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Lateral flow devices for nucleic acid analysis exploiting quantum dots as reporters

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Highlights

--Dipstick tests for DNA hybridization assays and genotyping of single-nucleotide polymorphisms
--Use of quantum dots as reporters
--Visual detection without the need for expensive instrumentation
--Simplicity and low-cost of the assays

Abstract

There is a growing interest in the development of biosensors in the form of simple lateral flow devices that enable visual detection of nucleic acid sequences while eliminating several steps required for pipetting, incubation and washing out the excess of reactants. In this work, we present the first dipstick-type nucleic acid biosensors based on quantum dots (QDs) as

reporters. The biosensors enable sequence confirmation of the target DNA by hybridization and simple visual detection of the emitted fluorescence under a UV lamp. The 'diagnostic' membrane of the biosensor contains a test zone (TZ) and a control zone (CZ). The CZ always fluoresces in order to confirm the proper function of the biosensor. Fluorescence is emitted from the TZ, only when the specific nucleic acid sequence is present. We have developed two general types of QD-based nucleic acid biosensors, namely, Type I and Type II, in which the TZ consists of either immobilized streptavidin (Type I) or immobilized oligodeoxynucleotides (Type II). The control zone consists of immobilized biotinylated albumin. No purification steps are required prior to the application of the DNA sample on the strip. The QD-based nucleic acid biosensors performed accurately and reproducibly when applied to *(a)* the visual detection of PCR amplification products and *(b)* visual genotyping of single nucleotide polymorphisms (SNPs) in human genomic DNA from clinical samples. As low as 1.5 fmoles of double-stranded DNA were clearly detected by naked eye and the dynamic range extended to 200 fmoles. The %CV were estimated to be 4.3-8.2.

1. Introduction

The detection and quantification of specific nucleic acid sequences are based on the hybridization (molecular recognition) of the target sequence

(analyte) with a complementary DNA sequence that is linked, directly or indirectly, to an appropriate label for signal generation. Presently, there is a growing interest in the development of lateral flow devices for nucleic acid analysis that enable visual detection of the analyte while eliminating several steps required for pipetting, incubation and washing out the excess of reactants.

Fluorometry is the most common technique used in biosensing because of its high sensitivity, simplicity and ability for multiplexing [1,2]. Nanomaterials are gradually replacing the organic fluorophores in the development of novel analytical methods because they exhibit several advantages such as brighter fluorescence, higher photostability and wider Stokes shift [3,4]. Furthermore, nanostructures provide a suitable solid substrate for the construction of biosensors that offer simplicity and higher sensitivity. Nanomaterials such as metallic, silica and carbon nanoparticles and nanotubes, as well as quantum dots (QDs) have been applied to the development of biosensors due to their unique physical, chemical, mechanical, magnetic and optical properties, enhancing the sensitivity and the specificity of detection [5].

QDs are nanometer-scale semiconductor nanocrystals that have been used successfully as reporters in biosensing and bioimaging [6] due to their excellent optical properties and the ability of attaching various molecules to their surface [7,8,9]. QDs have also been used for the development of immunoassays in a lateral flow strip format. The first electrochemical lateral flow test strip based on QDs was introduced in 2007 [10]. This was an immunosensor strip coupled with a disposable screen-printed electrode for the detection of prostate-specific antigen (PSA) in human serum. CdS-ZnS

core-shell QDs, captured on the strip, were determined by stripping voltammetry of the dissolved cadmium. More recently, QD-based fluorometric lateral flow immunoassays, for a variety of analytes, were reported. Both the 'sandwich' type (two-site) and competitive-type immunoassay configurations were exploited.

QD-based competitive-type fluorometric lateral flow immunoassays were developed for the detection of trichloropyridinol (the metabolite of the organophosphate insecticide chlorpyriphos) in rat plasma [11], the detection of mycotoxin ochratoxin A in red wines [12], and the detection of the antibiotic chloramphenicol in milk [13].

Sandwich-type fluorometric lateral flow immunoassays using QDs as reporters were developed for staphylococcal protein A (screening for syphilis) in human blood samples [14], nitrated ceruloplasmin (a biomarker for cardiovascular disease, lung cancer, and stress response to smoking) in spiked human plasma samples [15], alpha fetoprotein (tumor marker for hepatocarcinoma) in human serum samples [16], avian influenza virus in chicken serum samples [17] and anti-hepatitis B virus antibody in human serum [18].

Herein, we describe the first dipstick-type nucleic acid biosensors based on quantum dots. More specifically, this report refers to lateral flow tests exploiting streptavidin-functionalized CdSe-ZnS core-shell QDs as reporters. The biosensors enable sequence confirmation of the target DNA by hybridization and simple visual detection of the emitted fluorescence under a UV lamp. The QD-based nucleic acid biosensors were applied to *(a)* visual detection of PCR amplification products and *(b)* visual genotyping of single

nucleotide polymorphisms (SNPs) in human genomic DNA. The 'diagnostic' membrane of the biosensor contains a test zone (TZ) and a control zone (CZ). The CZ fluoresces always in order to confirm the proper function of the biosensor. Fluorescence is emitted from the TZ, only when the specific nucleic acid sequence is present. We have developed two general types of QD-based nucleic acid biosensors, namely, Type I and Type II. For Type I biosensors, the test zone contains immobilized streptavidin and the control zone consists of immobilized biotinylated albumin. For Type II biosensors, the TZ contains immobilized oligodeoxynucleotides and the CZ consists of immobilized biotinylated albumin. Moreover, in the Type II sensor, the oligonucleotides were either immobilized directly to the surface of the membrane (Type IIa) or indirectly through the immobilization of an albumin-oligo conjugate (Type IIb). No purification steps were required prior to the application of the DNA sample on the strip either the Type I or the Type II sensors.

