Disposable Nucleic Acid Biosensors Based on Gold Nanoparticle Probes and Lateral Flow Strip

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2009

Impact Factor: 5.64 · DOI: 10.1021/ac8024653 · Source: PubMed

Anal. Chem. **2009,** *81,* 1660–1668

Disposable Nucleic Acid Biosensors Based on Gold Nanoparticle Probes and Lateral Flow Strip

Xun Mao,[†] Yunqing Ma,[‡] Aiguo Zhang,[§] Lurong Zhang,[||] Lingwen Zeng,*[,⊥] and Guodong Liu*[,†]

Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, North Dakota 58105, DiaCarta LLC, 6519 Dumbarton Circle, Fremont, California 94555, Panomics, Inc., 6519 Dumbarton Circle, Fremont, California 94555, Department of Radiation Oncology, University of Rochester Medical Center, Rochester, New York 14642-8647, and Laboratory of Molecular Diagnostics, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, Guangdong, China, 510663

In this article, we describe a disposable nucleic acid biosensor (DNAB) for low-cost and sensitive detection of nucleic acid samples in 15 min. Combining the unique optical properties of gold nanoparticles (Au-NP) and the high efficiency of chromatographic separation, sandwich-type DNA hybridization reactions were realized on the lateral flow strips, which avoid multiple incubation, separation, and washing steps in the conventional nucleic acid biosensors. The captured Au-NP probes on the test zone and control zone of the biosensor produced the characteristic red bands, enabling visual detection of nucleic acid samples without instrumentation. The quantitative detection was performed by reading the intensities of the produced red bands with a portable strip reader. The parameters (e.g., the concentration of reporter probe, the size of Au-NP, the amount of Au-NP-DNA probe, lateral flow membranes, and the concentration of running buffer) that govern the sensitivity and reproducibility of the sensor were optimized. The response of the optimized device is highly linear over the range of 1−100 nM target DNA, and the limit of detection is estimated to be 0.5 nM in association with a 15 min assay time. The sensitivity of the biosensor was further enhanced by using horseradish peroxidase (HRP)-Au-NP dual labels which ensure a quite low detection limit of 50 pM. The DNAB has been applied for the detection of human genomic DNA directly with a detection limit of 2.5 μg/mL (1.25 fM) by adopting well-designed DNA probes. The new nucleic acid biosensor thus provides a rapid, sensitive, low cost, and quantitative tool for the detection of nucleic acid samples. It shows great promise for in-field and point-of-care diagnosis of genetic diseases and detection of infectious agents or warning against biowarfare agents.

The nucleic acid test is of central importance to the diagnosis and treatment of genetic diseases, to the detection of infectious agents, drug discovery, or warning against biowarfare agents.[1−4] Various strategies and technologies have been developed to identify unique DNA sequences. Northern, Southern, and Western blotting, agarose, and polyacrylamide gel electrophoresis have been used as standard procedures in research laboratories.[5] Such conventional methods fail to provide enough specific information and are very laborious and time-consuming, requiring up to 48 h of meticulous laboratory work. The advent of new methods and technologies including real-time polymerase chain reaction (RT-PCR),[6] DNA microarrays (gene chip),[7] the surface Plasmon resonance BIAcore instrument[8] and GeneXpert system[9] offer fast and sensitive tools to detect the nucleic acid sequences; however, high equipment cost and the requirement of highly trained personnel prevent their use in many laboratories. Continuing efforts have been made to seek an ideal tool for fast, sensitive, low-cost and easy-to-use detection of nucleic acids.[10,11] Nucleic acid biosensors may be good candidates to meet these standards. Various nucleic acid biosensors in connection with different transducers have been reported in the literature.[12,13] The sensitivities were further enhanced by the use of nanomaterial labels and novel signal amplification strategies labels.[14] Even copies of DNA or RNA can be detected without PCR amplification.[15,16] Most of these developed nucleic acid biosensors have not applied for routine use in the research laboratories or clinical diagnosis

* To whom correspondence should be addressed. E-mail: guodong.liu@ndsu.edu (G.L.), zeng6@yahoo.com (L.Z.). Phone: 1-701-231-8697(G.L.). Fax: 1-701-231-8831 (G.L.).

[†] North Dakota State University.

[‡] DiaCarta LLC.

[§] Panomics, Inc.

[||] University of Rochester Medical Center.

[⊥] Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences.

(1) Wang, J. *Chem.—Eur. J.* **1999**, *5*, 1681–1685.
(2) Palecek, E.; Fojta, M. *Anal. Chem.* **2001**, *73*, 75A–83A.
(3) Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2003**, *21*, 1192–1199.
(4) Gooding, J. J. *Electroanalysis* **2002**, *14*, 1149–1156.
(5) Nelson, D.; Cox, M. *Lehninger principles of biochemistry*; Worth Publishers: New York, 2000; pp 1132−1133.
(6) Kaltenboeck, B.; Wang, C. *Adv. Clin. Chem.* **2005**, *40*, 219–259.
(7) Brown, P. O.; Botstein, D. *Nat. Genet.* **1999**, *21*, 33–37.
(8) http://www.biacore.com.
(9) Petersen, K. E.; Kovacs, W. A.; Young, S. J. U.S. Patent 5958349, 1999.
(10) Piunno, P. A. E.; Krull, U. J. *Anal. Bioanal. Chem.* **2005**, *381*, 1004–1011.
(11) Hahn, S.; Mergenthaler, S.; Zimmermann, B.; Holzgreve, W. *Bioelectrochemistry* **2005**, *67*, 151–154.
(12) Odenthal, K. J.; Gooding, J. J. *Analyst* **2007**, *132*, 603–610.
(13) Leung, A.; Shankar, P. M.; Mutharasan, R. *Sens. Actuators, B* **2007**, *125*, 688–703.
(14) Wang, J. *Small* **2005**, *1* (11), 1036–1043.
(15) Zhang, Y.; Pothukuchy, A.; Shin, W.; Kim, Y.; Heller, A. *Anal. Chem.* **2004**, *76*, 4093–4097.
(16) Nam, J.; Stoeva, S. I.; Mirkin, C. A. *J. Am. Chem. Soc.* **2004**, *126*, 5932–5933.

