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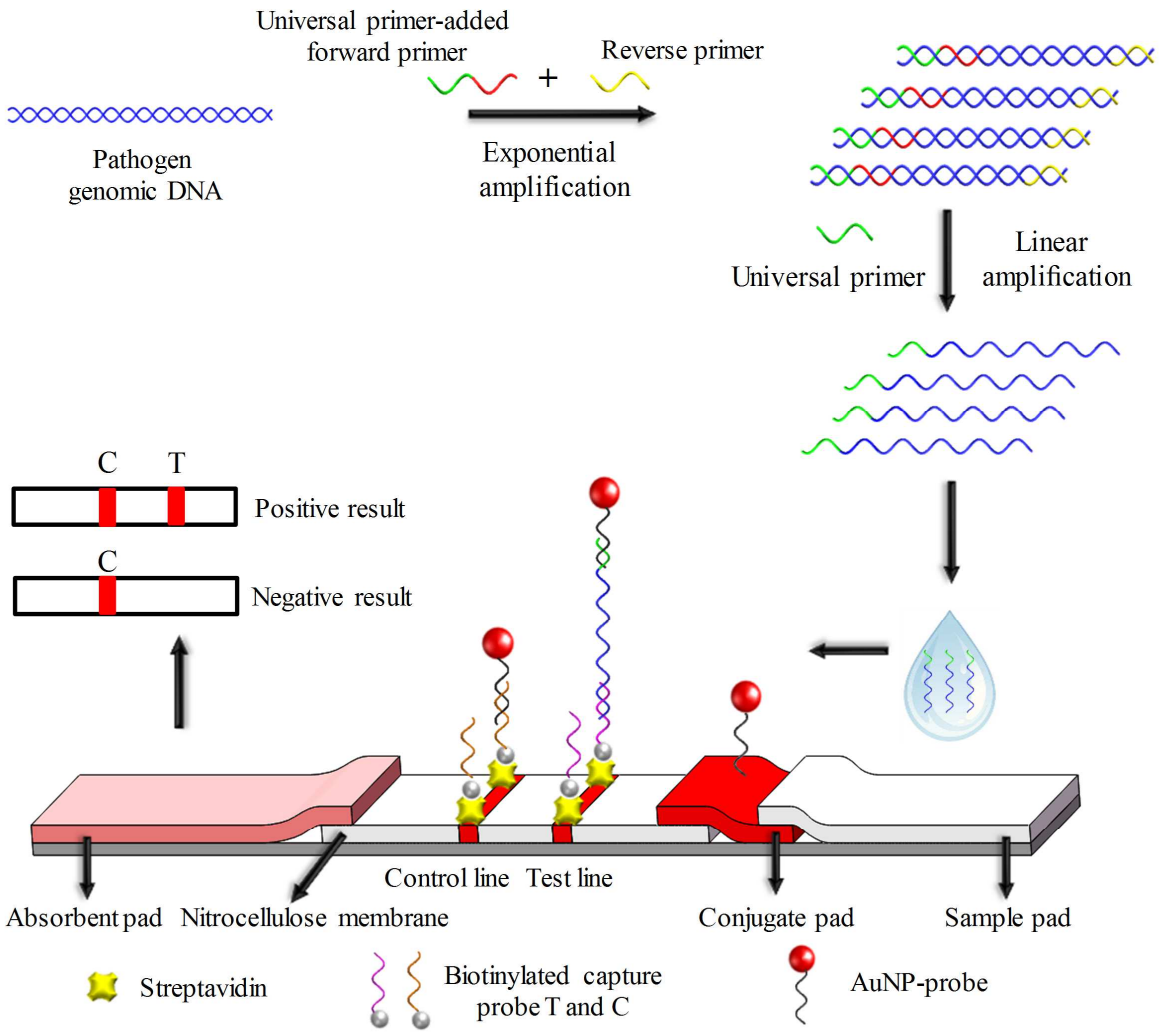

A gold nanoparticle-based lateral flow biosensor for sensitive visual detection of the potato late blight pathogen, *Phytophthora infestans*