2. Materials and Methods

2.1. Apparatus and reagents

PCR amplification and allele-specific primer extension reactions were performed in the MJ Research PTC-0150 thermal cycler (Watertown, MA). A digital camera, Konica Minolta DYNAX 5D (Konica Minolta Holdings Inc., Tokyo, Japan) was used for photographing the strips. A UV lamp (Philips HPW 125 W-T, Belgium) was used for the excitation of quantum dots on the strip. The Gel Analyzer software for DNA documentation was purchased from

Kodak (New York, NY). The TLC applicator, Linomat 5, and the software WinCats were from Camag (Muttenz, Switzerland).

Quantum dots (585 nm) conjugated with streptavidin (SA) were purchased from Invitrogen (CarlsBad, CA). The quantum dots consisted of CdSe as a core surrounded by a shell of ZnS. Terminal deoxynucleotidyl transferase (TdT) was from MBI Fermentas (Vilnius, Lithuania). *Taq* DNA polymerase was obtained from HyTest (Turku, Finland) and New England Biolabs (Beverly, MA). Vent (exo-) DNA polymerase was from New England Biolabs (Beverly, MA). Ultrapure 2'-deoxyribonucleoside 5'-triphosphates (dNTPs) were purchased from Invitrogen (CarlsBad, CA). Biotin-11-dUTP was from Applichem (Darmstadt Germany). Immunopore FP nitrocellulose membrane was purchased from Whatman (Florham Park, NJ). The wicking pad, glass-fiber conjugate pad, and absorbent pad were from Schleicher & Schuell (Dassel, Germany). Streptavidin from *Streptomyces avidinii* was purchased from Roche Diagnostics (Mannheim, Germany). EDTA was from Merck (Darmstadt, Germany). Anti-biotin antibody, Sephadex G-25, methanol, sucrose, sodium dodecyl sulfate (SDS) and all other common reagents were from Sigma (St. Louis, MO). Phosphate buffered saline (1×PBS) consisted of 140 mM NaCl, 2.7 mM KCl , 10 mM NaH_2PO_4, 1.7 mM KH_2PO_4, pH 7.4. Saline sodium citrate (SSC) buffer contained 0.3 M NaCl and 30 mM $Na_3C_6H_5O_7$, pH 7.0. The primers and probes used in this study were synthesized by MWG-Biotech (Ebersberg, Germany) and Invitrogen (Carsbad, CA). The NH_2-dT(30) probe used for the construction of the strip was 5´-NH_2-T_{30}-3´. The universal upstream and downstream primers, used for the amplification of a highly conserved region of the 23S rRNA of

Staphylococcus epidermidis, were 5´-GCGATTTC(CT)GAA(CT)GGGG(AG)AACCC-3´ and 5´-TTCGCCTTTCCCT CACGGTACT-3´, respectively [19]. The downstream primer was biotinylated at the 5´end. The specific probe for the identification of *S.epidermidis* was 5´-ACGGAGTTACAAAAGAACATGTTAGTTTTT-3´. The upstream and downstream primers, used for the amplification of TLR4 gene were 5'-GTAAAACGACGGCCAGTAGTCCATCGTTTGGTTCTGGGAGA-3' and 5'-CAGGAAACAGCTATGACGCCATTGAAAGCAACTCTGGTGTG-3', respectively. The specific primers used in the primer extension reaction for wild type and mutant allele for TLR4 gene were 5'-A_{24}GTGATTTTGGGACAAC-3' and 5'-A_{24}GTGATTTTGGGACAAT-3', respectively.

2.2 Construction of the dipstick-type DNA biosensor

The dry-reagent strip (4 mm × 70 mm) consisted of an immersion pad, a conjugate pad, a laminated membrane and an absorbent pad assembled on a plastic adhesive backing that provides the required rigidity. The four parts were positioned in such a way that their ends overlapped in order to ensure continuous flow (by capillary action) of the developing solution from the wicking pad up to the absorbent pad. Two types of DNA biosensors were constructed. **(a)** For Type I, streptavidin (1.6 µg) was immobilized by physical adsorption on the test zone of the strip. Similarly, biotinylated BSA (50 ng) was immobilized on the control zone of the strip. The membrane was dried at 80 ^{o}C for 1 h [20]. **(b)** For the construction of Type IIa biosensor, 10 pmol of

oligo(dT) strands were immobilized on the test zone of the biosensor, whereas 50 ng of biotinylated BSA were immobilized on the control zone. The membrane was dried at 80 ^{o}C for 2 h [21]. Type IIb biosensor was constructed by immobilizing a BSA-oligo(dT) conjugate (BSA-NH_2-dT(30)) on the test zone and biotinylated BSA (50 ng) on the control zone of the strip. The membrane was held at 80 ^{o}C for 1.5 h.

2.3 Visualization of the fluorescence

A simple UV light chamber was manufactured in-house for the visual detection of DNA sequences with the dipstick-type DNA biosensor using quantum dots as reporters. A schematic illustration of the UV chamber is presented in **Fig. 1**. The system consists of the following parts: (i) An excitation light source (a UV lamp, λ = 340-380 nm, λ_{max} = 365 nm). (ii) Position for sliding of the biosensor into the chamber. (iii) Mirrors and (iv) Position of the digital camera with an adjusted cut-off emission filter (HOYA 55mm Orange filter (G)) for seclusion of the emitted fluorescence.