10.1021/ac8024653 CCC: $40.75
Published on Web 01/21/2009

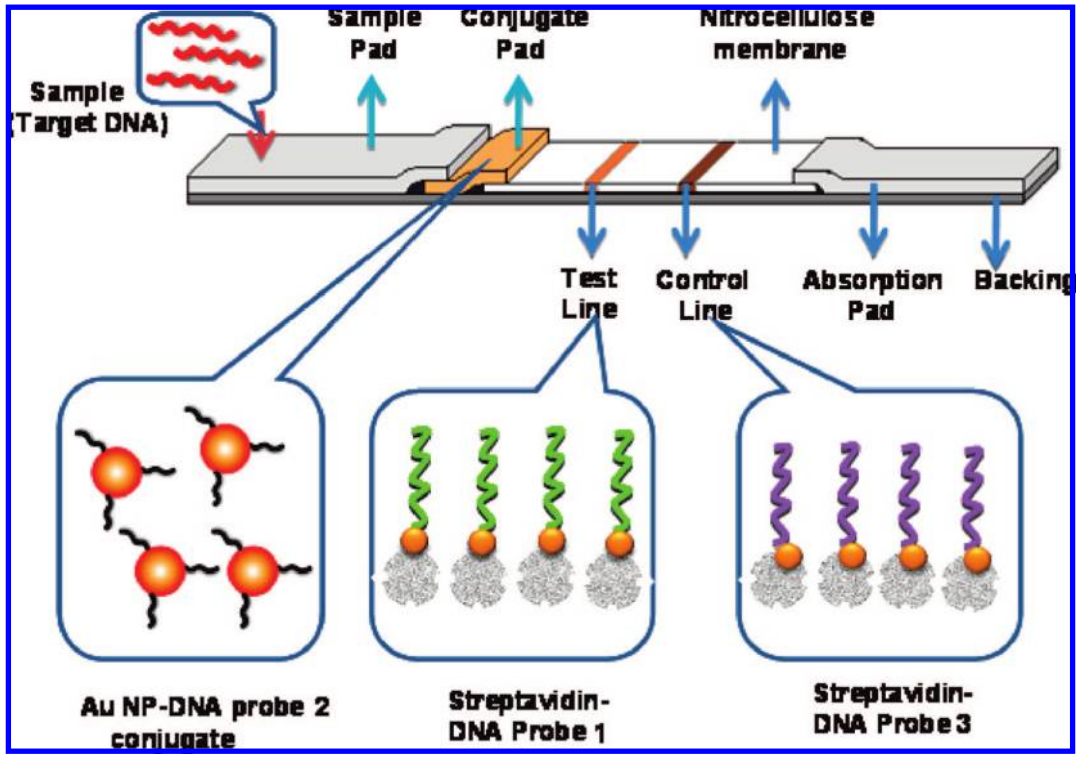

Figure 1. Schematic illustration of the DNAB.

applications because of their accuracy, reproducibility, and complex operations, such as multiple incubation and washing steps. For these nucleic acid biosensors to become widely used for both clinical and research applications, reduction in the complexity of the tests (i.e., number of steps and skill base required) together with a reduction in the instrumentation and cost per test are required. Particularly, for these nucleic acid biosensors to be applicable at the near patient (point of care or "POC"), or near process level, simple, easy-to-use, cost competitive systems are required.

Recently, research has concentrated on the development of POC nucleic acid biosensors. Emerging lateral flow nucleic acid (LFNA) test strips, also called dipstick test strips, offer a promising approach to realize POC nucleic acid detection. The idea to develop such LFNA test strips adopts from the well-developed immunochromatographic strip technology. Because the kinetics of nucleic acid hybridization in lateral flow are quite different and more complex as compared with the formation of the antigen−antibody complex used in common immunochromatography assay, most of the work to detect nucleic acid sequences with LFNA test strips are based on the formation of hapten-antibody or hapten-protein (e.g., biotin−avidin) complexes. For example, Fong et al.,[17] Piepenburg et al.,[18] and Corstjens et al.[19] made use of lateral flow immunoassays for the detection of DNA amplification products. Baeumer et al. reported a dipstick-type LFNA biosensors with nanomolar detection limit based on dye-encapsulating liposome labels.[20,21] The biosensors have been applied to detect Dengue virus in blood samples[22] and viable *Escherichia coli* in drink water.[23] Ioannou and Christopoulos's group reported a dry-reagent strip biosensor based on oligonucleotide functionalized gold nanoparticles (Au-NPs) for visual detection of DNA.[24] The biosensors have been used for visual detection of genetically modified organisms,[25] leukemia-related chromosomal translocations,[26] molecular diagnosis of bacterial infection,[27] and genotyping of single-nucleotide polymorphisms.[28] In these reported LFNA test strips, the actual hybridization reaction is generally performed before the flow. The pre-hybridization reaction time usually takes 10 to 30 min at desired temperatures. Some of tests required special and expensive hardware for quantitative detections.[19,29] Current formats of the reported LFNA test strips limit their in-field and POC applications.

Recently, we reported a dry-reagent strip biosensor based on DNA aptamer functionalized Au-NP probes for qualitative (visual)/

(17) Fong, W. K.; Modrusan, Z.; Mcnevin, J. P.; Marostenmaki, J.; Zin, B.; Bekkaoui, F. *J. Clin. Microbiol.* **2000**, *38*, 2525–2529.
(18) Piepenburg, O.; Williams, C. H.; Stemple, D. L.; Armes, N. A. *PLos Biol.* **2006**, *4*, 1115–1121.
(19) Corstjens, P.; Zuiderwijk, M.; Brink, A.; Li, S.; Feindt, H.; Niedbala, R. S.; Tanke, H. *Clin. Chem.* **2001**, *47*, 1885–1893.
(20) Baeumner, A. J.; Pretz, J.; Fang, S. *Anal. Chem.* **2004**, *76*, 888–894.
(21) Baeumner, A. J.; Jones, C.; Wong, C. Y.; Price, A. *Anal. Bioanal. Chem.* **2004**, *378*, 1587–1593.
(22) Baeumner, A. J.; Schlesinger, N. A.; Slutzki, N. S.; Romano, J.; Lee, E. M.; Montagna, R. A. *Anal. Chem.* **2002**, *74*, 1442–1448.
(23) Baeumner, A. J.; Cohen, R. N.; Miksic, V.; Min, J. *Biosens. Bioelectron.* **2003**, *18*, 405–413.
(24) Glynou, K.; Ioannou, P. C.; Christopoulos, T. K.; Syriopoulou, V. *Anal. Chem.* **2003**, *75*, 4155–4160.
(25) Kalogianni, D. P.; Koraki, T.; Christopoulos, T. K.; Ioannou, P. C. *Biosens. Bioelectron.* **2006**, *21*, 1069–1076.
(26) Kalogianni, D. P.; Bravou, V.; Christopoulos, T. K.; Ioannou, P. C.; Zoumbos, N. *Nucleic Acids Res.* **2007**, *35*, e23.
(27) Kalogianni, D. P.; Goura, S.; Aletras, A.; Christopoulos, T. K.; Chanos, M. G.; Christofidou, M.; Skoutelis, A.; Ioannou, P. C.; Panagiotopoulos, E. *Anal. Biochem.* **2007**, *361*, 169–175.
(28) Litos, I. K.; Ioannou, P. C.; Christopoulos, T. K.; Traeger-Synodinos, J.; Kanavakis, E. *Anal. Chem.* **2007**, *79*, 395–402.
(29) Carter, D. J.; Cary, R. B. *Nucleic Acids Res.* **2007**, *35*, e74.