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Abstract

Phytophthora infestans, the causal agent of late blight in potatoes and tomatoes, is the most important and ongoing pathogenic threat to agricultural production worldwide. Rapid and early identification of *P. infestans* is an essential prerequisite for countering the further spread of infection. In this study, a novel method for visual detection of *P. infestans* has been developed by integrating universal primer mediated asymmetric PCR with gold nanoparticle (AuNP)-based lateral flow biosensor. We employed asymmetric PCR to generate large amounts of single-stranded DNA (ssDNA) by amplifying a region of *P. infestans*-specific repetitive DNA sequence. The ssDNA products were then applied to the lateral flow biosensor to perform a visual detection using sandwich-type hybridization assays. In the presence of target DNA, sandwich-type hybridization reactions among the AuNP–probe, target DNA and capture probe were performed on the test line of the biosensor, and then a characteristic red band was produced for the accumulation of AuNPs. Quantitative analysis obtained by recording the optical intensity of the red band demonstrated that this biosensor could detect as little as 0.1 pg μL^{-1} genomic DNA. Furthermore, the specificity of the biosensor was confirmed by detecting three other *Phytophthora* species and two pathogenic fungi. We believe this method has potential application in early prediction of potato late blight disease and instigation of management actions to reduce the risk of epidemic development.

Keywords: Gold nanoparticle-based biosensor, Visual detection, Universal primer, Asymmetric amplification, *Phytophthora infestans*

1. Introduction

Plant diseases, such as the devastating potato late blight disease which can lead to severe or complete potato yield loss [1-3], pose a great threat to the sustainability of the world food supply. The annual economic cost generated by late blight through lost potato production and increased control management costs is conservatively estimated to be $6.7 billion annually, worldwide [4]. *Phytophthora infestans*, the causal agent of potato late blight, is the most notable plant pathogen within the oomycetes. Its epidemiological success is generally attributable to rapid replacement of pathogen population generated by a mixed mode of reproduction although the incidence of sexual reproduction remained very low in most parts of the world with *P. infestans* reproducing predominately via its asexual lineages [5-7]. In its asexual mode, sporulation occurs on the surface of infection lesions with a single lesion potentially producing thousands of sporangia, which germinate directly to form infection hyphae or release multiple motile zoospores that infect, colonize and produce new sporangia [8,9]. The massive numbers of sporangia are easily dispersed to new hosts by wind or rain-splash thereby leading a very rapid increase of late blight that can completely destroy entire fields of crops within just a few days [10-12].

Management of late blight is a continuing global challenge as the pathogen rapidly response to the deployment of novel R genes through the evolution of new virulence traits or the release of novel fungicides through the development of resistance or tolerance within pathogen populations [13-16]. In the fight against late blight, early detection is critical in ensuring the most appropriate and efficacious use of control mechanisms like fungicides. To this end it is critical to develop a simple, inexpensive, specific and sensitive method to detect

P. infestans at the earliest possible stage in epidemic development.

The most common and widely utilized techniques for the routine identification of *P. infestans* are enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) [17-20]. ELISA is a serological technique widely used in the routine identification of plant pathogens, but it is labor-intensive and time-consuming due to the requirement of multiple steps and long incubation periods. PCR provides high specificity, sensitivity, efficiency and simplicity, which gives a reliable detection method but often requires additional steps of laborious gel electrophoresis and visual analysis of the resulting bands. To overcome these limitations, there is increasing interest by pathologists in the development of new detection methods.

Lateral flow assay (LFA), which combines chromatography with conventional immunoassay, has attracted considerable attention in the biomarker analysis and diagnostic fields because of its low cost, simplicity of use, rapid on-site responsiveness and simple assessment based on visual assessment [21-23]. Generally, particulate labels in the form of colored or fluorescent nanoparticles, such as gold nanoparticles (AuNPs), colored latex beads, magnetic particles (MPs), carbon nanotubes (CNTs) or quantum dots (QDs), are obligatory for visual inspection in LFA and AuNPs are the most commonly used optical label [24-27]. LFA is usually combined with nucleic acid amplification techniques to achieve high detection efficiency [28-31]. It is noteworthy that asymmetric PCR is especially appropriate to combine with LFA, because it produces single stranded DNA targets that can be directly used on the lateral flow platform without the need for sample denaturation steps following amplification.