2.4 Tailing of oligonucleotides with dATP and dTTP

Tailing reactions were carried out in a final volume of 20 µL containing 0.2 M potassium cacodylate, 25 mM Tris (pH 7.2), 1 mM $CoCl_2$, 0.1 ml/L Triton X-100, 2 mM dATP (or 3.5 mM dTTP), 400 pmol of specific for *S.epidermidis* probe (1nmol of dT_{30} probe) and 30 U terminal transferase. The reaction was incubated for 1 h at 37 °C and then was stopped by adding 2 µL

of 0.5 M EDTA, pH 8.0. Methylmaleimide was also added at a final concentration of 2.5 mM in the case of the tailing reaction with dATP.

2.5 Conjugation of bovine serum albumin (BSA) with dT_{30} oligonucleotide

The reaction was carried out in a total volume of 50 µL containg 5 nmol of dT_{30} that carried an amino-group at 5´ end, BS3 (500 nmol in DMSO), 0.1 M $NaHCO_3$, pH 8.0. The reaction was incubated at room temperature for 15 min, in the dark. The product was purified twice from the excess of BS3 by size exclusion chromatography (Sephadex G-25). Then, 17 µL of a BSA solution (1 g L^{-1} in PBS supplemented with 5 mM EDTA) and 5 µL of EDTA (50 mM in 10×PBS) were added and the mixture was incubated for 12 h at 4 °C, in the dark.

2.6 Detection of PCR amplification products

Sample collection and DNA isolation were carried out as described in ref. 23. PCR amplification of the highly conserved 23S rRNA sequence of *Staphylococcus epidermidis* was carried out in a total volume of 50 µL. The reaction mixture contained 67 mM Tris–HCl, pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 1 mL L^{-1} Tween 20, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, 1.25 U *Taq* DNA polymerase and 10 µL of extracted DNA. The reaction included the following steps: 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. At the end, the solution was held at 72 °C for 10 min [22,23].

The PCR products were first detected and quantified by 2% agarose gel electrophoresis and ethidium bromide staining, using the molecular weight markers ΦX174 DNA, *Hae*III digest as calibration standards for the quantification.

2.7 Visual detection of the PCR products

The amplification products, that were biotinylated at the 5´ end (during PCR), were first hybridized with a (dA)-tailed specific probe. The hybridization reaction contained 10 μL PCR product, 90 mM NaCl and the 1 pmol specific probe. The mixture was heated at 95 °C for 2 min and then incubated at 37 °C for 5 min. The reaction took place in the thermal cycler.

For the detection, a 5-μL aliquot of 5´ biotinylated oligo(dT) solution (5 pmol) was applied to the conjugate pad of the biosensor. Then, 5 μL of the hybridization reaction mixture was also applied to the conjugate pad followed by the addition of 5 μL of 100 nM QD-SA conjugate. The biosensor was then immersed into 300 μL of the developing solution containing 40 mL L^{-1} glycerol, 0.5 g L^{-1} SDS, 10 mL L^{-1} Tween-20, BSA 1 g L^{-1} in 2×SSC, pH 7.0. The strip was removed from the developing solution after 10 min and inserted into the UV chamber. The immobilized QDs at the test zone and the control zone of the biosensor were excited at λ = 340-380 nm. The emmited fluorescence was captured by the digital camera. A fluorescent line (orange) is formed at the test zone of the biosensor in the presence of the PCR product. A second orange line is formed at the control zone to confirm the proper function of the biosensor.

2.8 Visual genotyping of single nucleotide polymorphisms (SNPs)

Sample collection and DNA isolation were carried out as described in ref. 24. A 634-bp segment flanking the A896G and C1196T polymorphic sites of the human TLR4 gene was amplified by PCR from genomic DNA using the Qiagen Hotstar Taq master mix kit (Hilden, Germany). The amplification reaction was carried out in a total volume of 50 µL containing 25 µL master mix (0.05 U µ L^{-1} Hotstar DNA polymerase, 1.5 mM $MgCl_2$ and 200 µM dNTPs), 0.4 µM of each primer and 60–120 ng of genomic DNA. The thermal cycling protocol included the following steps: 95 °C for 15 min, followed by 35 cycles of 95 °C for 1 min, 65 °C for 1 min, 72 °C for 1 min, and a final extension step at 72 °C for 8 min [24].

The PCR-amplified DNA was subjected to two separate allele-specific primer extension reactions, one with the normal (N) primer and the other with the mutant (M) primer. Both primers contained a $(dA)_{24}$ segment at the 5' end. The extension reaction was carried out in a total volume of 20 µL contained 20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 0.1 mL L^{-1} Triton X-100, pH 8.8, 0.5 U Vent (exo-) DNA polymerase, 1-2 µL of amplified DNA, 1 pmol of the appropriate primer, 2 mM $MgSO_4$, 2.5 µM each of dATP, dCTP, dGTP, 1.25 µM dTTP and 1.25 µM biotin-dUTP. The extension reactions were performed in the thermal cycler as follows: 95 ^oC for 5 min and three cycles of 95 ^oC for 15 s, 55 ^oC for 10 s, 72 ^oC for 15 s. The reaction products were subjected to a final denaturation step at 95 °C for 5 min and placed immediately on ice. For detection, a 5-µL aliquot of the denatured product was applied onto the conjugate pad next to the deposited QD-SA conjugate (5 µL,

100 nM). The strip was then dipped into a microcentrifuge tube containing 300 μL of the developing solution. After 10 min, the strip was removed from the developing solution and placed into the UV chamber, where it was excited at 340-380 nm and the fluorescence emitted from the test and the control lines was captured by a digital camera. An orange line at the test zone of the biosensor denotes the presence of the allele that corresponds to the primer N or M. A second orange line at the control zone confirms the proper function of the biosensor.