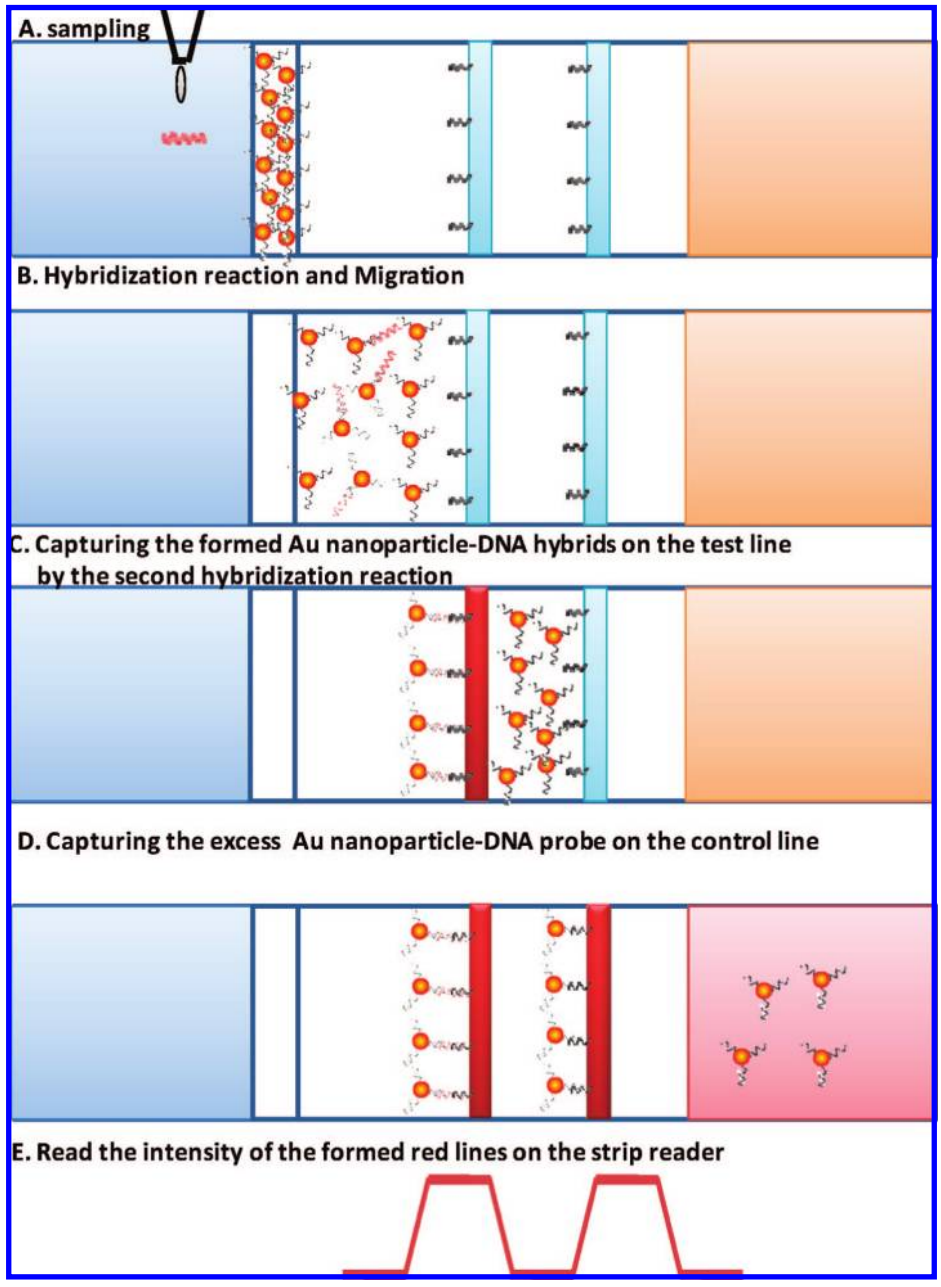

Figure 2. Measurement principle of the DNAB.

quantitative detection of protein within minutes.[30] In this article, we present a disposable nucleic acid biosensor (DNAB) based on the oligonucleotide functionalized Au-NPs and lateral flow device for fast, sensitive, and POC detection of nucleic acid samples. Combining the unique optical properties of Au-NPs and high efficiency of chromatographic separation, sandwich-type DNA hybridization reactions were performed on the lateral flow strips, which avoid the prehybridization procedure in the reported LFNA and multiple incubation, separation, and washing steps in the conventional nucleic acid biosensors. Qualitative judgment can be performed by observing the color change of the test line, and quantitative detection can be realized by recording the intensity of the test line with a portable strip reader. The total assay time for a nucleic acid sample is 15 min. The sensitivity of the biosensor was further enhanced by using horseradish peroxidase (HRP) and Au-NP dual labeled probes (HRP-Au-NP-DNA) which ensure a quite low detection limit of 50 pM. The feasibility of the biosensor was evaluated by detecting the human genomic DNA samples directly with a detection limit of 2.5 μg/mL (1.25 fM). The promising properties of the biosensor are reported in the following sections.

(30) Xu, H.; Mao, X.; Zeng, Q. X.; Wang, S. F.; Kawde, A. N.; Liu, G. D. *Anal. Chem.* **2009**, Article ASAP.

EXPERIMENTAL SECTION

Apparatus. Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator, and the Guillotine cutting module CM 4000 were from Biodot LTD (Irvine, CA). Portable strip reader DT1030 was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China).

