve of a LFB with different concentrations of target DNA. Error bars represent standard deviation, $n = 3$.

realized by recording the optical density of the test line with a portable strip reader. While in the presence of synthetic mutant target DNA (TTTTGGGCACTCTTGCCTACGC-CAACAGCTCCAACTACCACAAGCCCC), because of the specificity of T4 DNA ligase, 5′-PO_4 of the hairpin probe and 3′-OH of the reporter probe cannot be ligated. The short primer triggers a polymerization reaction in the presence of dNTPs and polymerase and the target is displaced from the hairpin probe. No biotinylated digoxin-modified DNA sequence is synthesized. Therefore no test line but a control line is observed when the reaction product is loaded to the LFB.

The sensitivity of LFB was evaluated by using different concentrations of wild-type target DNA. Seven different concentrations of synthetic wild-type target DNA were used for sensitivity analysis (10 pM, 1 pM, 100 fM, 10 fM, 1 fM, 0.1 fM, 0.01 fM, 0 fM (negative control)). For each test, 1 μL of synthetic target at different concentrations was added to 24 μL of reaction solution. After being incubated at 25 °C for 2 h, the reaction mixture was mixed with 50 μL of 4× SSC and loaded to the LFB. Semi-quantitative analysis was performed by recording the intensities of test lines with a portable strip reader. Through the portable strip reader, well defined peaks were observed and were recorded as signals. As shown in Fig. 2A, the signal increases along with the concentration of the wild-type target DNA. The signal can be detected when the concentration of the wild-type target DNA is as low as 0.01 fM. At this concentration, the test line can also be observed with the naked eye. This concentration is considered as the limit of detection of this assay for synthetic wild-type target nucleic acids. The resulting calibration plot of the peak areas *versus* target DNA concentration is linear between 1 fM and 100 fM (Fig. 2B).

For specificity analysis, 1 fM wild-type target, 10 μM mutant target and 10 μM of three synthetic random sequences were tested. Fig. 3A shows that a visible red line was observed on the test zone of the biosensor when wild-type target DNA was tested, but no test line was observed when synthetic mutant DNA and three DNA samples of different random sequences were tested. Only the wild-type target yielded positive results on test lines, demonstrating high specificity of the LFB. The biosensor can clearly differentiate one base mutation between wild-type target DNA and mutant target DNA.

To determine the reproducibility of the LFB, each of the three samples with concentrations of 10, 100, and 1000 fM of synthetic wild-type target DNA was loaded to 20 LFBs. The test line intensities of the LFBs were recorded using the strip reader, and the CVs for the three samples were calculated to be 10.8%, 9.54%, and 7.53%, respectively.

To evaluate the performance of the LFB for clinical sample analysis, human genomic DNAs were tested. Human genomic

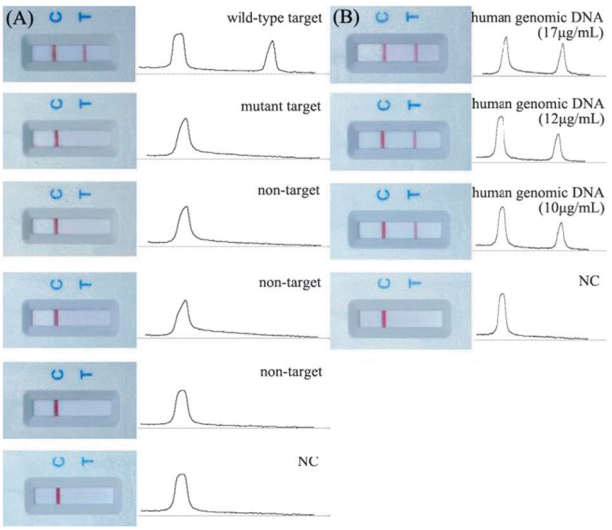

Fig. 3 (A) Photo images (left) and corresponding intensities (right) of the LFB for specificity analysis with wild-type target DNA, single-base mutant DNA, non-target DNA (DNA with random sequences) and negative control. (B) Photo images (left) and corresponding intensities (right) of the LFB tested with three extracted human genomic DNA and negative control.

DNAs of three wild-type clinical blood samples were extracted using a commercial DNA extraction kit. Before the test, the extracted human genomic DNA was denatured by treating with boiling water for about 5 min, and then immediately cooled in ice for another 15 min. One microliter of denatured human genomic DNA was mixed with 24 μL of CSDPR reaction solution, and incubated at 25 °C for 2 h. The resulting mixture was mixed with 50 μL of 4× SSC and then loaded to the LFB. Fig. 3B shows that a visible red line was observed when human genomic DNA was used, but no test line was observed in the absence of human genomic DNA. The same results were obtained when the same three samples were tested with PCR assay (Fig. S2, ESI†).

In summary, a lateral flow biosensor assay for detection of SNPs has been developed. This assay takes advantage of three techniques: T4 DNA ligase, CSDPR and AuNPs. T4 DNA ligase provides high specificity recognizing single-base mismatch. CSDPR provides high sensitivity with signal amplification and AuNP transforms the product nuclei acid signal into color change that allows visual detection. The developed LFBs were examined with both synthesized wild-type target DNA and mutant target DNA. Owing to the signal amplification implemented by both CSDPR and AuNPs, it is very sensitive. The limit of detection for synthetic wild-type target is as low as 0.01 fM, which is better than or comparable to previously reported methods for SNP detection.[6,14–16] The specificity was also confirmed by single base mismatch mutant target DNA and other non-target DNAs. No false positive result was produced upon testing mutant target DNA and other non-target DNAs. The performance of the LFB was further confirmed using human genomic DNA extracted from human clinical blood samples and shown to be excellent. This assay can be performed at room temperature in 2.5 hours, requiring no complex or expensive instruments. It is easy to use and no trained personnel are needed. What's more, by using AuNPs instead of fluorescent dyes or radioisotopes, the result can be directly observed by the naked eye, and can also be recorded by a convenient portable strip reader when semi-quantitative analysis is required. Compared with PCR, realtime-PCR and other methods using fluorescence, it requires no complicated machines. It is suitable for point of care diagnosis. Therefore, this simple, cost effective, robust and promising LFB for SNP detection has great potential for the detection of genetic diseases, in personalized medicine, cancer related mutations, and drug resistant mutations of infectious agents.

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