3. Results and Discussion

We constructed three types of dipstick-type DNA biosensors that use quantum dots as reporters for *(a)* visual detection of PCR products and *(b)* visual genotyping of single nucleotide polymorphisms (SNP). We used streptavidin-functionalized CdSe QDs with ZnS as a shell.

3.1 Optical detection of PCR products

In **Fig. 2**, a schematic illustration of the principle of the Type I biosensor for the visual detection of PCR products is presented. Type I biosensor was constructed by immobilizing streptavidin at the test zone and biotinylated BSA (B-BSA) at the control zone of the membrane of the strip. The QD-SA conjugate and a biotinylated oligo(dT) were deposited on the conjugate pad of the strip. The biotinylated PCR products were first hybridized (<10 min) with a specific, for *S.epidermidis*, probe that carried a poly(dA) tail

and then applied to the conjugate pad of the biosensor. As the developing solution migrates upward due to capillary action, the QDs bind to the hybrids and the entire macromolecular complex is captured by the immobilized streptavidin at the test zone of the biosensor. As a consequence there is an accumulation of QDs and an orange fluorescent line at the test zone only when the PCR product is present. A second fluorescent line is formed as the excess of the QD-SA conjugate is captured from the immobilized B-BSA at the control zone of the biosensor to confirm the proper function of the test.

To study the ability of the biosensor to detect double-stranded DNA, we applied various amounts of PCR products ranging from 0 to 200 fmol (**Fig. 3**). We observe that as low as 1.5 fmol can be clearly detected by naked eye. The intensity of the test zone increases with the amount of the PCR product. A negative controls (no target) is included in Fig, 3 and they give no line at the test zone.

The reproducibility of the biosensor was determined by testing, in triplicate, two PCR products containing 6.25 and 50 fmol of amplified DNA (**Fig. 3**). The biosensors were photographed using the digital camera and the color intensity of the test zone was determined densitometrically. The CVs obtained were 8.2% and 4.3% at the 6.25 fmol and the 50 fmol level, respectively.

3.2 Visual genotyping of single nucleotide polymorphisms (SNPs)

Fig. 4 illustrates the principle of the biosensor that exploits QDs functionalized with streptavidin as reporters for visual genotyping of SNPs.

PCR-amplified DNA is subjected to two separate primer extension reactions, each with an allele-specific primer. Both primers contain a $(dA)_{24}$ segment at the 5′ end and differ only in one base at the 3′ end. Due to the specificity of DNA polymerase, the primer is extended only if it is perfectly complementary to the target sequence. Biotin-dUTP is incorporated during the extension step. After denaturation, the extended products are applied onto the conjugate pad of the sensor and the biosensor is dipped into the developing solution. Type IIa biosensor was used in this study that was constructed by a direct immobilization of oligo(dT) at the test zone and biotinylated BSA (B-BSA) at the control zone of the membrane. As the sample migrates along the strip, the extension product is captured by immobilized oligo(dT) strands at the test zone via (dA)/(dT) hybridization. The streptavidin modified QDs are captured to the hybrids via streptavidin-biotin interaction, thus creating a fluorescent line. Excess of QD-SA conjugate is captured by immobilized biotinylated albumin at the control zone. The genotype is obtained by taking the results of the two biosensors corresponding to the extension reaction of the normal primer (N) or mutant primer (M). Thus, a normal genotype (N/N genotype) gives a fluorescent line only for the extension of the N-primer. On the other hand, a homozygote for the mutation (M/M genotype) gives a fluorescent line only for the extension of the M-primer. A heterozygote (N/M genotype) gives a positive result with both biosensors. Thus, the negative controls for the genotyping reactions are estimated from the samples themselves, when no zone is formed for the extension reaction of a normal sample with the mutant primer or of a mutant sample with the normal primer.

As a model, we have used the biosensor for genotyping the C1196T polymorphism of toll-like receptor 4 (TLR4) gene in 15 samples of human genomic DNA. Genomic DNA from samples that were previously genotyped [25,26] was used in order to confirm the accuracy of the biosensor. The results are presented in **Fig. 5**. The genotyping results obtained from the biosensor were compared to the genotypes found, for the same samples, in previous work [25,26]. In all cases the biosensor generated the correct genotypes for each sample.

The reproducibility of the biosensor for the visual genotyping of SNPs was assessed by analyzing in triplicate the extension products of a normal (N/N), a heterozygote (N/M) and a homozygote for the mutation (M/M) sample (**Fig. S1**, Supplement). An amount of 100 fmol of each PCR product was subjected to two separate primer extension reactions and applied to the biosensor in triplicate. The CVs were 2.7% for the N/N sample for the extended product of the N primer and 1.1% for the M/M sample for the extended product of the M primer.

The genotyping study was also performed by using the Type IIb biosensor that was constructed by the indirect immobilization of oligo(dT) at the test zone through the application of bovine albumin-oligo $(dT)_{30}$ conjugate. Biotinylated BSA was immobilized at the control zone. **Fig. S2** (Supplement) presents the results of genotyping the C1196T polymorphism (TLR4 gene) in the 15 genomic DNA samples using Type IIb biosensor. We observed that the intensity of the fluorescent lines at the test zones of Type IIb biosensor were slightly weaker than the ones obtained by Type IIa but this did not affect the performance of the test since SNP genotyping is a qualitative test for the

presence or absence of alleles. The slight decrease in the intensity of the test zone in type IIb sensor might be due to the interaction of the the oligo with the BSA that might interfere slightly with the hybridization.