Reagents. Streptavidin from *Streptomyces avidinii*, $HAuCl_4$, sucrose, hydroxylamine, Tween 20, dithiothreitol (DTT), Triton

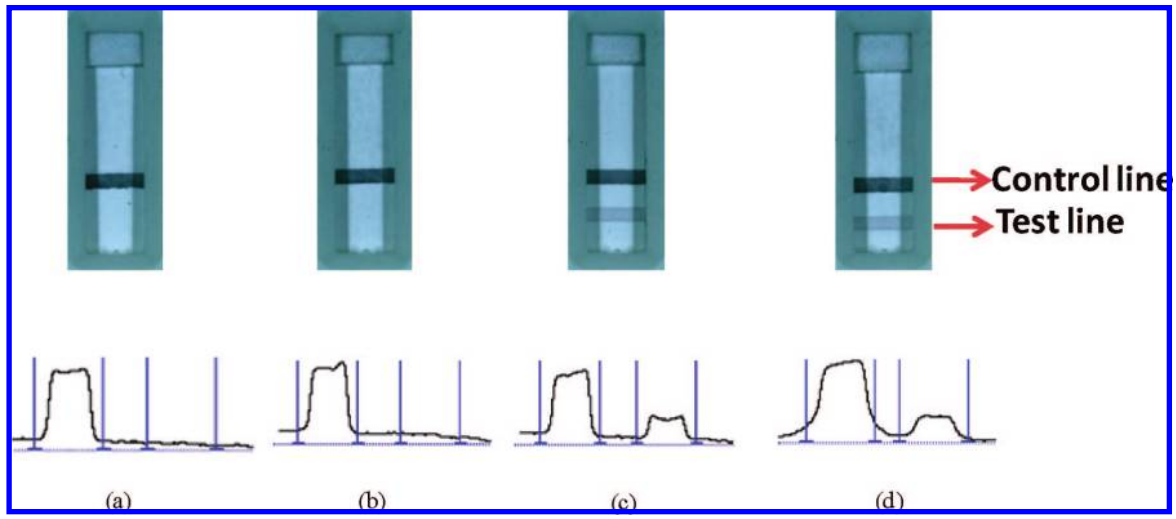

Figure 3. Typical photo images (top) and recorded response signals of DNAB with a portable strip reader after applying the sample solutions (bottom, left: optical response of the control line; right: optical response of the test line). (a): 0 nM target DNA; (b): 1000 nM noncomplementary DNA; (c): 100 nM target DNA; (d) 1000 nM noncomplementary target DNA+100 nM complementary target DNA. Assay time: 15 min; all sample solution were prepared with 4×SSC buffer.

X-100, trisodium citrate, bovine serum albumin (BSA), and sodium chloride-sodium citrate (SSC) Buffer 20× concentrate (pH 7.0), phosphate buffer saline (PBS, pH 7.4, 0.01 M) were purchased from Sigma-Aldrich. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100), and nitrocellulose membranes (HFB18004 and HFB 24004) were purchased from Millipore (Billerica, MA). Human Genomic DNA (174 μg/mL) was obtained from Promega Corporation. DNA oligonucleotides were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and had the following sequence:

Target DNA: 5′-TTCCCTAGCCCACCCAGTGTGCAAGGGC-AGTGAAGACTTGATTGTACAAAATACGTTTTG-3′

DNA probe 1 (on the test zone of the biosensor): 5′ - ACA CTG GGT GGG CTA GGG AA/Biotin/ - 3′

DNA probe 2(conjugated with Au-NP): 5′-ThioMC6-D/CAA AAC GTA TTT TGT ACA AT - 3′

DNA probe 3 (on the control zone of the biosensor): 5′-Biotin/ ATT GTA CAA AAT ACG TTT TG - 3′

Non-complementary DNA: 5′-ATG GCA TCG CTT AGC TGC CAG TAC ACT GAT TGA AGA CAT CAT AGT GCA GAC AAG CAT ATC - 3′

The sequences of the oligonucleotides used for human genomic DNA test:

DNA probe 4 (used to prepare the test zone of the biosensor): 5′-Biotin/CGCCCGGCTAATTTTTTGTATTTTTAGTAGAGAC - 3′

DNA probe 5 (used to prepare the Au-NP-DNA conjugates): 5′- ThioMC6/GGG ACT ACA GGC GCC CGC CAC CA - 3′

DNA Probe 6 (used to prepare the control zone of the biosensor): 5′-Biotin/TGGTGGCGGGCGCCTGTAGTCCC-3′

All chemicals used in this study were analytical reagent grade. All other solutions were prepared with ultrapure (>18 MΩ) water from a Millipore Milli-Q water purification system (Billerica, MA).

Preparation of Gold Nanoparticles (Au-NP), Au-NP-DNA and HRP-Au-NP-DNA Conjugates. Au-NPs with average diameter 15 nm ± 3.5 nm were prepared according to the reported methods with slight modifications.[31,32] All glassware used in this preparation was thoroughly cleaned in *aqua regia* (three parts HCl, one part HNO_3), rinsed in doubly distilled water, and oven-dried prior to use. In a 500 mL round-bottom flask, 100 mL of 0.01% $HAuCl_4$ in doubly distilled water was brought to a boil with vigorous stirring. To this solution was added 4.5 mL of 1% trisodium citrate. The solution turned deep blue within 20 s, and the final color changed to wine-red occurred 60 s later. Boiling was pursued for an additional 10 min, the heating source was removed, and the colloid solution was stirred for another 15 min. The resulting Au-NP solution was stored in dark bottles at 4 °C and was used to prepare Au-NP-DNA conjugate. The resulting solution of Au-NPs was characterized by an absorption maximum at 520 nm. The Au-NPs with 50, 100, and 200 nm diameters were purchased from British Biocell International Research Inc. (Madison, WI).

A thiolated oligonucleotide (DNA probe 2) was used for conjugation with Au-NPs. Before the conjugation, the thiolated oligonucleotide was activated by the following procedure: 98 μL of thiolated oligonucleotide probes (1.0 OD) was mixed with 2 μL of triethylamine and 7.7 mg of DTT to react for 30 min at room temperature (RT), then the excess DTT was removed by four times extraction with 400 μL of ethylacetate solution. Conjugation reactions were carried out by adding the activated DNA probe solution to 1 mL of the 5-fold concentrated Au-NP solution. After standing at 4 °C for 24 h, the solution was subjected to "aging" by the addition of NaCl up to a concentration of 150 mM, and a certain quantity of 1% sodium dodecyl sulfate (SDS) was added to reach a final concentration of 0.01%. The solution was allowed to stand for another 24 h at 4 °C, and the excess of reagents were removed by centrifugation for 12 min at 12000 rpm. The supernatant was discarded, and the red pellet was redispersed in 1 mL of eluent buffer containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween, and 10% sucrose.

(31) Hayat, M. A. *Colloidal Gold: Principles, Methods and Applications*; Academic Press: San Diego, CA, 1991.