In the present work, we develop a new strategy for rapid, sensitive and visual detection

of *P. infestans* by integrating universal primer-mediated asymmetric PCR (UP-APCR) with gold nanoparticle-based lateral flow biosensor. Traditional asymmetric PCR utilizes conventional PCR primers at unequal concentrations to generate single-stranded DNA (ssDNA) amplicons. Unfortunately, that approach requires extensive optimization of the conditions, such as forward to reverse primer ratios, number of amplification cycles and annealing temperature, and tends to promote non-specific amplification [32-34]. Compared with traditional asymmetric PCR, UP-APCR contains one more primer at a normal concentration, a universal primer, and overcomes shortcomings in traditional asymmetric PCR [35]. The product of UP-APCR is primarily ssDNA, facilitating a rapid test using the gold nanoparticle-based lateral flow biosensor. This allows visual detection of the amplified products within several minutes. Using this lateral flow biosensor, we amplified and detected *P. infestans* genomic DNA successfully with high sensitivity. This approach shows great promise in practical applications for plant quarantine and disease diagnosis use.

2. Materials and methods

2.1 Source of isolates and DNA extraction

All oomycete and fungal species used in this study are listed in Table S1. *Phytophthora infestans* samples were collected from different geographic areas of China. Other *Phytophthora* and fungal strains were available in our laboratory or kindly provided by Dr. W. Ye in Nanjing agricultural university, China. All strains were carefully stored on rye agar, potato dextrose agar or V8 agar slants.

To extract total DNA, these pathogens were incubated on rye or potato dextrose agar topped with sterile cellophane sheets before inoculation to facilitate collection of the mycelia.

After harvesting, mycelia were transferred into sterile, 2 mL centrifuge tubes and lyophilized with a vacuum freeze dryer (Alpha1-2, Christ, Germany). The lyophilized mycelia were ground to a powder with a mixer mill (MM400, Retsch, Germany). Then genomic DNA was extracted using a DNA extraction Kit (D3195-02, Omega Bio-tek, Beijing, China) following the manufacturer's instructions. In addition, total DNA was extracted from both *P. infestans*-infected and healthy potato tissues (leaf and stem) using the Cetyl-Trimethylammonium Bromide (CTAB) method, as described earlier [36]. The DNA was quantified by measuring the optical density at 260 nm with a spectrophotometer (Eppendorf BioPhotometer, Hamburg, Germany) and stored at -20 ℃ for further use.

2.2 Functionalization of gold nanoparticles (AuNPs)

The 15 nm AuNPs were purchased from BBI Solutions (BBI Solutions, Cardiff, UK). AuNP-probe labeling was completed according to published protocols with slight modifications [37]. Briefly, 10 µL of 200 µM thiolated detection probe was activated with 3.3 µL of 50 mM acetate buffer (pH 5.2) and 5 µL of 1 mM TCEP. After 1 h of incubation at room temperature, 1 mL of the AuNPs was transferred into a microcentrifuge tube, and then the TCEP-treated thiol detection probe also was added. The mixture was incubated for at least 16 h under gentle shaking. Then 10 µL of 500 mM Tris acetate (pH 8.2) buffer was added drop-wise to the tube with gentle shaking. The final Tris acetate concentration should be 5 mM. The solution was aged by slow addition of 100 µL NaCl (1 M) with rapid stirring and allowed to stand for at least 24 h. After centrifugation at 12,000 rpm for 15 min at room temperature (23−25 °C), the supernatant was discarded and the soft sediment of AuNP-thiolated detection probe conjugates rinsed by resuspension in 1 mL of 10 mM

Tris-HCl buffer (pH 7.4) and collected after a second centrifugation at 12,000 rpm for 15 min. Finally, the detection probe-modified AuNPs were resuspended in a solution containing 20 mM sodium phosphate, 0.25 % Tween 20, 10 % sucrose, and 5 % BSA. The conjugates were stored at 4 °C before further use.