Several lateral-flow assays for nucleic acid sequences have been described. In Table 1, we present the performance characteristics of these assays with respect to type of label, assay configuration, type of target sequence, detectability, required instrumentation, time, and target pretreatment. Lateral-flow assays that involve hybridization of the target sequence with complementary probes offer the advantage of sequence confirmation. The probe-target hybridization is more effective when simple oligonucleotides, or any single-stranded DNA (ssDNA), are used as targets rather than double-stranded DNA (dsDNA). This is because in dsDNA, the reannealing of target strands competes with probe hybridization. As a consequence, higher detectabilities are expected with ssDNA rather than dsDNA targets. It should be emphasized, however, that in real world samples the target is dsDNA, usually PCR-amplified sequences. Gold nanoparticles, colored polymer particles and carbon nanostrings offer visual detection of the hybrids whereas upconverting phosphors offer higher detectability but require a fluorometric scanner for detection. The QD-based biosensor developed in the present work enables visual (by naked eye) detection of 1.5 fmol PCR products which is among the highest detectabilities of lateral-flow devices for dsDNA. There is no need for a fluorometric scanner. Although the performance of the sensor was demonstrated with a single type of QDs, we believe that the ability to tune the emission wavelength of the QD is in fact the most attractive feature of the

proposed biosensor. Because the emission wavelength is size dependent, it is easy to prepare QDs that emit at a wide range, from blue to red. The use of QD labels with different emission wavelengths offers the potential of multianalyte testing enabling the simultaneous detection of several target sequences and/or the genotyping of several SNPs with a single lateral-flow device.

4. Conclusions

We have developed two types of nucleic acid biosensors that exploited streptavidin-functionalized quantum dots as efficient reporters for: *(a)* visual detection of double-stranded DNA (PCR products) and *(b)* visual genotyping of SNPs. The biosensors were applied successfully to real clinical samples. The developed DNA biosensor is extremely simple and enables visual detection and sequence confirmation of PCR products, as well as visual genotyping of even single nucleotide changes in the human genome. The assay with the biosensor is rapid and eliminates multiple incubation and washing steps. The cost of the assay is low since it does not require specialized instrumentation and highly qualified personnel. The cost of the sensor is less than 2 €. The detection is completed within 15 min and as low as 1.5 fmol of PCR product can be detected easily by naked eye. The products of the allele-specific primer extension reaction are detectable after three cycles only (7 min). Moreover, the PCR products and the extension products are applied directly to the biosensor without prior purification of the DNA after the PCR step or after the primer extension reaction step.

Appendix A. Supplementary data

The following are Supplementary data to this article:

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Legends

Fig. 1
Schematic illustration of the home-built UV chamber for the visual detection of DNA sequences with the dipstick-type DNA biosensor using quantum dots as reporters.

1: UV chamber, 2: Digital camera, 3: Mirrors, 4: Position of the biosensor, 5: UV lamp