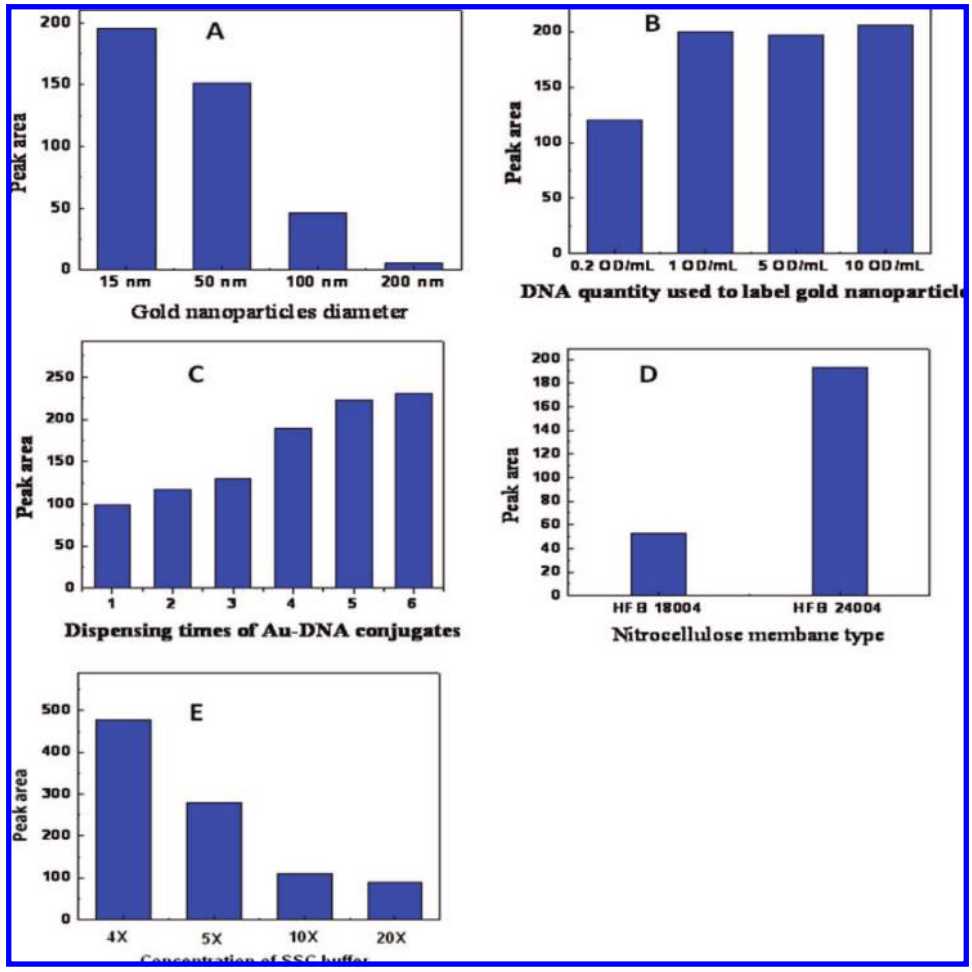

Figure 4. (A) Effect of the Au-NP diameter on the response of 100 nM target DNA at the DNAB; (B) The effect of DNA probe concentration used during the preparation of Au-NP-DNA conjugate on the response of 100 nM target DNA at the DNAB, Au-NP diameter: 15 nm; (C) The effect of dispensing times of Au-NP-DNA conjugates on the response of 100 nM target DNA at the DNAB, diameter of Au-NP is 15 nm, the concentration of DNA probe for the preparation of Au-NP-DNA is 1.0 OD/mL. Assay time: 15 min, all DNA sample solutions were prepared with 20×SSC buffer; (D) Effect of nitrocellulose membrane sources on the response of 100 nM target DNA at the DNAB; (E) Effect of SSC concentration on the response of 100 nM target DNA at the DNAB, SSC buffer with different concentrations were prepared with by diluting the 20 times concentrated SSC buffer. Assay time: 15 min; dispensing times of Au-NP conjugate on the conjugate pad: 6; concentration of DNA probe for the preparation of Au-NP-DNA is 1.0 OD/mL.

For the preparation of the HRP-Au-NP-DNA conjugates, the resulting red pellet above was redispersed in 1 mL of H_2O and 25 μL of 5 mg/mL HRP solution was added. The mixture was incubated for 1 h. The excess reagents were removed and washed twice by centrifugation for 12 min at 12000 rpm; the red pellet was redispersed in 1 mL of eluent buffer.

Preparation of the DNABs. A schematic diagram of the DNAB is shown in Figure 1. The DNAB consists of four components: sample application pad, Au-NP-DNA conjugate pad, nitrocellulose membrane, and absorbent pad. All of the components were mounted on a common backing layer (typically an inert plastic, e.g., polyester) using the Clamshell Laminator (Biodot, CA, U.S.A.). The sample application pad (17 mm × 30 cm) was made from glass fiber (CFSP001700, Millipore) and saturated with a buffer (pH 8.0) containing 0.25% Triton X-100, 0.05 M Tris-HCl, and 0.15 M NaCl. Then it was dried and stored in a desiccator at RT. The conjugate pad (8 mm × 30 cm) was prepared by dispensing a desired volume of Au-NP-DNA (or HRP-Au-NP-DNA) conjugates solution onto the glass fiber pad with the dispenser Airjet AJQ 3000, and then drying it at RT. The pad was stored in a desiccator at 4 °C. Nitrocellulose membrane (25 mm × 30 cm) was used to immobilize the capture DNA probes (DNA probe 1) and control DNA probes (DNA probe 3) at different locations to form test zone and control zone, respectively. To facilitate its immobilization on the nitrocellulose membrane, streptavidin was used to react with the biotinylated DNA probes to form the streptavidin−biotin DNA conjugates. Briefly, 60 μL of 1 mM biotinylated DNA probes and 140 μL of PBS were added to 300 μL of 1.67 mg/mL streptavidin solution, and the mixture was incubated 1 h at RT. The excess DNA probes were removed by centrifugation for 20 min with a centrifugal filter (cutoff 30000, Millipore) at 6000 rpm. The resulting conjugates were washed three times with 1 mL of PBS in the same centrifugal filter. Finally, 500 μL of PBS was added into the remaining solution in the filter. The conjugates were then dispensed on the nitrocellulose membrane with the dispenser Biojet BJQ 3000. The distance between the test zone and control zone is around 2 mm. The DNA probe loaded membrane was then dried at RT for 1 h and stored at 4 °C

(32) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. *J. Anal. Chem.* **1995**, *67*, 735–743.