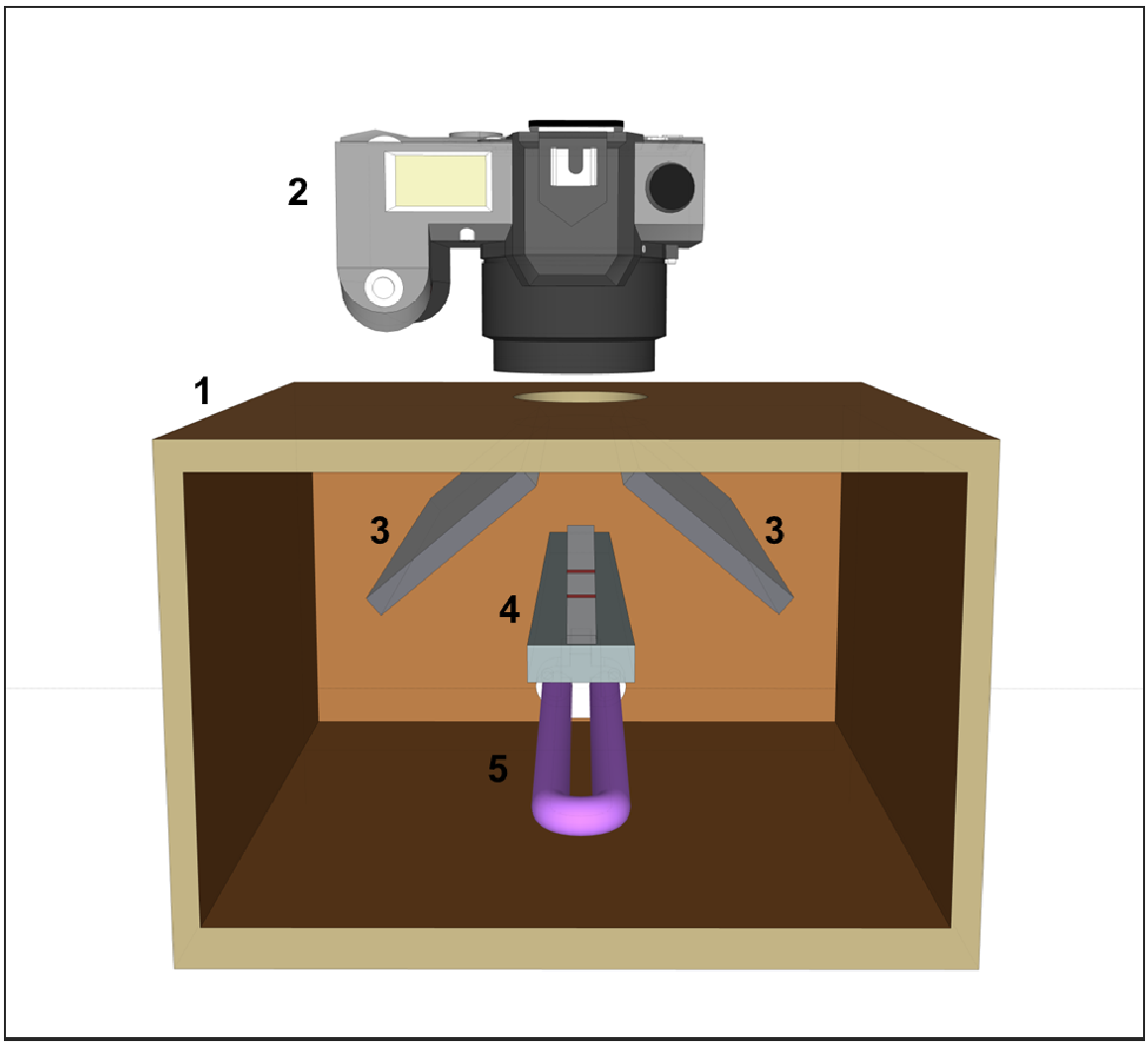

Fig. 2

Design and principle of the nucleic acid biosensor (Type I) for the visual detection of PCR products exploiting functionalized quantum dots as reporters. The arrow shows the direction of the flow.

TZ: test zone, CZ: control zone, IP: immersion pad, CP: conjugate pad, M: membrane, AP: absorbing pad, SA: streptavidin, B: biotin, BSA: bovine serum albumin

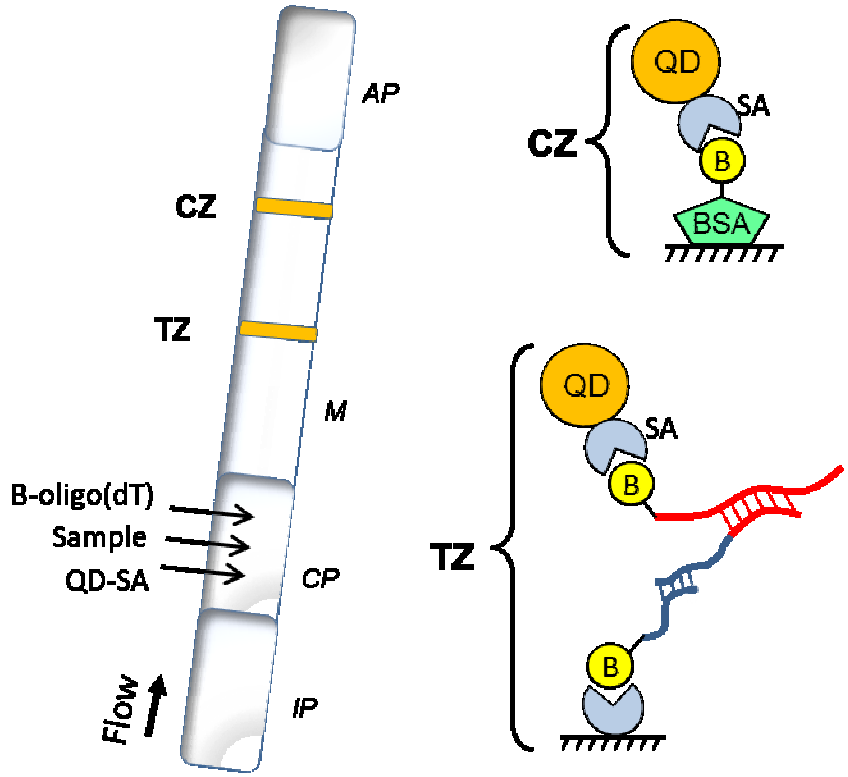

Fig. 3

(A) Performance of the quantum dot-based lateral flow hybridization tests for visual detection of PCR products. The amount of PCR product, in fmoles, is shown above the strips. (B) Reproducibility of the DNA biosensor type I for 50 and 6.25 fmol PCR product, respectively. CZ: Control zone; TZ: Test zone.

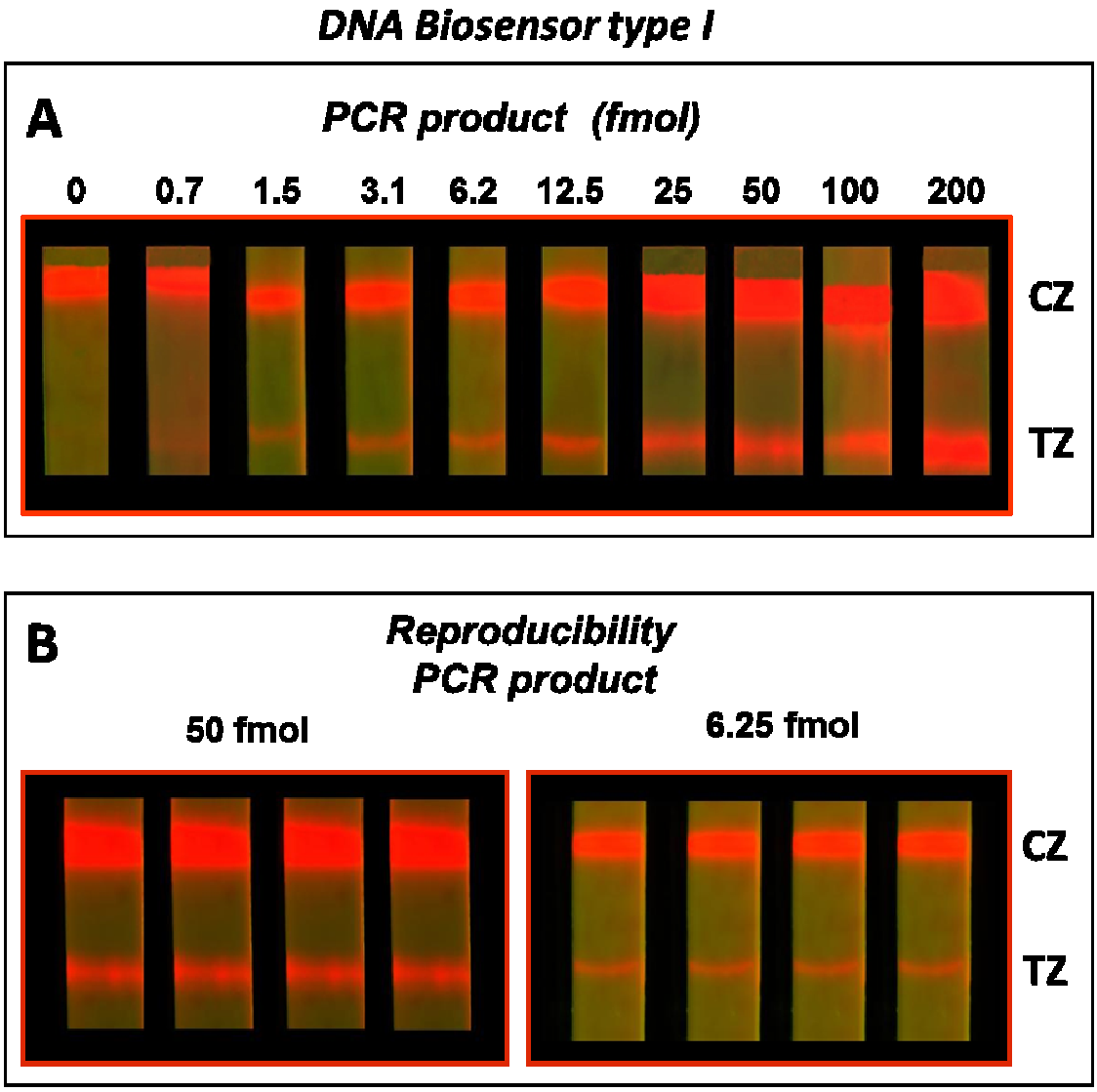

Fig. 4

(A) The allele-specific primer extension reaction. (B) Biosensor design and the principle of the lateral flow hybridization tests for visual genotyping of single nucleotide polymorphisms by exploiting functionalized quantum dots as reporters.

TZ: test zone, SA: streptavidin, B: biotin, BSA: bovine serum albumin.

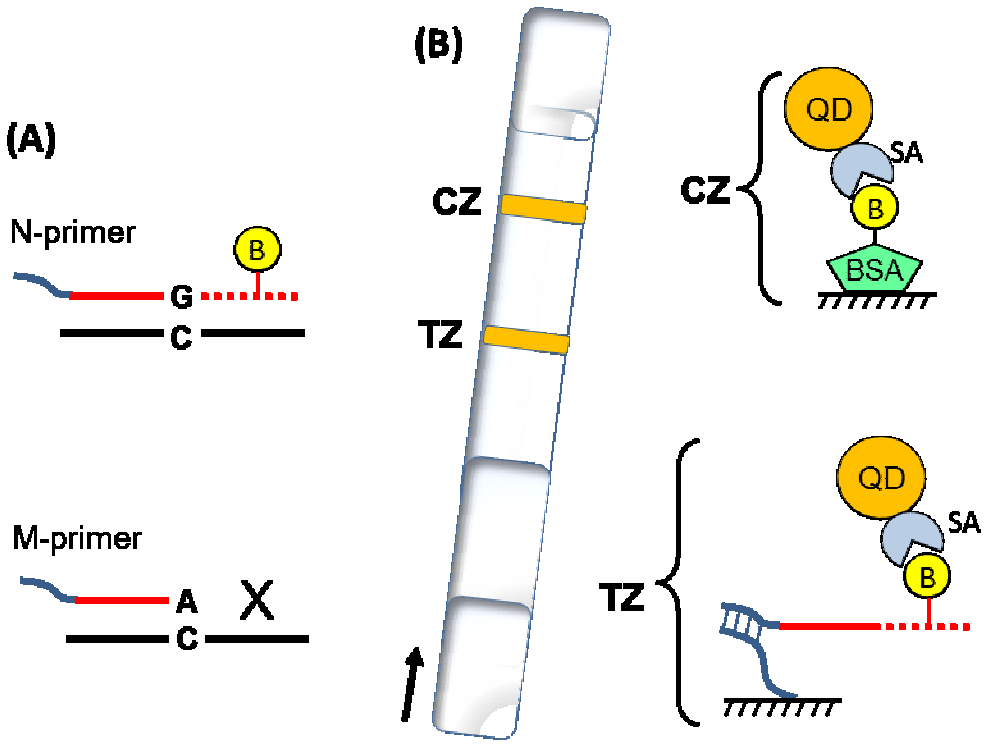

Fig. 5

Visual genotyping of a single nucleotide polymorphism in 15 samples (S1-S15) of human genomic DNA by lateral flow hybridization tests (DNA biosensor type IIa) based on quantum dot reporters. The allele (N: Normal, M: Mutant) is shown above the strips. CZ: Control zone; TZ: Test zone; N: Normal sample; H: Heterozygote sample; M: Mutant sample.