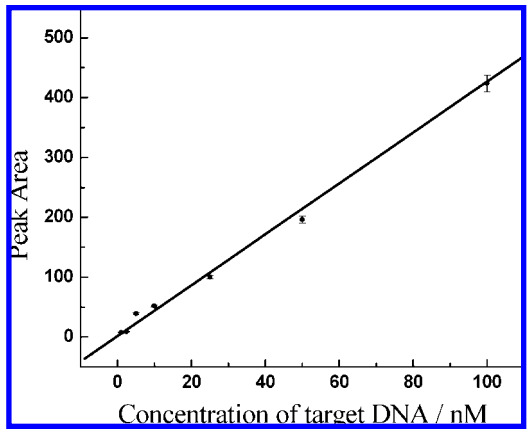

Figure 5. Calibration curve of the DNAB with different concentrations of target DNA. Experimental conditions, same as in Figure 4E. Error bars represent standard deviation, $n = 6$.

in a dry state. Finally, the sample pad, conjugate pad, nitrocellulose membrane, and absorption pad were assembled on a plastic adhesive backing (60 mm × 30 cm) using the clamshell laminator. Each part overlaps 2 mm to ensure the solution is migrating through the strip during the assay. Strips with a 4 mm width were cut by using the Guillotin cutting module CM 4000.

Sample Assay Procedure. For the optimization experiments, the procedure of DNAB test is the following: 130 μL of sample solution containing a desired concentration of target DNA in 4 × SSC buffer was applied to the sample application zone. After waiting for a desired time (for example, 10 min), another 50 μL 4×SSC buffer was applied to wash the strip. The bands were visualized within 15 min. For quantitative measurements, the strip was inserted into the strip reader DT1030, the optical intensities of the test line and control line could be recorded simultaneously by using the "GoldBio strip reader" software.

Signal Amplification with HRP-Au-NP Dual Labels. In the case of signal amplification, the conjugate pad was prepared by dispensing HRP-Au-NP-DNA conjugates on the glass fiber, and other preparations are following the above procedures. After a complete assay as described above, 90 μL of "signal amplification solution" containing 0.05% 3-amino-9-ethyl-carbazole (AEC), 0.015% H_2O_2 in 0.05 M sodium acetate buffer (pH 5.5) was applied to the sample pad. The enzymatic reaction proceeded for 5 min to deposit red enzymatic products on the test zone and control zone of the DNAB. The intensities of test line and control line were recorded with the strip reader.

Detection of Human Genomic DNA with the DNAB. Another set of oligonucleotide probes (DNA probe 4, DNA probe 5, and DNA probe 6) were used to prepare the DNAB for the detection of human genomic DNA. DNA probe 4 was used to prepare the test zone of the DNAB. HRP and DNA probe 5 were conjugated with the Au-NPs following the procedure mentioned above. DNA probe 6 was used to prepare the control zone of the DNAB. Before the test, the sample solution containing certain quantity of human genomic DNA (for example, 10 μL) was first denatured by treating with boiling water for 5 min, and then transferred to a beaker filled with ice water for 15 min. The denatured genomic DNA sample solution was then applied to the sample pad of the DNAB and washed with 120 μL of 6×SSC (1% BSA, 0.2% Tween-20, 0.01% SDS) buffer. Following the signal amplification, the signal of the DNAB was recorded with the strip reader.

RESULTS AND DISCUSSION

Principle of DNAB Measurement. The principle of the DNAB measurement is based on an on-strip sandwich DNA hybridization reaction, and the protocol is illustrated in Figure 2. In this study, three DNA probes were used: DNA probe 1 and DNA probe 3 were immobilized on the nitrocellulose membrane to form the test zone and control zone, respectively. DNA probe 2 was attached to the Au-NPs and dispensed in the conjugate pad (glass fiber). The sample solution containing target DNA is applied on the sample application pad (Figure 2 A). The solution migrates by capillary action and passes the conjugate pad, and then rehydrates the Au-NP-DNA probe 2 conjugates. The target DNA hybridizes with DNA probe 2 of the Au-NP-DNA conjugates to form the complex and continue to migrate along the strip (Figure 2 B). The hybrids are captured on the test zone by the second hybridization between the target DNA and the immobilized DNA probe 1 (Figure 2 C). The accumulation of Au-NPs in the test zone of the nitrocellulose membrane is visualized as a characteristic red band. The excess of Au-NP-DNA probe 2 conjugates continue to migrate and pass the control zone, in which the DNA probe 3 is immobilized. Then the excess of Au-NP-DNA probe 2 conjugates are captured by the hybridization events between the DNA probe 2 and the DNA probe 3, thus forming a second red band (Figure 2 D). In the absence of target DNA, no red band is observed in the test zone. In this case, a red band (control line) shows that the DNAB is working properly. Qualitative analysis is simply performed by observing the color change of the test zone, and quantitative analysis is realized by reading the optical intensity of the test line with the portable strip reader.

Figure 3 presents the typical photo images and corresponding optical responses of 0 nM target DNA (control, a), 1000 nM noncomplementary DNA (b), and 100 nM target DNA (c) and the mixture of 1000 nM noncomplementary DNA and 100 nM target DNA (d). Two red bands were observed in the presence of target DNA (Figure 3c), and only one band (control line, top) was observed in the absence of target DNA (Figure 3a) and presence of excess noncomplementay DNA (Figure 3b). The presence of excess noncomplementary DNA does not affect the signal of target DNA (Figure 3d). The intensities of the bands were recorded by the strip reader shown on the bottom of the figures. Well-defined peaks were observed, and the peak areas were proportional to the captured Au-NPs in the test line (right side) and control line (left side).

Optimization of DNAB Fabrication and Assay Parameters. In the current study, Au-NPs were used as labels for tracing DNA hybridization events. The accumulation of Au-NPs in the test and control zones are visualized as characteristic red bands, which could be used for qualitative and quantitative analysis of target DNA concentration. The size of Au-NP, the amount of DNA probes on Au-NP, and the amount of Au-NP-DNA conjugates on the conjugate pad would affect the response of target DNA on the DNAB. First we investigated the effect of the size of the Au-NP on the response of target DNA. The Au-NPs with diameters of 15, 50, 100, and 200 nm were used to prepare conjugates for the DNAB test. Figure 4 A presents the histogram of 100 nM target DNA responses on the DNAB prepared with different sizes of