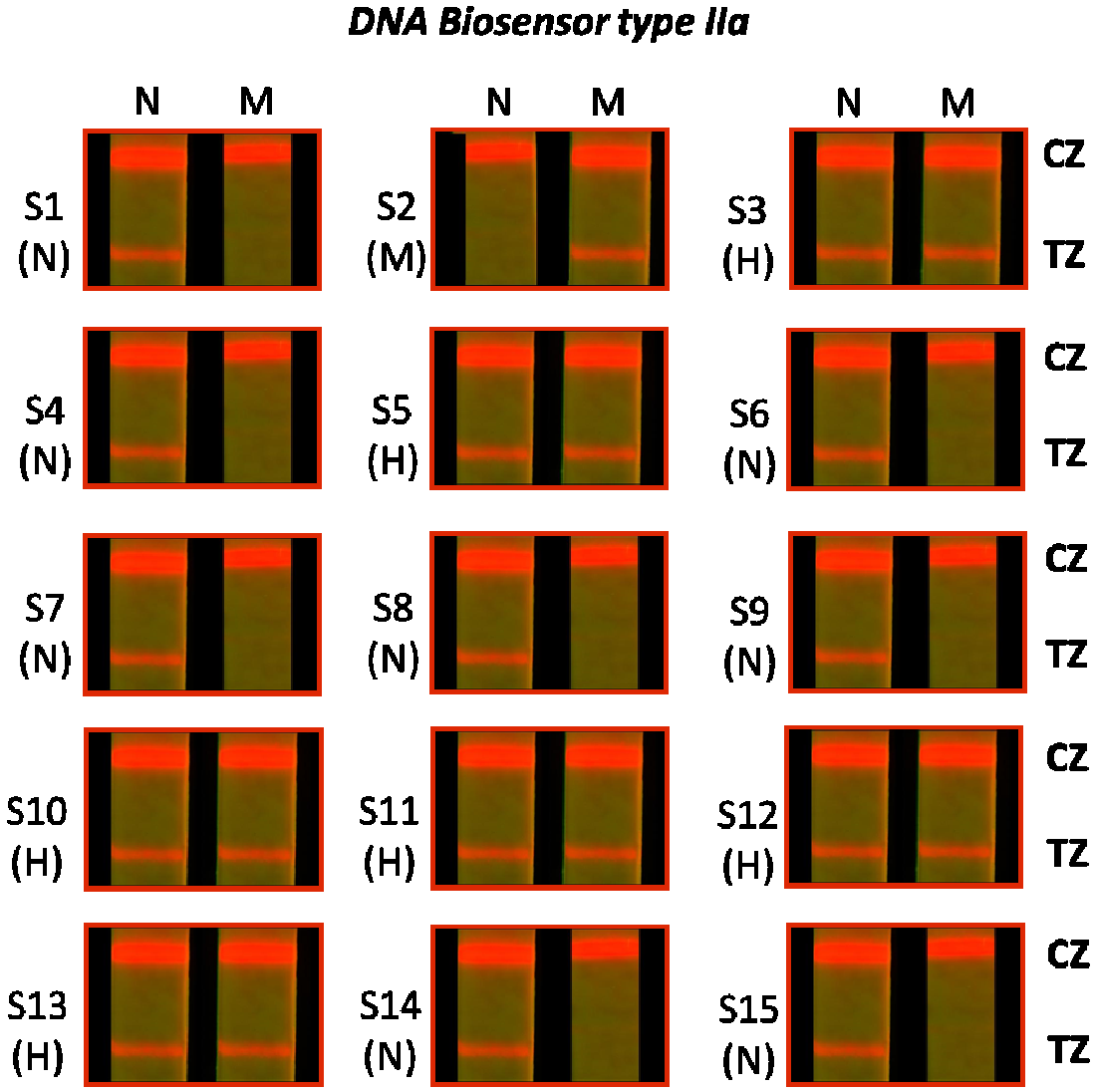

Table 1. Comparison of various lateral-flow assays for nucleic acid sequences

Label	Assay type	Target	Detectability (fmoles)	Detection Instrument	Time[1] (min)	Target pre-treatment	Ref.
Gold nanoparticles	Hybridization	dsDNA 233 bp	2	None (naked eye)	7	NO	27
Gold nanoparticles	Capture & detection of double-labeled DNA	dsDNA 237 bp	32	None (naked eye)	-	NO	28
Gold nanoparticles	Hybridization	ssDNA 91 nt	10	None (naked eye)	-	NO	29
Gold nanoparticles	Hybridization	ssDNA 60 nt	32.5	None (naked eye)	-	NO	30
Gold nanoparticles & Peroxidase	Hybridization	ssDNA 38 nt	0.0008	None (naked eye)	-	NO	31
Liposomes	Hybridization	RNA	10	Reflectometer	20-30	NO	32

Upconverting phosphors	Hybridization	dsDNA 452 or 560 bp	0.03	Fluorometric scanner	60	NO	33
Upconverting phosphors	Hybridization	ssDNA 688 or 93 nt	0.7 (688 nt) 0.1 (93 nt)	Fluorometric scanner	30	YES	34
Polymer microspheres	Hybridization	dsDNA 225 bp	2.5	None (naked eye)	7	NO	35
Polymer microspheres	Hybridization	ssDNA 60 nt	32	None (naked eye)	-	NO	36
Carbon nanoparticles	Hybridization	dsDNA 225 bp	1	None (naked eye)	7	NO	37

[1]Time required for hybridization of the target before the application to the strip.

Table 1. Comparison of various lateral-flow assays for nucleic acid sequences

Label	**Assay type**	**Target**	**Detectability (fmoles)**	**Detection Instrument**	**Time[1] (min)**	**Target pre-treatment**	**Ref.**
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Polymer microspheres	Hybridization	ssDNA 60 nt	32	None (naked eye)	-	NO	36
Carbon nanoparticles	Hybridization	dsDNA 225 bp	1	None (naked eye)	7	NO	37

[1]Time required for hybridization of the target before the application to the strip.