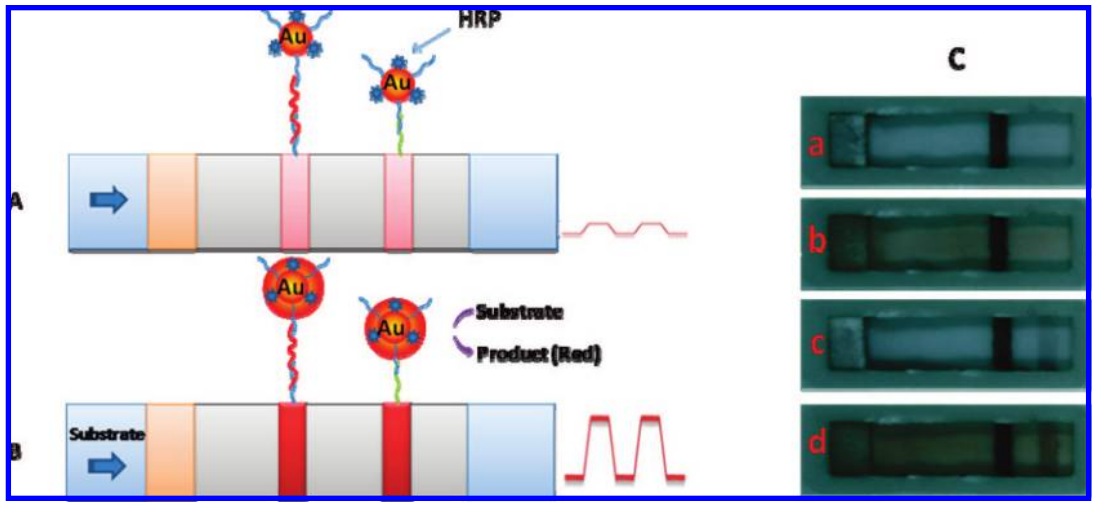

Figure 6. Schematic illustration of the signal amplification protocol of DNAB based on the use of HRP-Au-NP dual labels. (A) without amplification; (B) signal amplification by depositing insoluble enzymatic product (red) on the captured Au-NPs; (C) Typical photo images of the DNAB without (a, c) and with signal amplification (b, d). (a) 0 nM target DNA without signal amplification; (b) 0 nM target DNA with signal amplification; (c) 10 nM target DNA without signal amplification; (d) 10 nM target DNA with signal amplification. The signal amplification of the DNAB was performed by applying 90 μL of "signal amplification solution" containing 0.05% 3-amino-9-ethyl-carbazole (AEC), 0.015% H_2O_2 in 0.05 M sodium acetate buffer (pH 5.5) to the sample pad. The enzymatic reaction time is 5 min; other conditions are the same as in Figure 5.

Table 1. Summary of the Analytical Performances of the Detection of Human Genomic DNA with Different Methods

method	detection limit and assay time	reference
microarray	200 fM, unknown	34
colorimetric scatter	333 zM, 2 h	35
microarray	50 fM, 1 h	36
Surface Plasmon Resonance Imaging	1 pM, > 2 h	37
piezoelectric sensor	~10 fM, >30 min	38
DNAB	1.25 fM, ~20 min	current method

Au-NP labels. It can be seen that the responses of the DNABs decrease with the increase of diameters of the Au-NPs. A bright red band was observed with 15 nm Au-NPs, and the color of the bands change from red to purple; the intensities of the bands decrease when the particle size increases from 50 to 200 nm (results not shown). As particle size increases, the wavelength of surface Plasmon resonance related absorption shifts to longer, redder wavelengths. It means that red light is adsorbed, and bluer light is reflected, yielding Au-NPs with a pale or purple color. It was also found the migration speed of Au-NP-DNA conjugates on the nitrocellulose membrane decreased with the increase of the size of Au-NPs, resulting in a long assay time. So the Au-NP with a diameter of 15 nm was used for the following experiments.

Secondarily, the amount of DNA probe on the Au-NP surface would affect the response of the DNAB. Considering the short hybridization time on the test, multiple DNA probes attached gold nanoparticle may increase the hybridization efficiency and sensitivity. We studied the effect of DNA probe concentration during the preparation of conjugates on the signal of the DNAB (Figure 4B). The response signal (peak area) increases upon raising the DNA concentration in the conjugate solution from 0.2 OD/mL to 1.0 OD/mL, and then it starts to level off, which corresponds to the saturation of DNA probes on the Au-NP surface. A DNA probe concentration of 1.0 OD/mL in the conjugate solution was used to prepare the Au-NP-DNA conjugates for the following experiments. The number of DNA probe per Au-NP was estimated to be 110 with the reported method.[33]

In the current study, the Au-NP-DNA conjugates were immobilized on the glass fiber by physical adsorption. The intensities of test line and control line depend on the amount of Au-NP-DNA conjugate captured on the lines, which in turn correspond to the amount of conjugates in the conjugate pad. The amount of Au-NP-DNA conjugates on the conjugate pad was controlled by the dispensing volume of the conjugate solution. Figure 4C presents the histogram of the recorded intensity of test line for 100 nM of target DNA test with the different amount of Au-NP-DNA conjugate loaded conjugate pads. The responses of the DNAB increased up to four dispensing times on the conjugate pad and then tended to be stable after six times dispensing times, which was used as the optimal dispensing times for most of the experiments. The further increase of the dispensing times of Au-NP-DNA conjugates caused an increasingly nonspecific adsorption and the assay time.

In the current study, sandwich-type DNA hybridization reactions were performed on the DNAB; the hybridization time, which depends on the migration time of buffer in the nitrocellulose membrane, plays an important role for the sensitivity of the test. Two nitrocellulose membranes including HFB 18004 and HFB 24004 (Millipore) were chosen to prepare the DNAB. According to the instruction of the manufacturer, the migration times of the buffer in HFB 18004 and HFB24004 membranes are 3 and 4 min, respectively. Figure 4D presents the intensities of the test line of 100 nM target DNA with the DNABs prepared by the above nitrocellulose membranes. One can see the intensity of the test line with the HFB 24004 nitrocellulose membrane is significantly higher (around 4 times) than that prepared with the HFB 18004.

(33) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A.; Letsinger, R. L.; t Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535–5541.

The whole assay time with HFB 24004 is around 15 min. So the HFB 24004 nitrocellulose membrane was used to prepare the DNABs.

Another factor to affect the sensitivity and reproducibility of the DNAB test is the use of various buffers. Appropriate buffers would minimize the nonspecific adsorption and increase the sensitivity and reproducibility of the DNAB. In the current study, four kinds of buffers were used in the DNAB fabrication and test: (1) the buffer (pH 8.0) containing 0.25% Triton X-100, 0.05 M Tris-HCl and 0.15 M NaCl was used to saturate the sample pad. This treatment would facilitate to transport the target DNA into a downstream portion of the lateral flow device and reduce entrapment of target DNA in the sample pad. (2) During the preparation of Au-NP-DNA conjugates, the Au-NP-DNA conjugate pellets were dispersed in the buffer containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween, and 10% sucrose. The addition of BSA, Tween 20, and sucrose is to stabilize the Au-NPs and facilitate the release of the Au-NP-DNA conjugate from the conjugate pad. Another important function of these buffer components is to reduce the nonspecific adsorption of Au-NP-DNA on the nitrocellulose membrane. After rehydrating the nanoparticle conjugates, the components (BSA, Tween 20 and sucrose) are dispersed in the running buffer, migrate along the strip, and will block the nitrocellulose membrane naturally without additional block steps. (3) A 0.01 M PBS buffer (pH 7.4) was used to prepare streptavidin−biotinylated DNA probe solutions, which were used to prepare the test zone and control zone. (4) Running buffer in the test affects critically the performance of the sensors. We compared the performances of DNAB tests with different running buffers including PBS, PBS+0.1% BSA, PBS+0.1% Tween 20 and SSC buffers, and found the SSC buffer exhibited the best performance (results not shown). Furthermore, we found that the concentration of SSC had a significant effect on the intensity of the test line (Figure 4 E). It can be seen that the intensity of the test line is increasing with the increase of the concentration of SSC (20× to 4×), further increasing of the SSC concentration (3×, 2×) leads to the increase of the background signal and assay time, so a 4×SSC buffer was used for the following experiments.

Analytical Performance. Under optimal experimental conditions, we examined the performance of the DNAB with different concentrations of target DNA. For qualitative analysis, the red bands in the test zone were observed with as low as 5 nM of target DNA. Quantitative detections were performed by recording the intensities of the test lines with the portable strip reader. Well-defined peaks were observed, and the peak areas increased with the increase of DNA concentration. The resulting calibration plot (Figure 5) of the peak areas versus target DNA concentration is linear over the 1 to 100 nM range and is suitable for quantitative work. The detection limit of 0.5 nM (based on S/N = 3) was estimated in connection with the 15 min assay time. This detection limit corresponds to 65 fmol in the 130 μL sample solution.

Signal Amplification of the DNAB. To improve the visual effect of the test line and the sensitivity of quantitative nucleic acid detection, the signal of the DNAB was amplified by using HRP-Au-NP dual labels. The signal amplification protocol is illustrated in Figure 6A,B. In this case, HRP is used as co-label to prepare HRP-Au-NP-DNA conjugates. After a complete assay, the HRP-Au-NP-DNA conjugates are captured in the test zone and control zone of the DNAB to produce relative weak red bands (Figure 6A). Followed by applying a "signal amplification solution" (0.05% 3-amino-9-ethyl-carbazole (AEC), 0.015% H_2O_2 in 0.05 M sodium acetate buffer (pH 5.5)), the enzymatic reactions between the captured HRP on the DNAB and AEC in the presence of H_2O_2 will produce insoluble red products, which deposit on the Au-NP surface to enhance the visual effect and the intensities of red bands (Figure 6B). Figure 6C presents the typical photo images of a control sample (0 nM target DNA) and 10 nM DNA sample before (a, c) and after (b, d) signal amplification. It can be seen that the visibility of the test line for the 10 nM DNA sample enhanced significantly. Such enhancement is similar with the conventional silver/gold staining. No enhancement was observed with the control sample (a and b). The test line band became quite visible even at 0.2 nM target DNA, and the detection limit of quantitative detection with the strip reader was improved to be 50 pM (6.5 fmol), which is comparable to that of the reported "dipstick" test.[20,24] The sensitive and specific response was coupled with high reproducibility. A series of measurements of 1 nM DNA with 8 DNABs yield reproducible signal with a RSD of 6.4% (data not shown).

Detection of Genomic DNA with the DNAB. To demonstrate the feasibility of the DNAB for practical applications, the DNAB was then applied to detect human genomic DNA directly by adopting well-designed DNA probes (see the sequences in the experimental sections) and HRP-Au-NP dual labels. We noticed the whole assay time (20 min) of human genomic DNA on the DNAB with 4×SSC as running buffer was longer than that of the synthesized DNA target with 60 bases. Such longer assay time may be caused by the large molecular weight of genomic DNA, resulting a low migration rate on the nitrocellulose membrane. So we optimized the concentration and components of the running buffer and found the assay would be completed in 15 min with 6×SSC buffer containing 1% BSA, 0.2% Tween-20, 0.01% SDS. The resulting calibration plot (see Supporting Information, Figure S1) of the peak areas versus the logarithm of human genomic DNA concentration is linear over the 5 to 100 μg/mL range and is suitable for quantitative work. The logarithm relationship between the human genomic DNA concentration and the responses (peak area) of the DNAB may be caused by the repeated complementary sequences of the genomic DNA, which binds with multiple Au NPs. The detection limit of the DNAB was estimated to be 2.5 μg/mL (S/N = 3). This detection limit corresponds to 1.25 fM, which is much lower than that obtained with other methods [34−38] (Table 1). The assay time with the DNAB is also less than most of the reported methods, which require 30 min to several hours. The sensitive and specific response was coupled with high reproducibility. A series of measurements of 25 μg/mL human

(34) Storhoff, J. J.; Marla, S. S.; Bao, P.; Hagenow, S.; Mehta, H.; Lucas, A.; Garimella, V.; Patno, T.; Buckingham, W.; Cork, W.; Müller, U. R. *Biosens. Bioelectron.* **2004**, *19*, 875–883.

(35) Storhoff, J. J.; Lucas, A. D.; Garimella, V.; Bao, Y. P.; Müller, U. R. *Nat. Biotechnol.* **2004**, *22*, 883–887.

(36) Bao, Y. P.; Huber, M.; Wei, T. F.; Marla, S. S.; Storhoff, J. J.; Müller, U. R. *Nucleic Acids Res.* **2005**, *33*, e15.

(37) Li, Y.; Wark, A. W.; Lee, H. J.; Corn, R. M. *Anal. Chem.* **2006**, *78*, 3158–3164.

(38) Minunni, M.; Tombelli, S.; Fonti, J.; Spiriti, M. M.; Mascini, M.; Bogani, P.; Buiatti, M. *J. Am. Chem. Soc.* **2005**, *127*, 7966–7967.

genomic DNA with 8 DNABs yield a reproducible signal with an RSD of 6.8% (data not shown).

CONCLUSIONS

We have successfully developed a DNAB for rapid, sensitive, and quantitative detection of nucleic acid samples. Under optimal conditions and signal amplification, the DNAB was capable of detecting minimum 50 pM target DNA. The biosensor has been applied for the detection of human genomic DNA directly with a detection limit of 2.5 μg/mL (1.25 fM) by adopting well-designed DNA probes. Further work will be targeted at the selectivity of the DNAB toward the mismatched DNAs. The new nucleic acid biosensor thus provides a rapid, sensitive, low cost, and quantitative tool for the detection of nucleic acid samples. It shows great promise for in-field and point-of-care diagnosis of genetic diseases and detection of infectious agents or warning against biowarfare agents.

ACKNOWLEDGMENT

G.L. acknowledges the financial support from North Dakota Experimental Program to Stimulate Competitive Research (EPSCoR) and new faculty startup funds of North Dakota State University.

SUPPORTING INFORMATION AVAILABLE

Further details are given in Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review November 20, 2008. Accepted December 22, 2008.

AC8024653