2.3 Assembly of the gold nanoparticle-based lateral flow biosensor

The lateral flow biosensor used in the present work consisted of four components: sample pad, conjugate pad, nitrocellulose membrane (NC membrane) and absorbent pad (Fig. 1). The sample pad, which was made of glass fiber, was saturated with saline - sodium citrate (SSC) buffer (4 ×). Subsequently, the pad was dried at 37 ℃ for 2 h and stored in a desiccator at room temperature before use. The conjugation pad was used as storage for the AuNP-probes. A desired volume of the AuNP-probes prepared previously was dispensed onto the conjugation pad, dried at room temperature, and then stored in a desiccator before further use. The NC membrane was used to form test line and control line with streptavidin-biotinylated capture probe T and streptavidin-biotinylated capture probe C solutions. In brief, 50 μL of 100 μM biotinylated probes were added to 150 μL of 1 mg mL^{-1} streptavidin solution, and the mixture was incubated for 1 h at room temperature. Subsequently, the solutions were separately immobilized on the NC membrane with the HM3020 X-Z dispenser. The distance between the test line and control line was around 6 mm. The membrane was dried at room temperature and stored at 4 ℃ in a dry state. Finally, the sample pad, conjugate pad, NC membrane and absorbent pad were assembled with a 2 mm overlap between each component on a plastic adhesive backing, and the whole assembled plate was cut into 4-mm width strips with programmable shears and stored in a desiccator for the subsequent assays.

2.4 Universal primer mediated asymmetric PCR

Asymmetric PCR mixtures (25 μL) contained 1×PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 200 μM deoxynucleotide triphosphates (dNTPs), 0.25 U of Taq DNA polymerase, 30 nM forward primer (PiO8-U-F, Table 1), 30 nM reverse primer (PiO8-R, Table 1), 300 nM universal primer, 1 μL DNA template, and deionized water. PCR was carried out in an Eppendorf AG thermal cycler with the following program: 94 ℃ for 3 min, 40 cycles at 94 ℃ for 30 s, 52 ℃ for 30 s, 72℃ for 30 s, and 72 ℃ for 10 min; 4℃ hold. The amplification product was snap-cooled on ice before proceeding with the gold nanoparticle-based lateral flow biosensor.

2.5 Visual detection of amplification product with the lateral flow biosensor

25 μL running buffer (4 ×SSC) was mixed with 25 μL asymmetric PCR product. The mixture was then dispensed onto the sample pad of the gold nanoparticle-based lateral flow biosensor for rapid detection. After waiting for 10 min, 50 μL of running buffer was applied to wash the biosensor. The red bands were visualized within 5 min. The results were photographed and quantitative measurements were performed by determining the peak area of test line using ImageJ.

3. Results and discussion

3.1 The principle of universal primer mediated asymmetric PCR (UP-APCR) based lateral flow biosensor for detecting plant pathogen

The operating principle of the lateral flow biosensor for detecting *P. infestans* is illustrated in Fig. 1. Total genomic DNA was extracted from *P. infestans* mycelia or late blight-infected potato field samples. Then, three primers, including universal primer (UP) at a normal

concentration and specific primer pairs (forward and reverse primer) at a limiting concentration, were introduced into the UP-APCR cycling. UP was a random DNA sequence which was not present within the target genome's DNA sequence and therefore made no amplification. Specific primer pairs were used to specifically amplify well-conserved *P. infestans* genes. Unlike traditional PCR, the forward primer in this method contained an extra specific sequence at its 5' end; this was the same as the sequence of the UP. We called this primer UP-added forward primer (UAFP). In the initial phase of the amplification process, double-stranded DNA (dsDNA) amplicons were generated by the symmetrical UAFP primer pairs (UAFPs and reverse primers), while the UPs made no amplification because of the absence of a target template. Subsequently, when the limiting UAFP primer pairs were used up, the resulting dsDNA products were served as templates for the excess UP to initiate linear amplification and yield a large quantity of ssDNA products, which contained UP sequence at their 5'-ends. The resultant PCR products were then applied to the sample pad of the lateral flow biosensor without any pretreatment. The PCR products migrated through capillary action to the conjugate pad, where AuNP–probes designed to hybridize with the universal primer sequence of ssDNA were immobilized. The target ssDNA hybridized with the detection probe on AuNP to form a complex and continued migrating to nitrocellulose membrane, onto which streptavidin-biotinylated capture probe T and streptavidin-biotinylated capture probe C solutions were sprayed to form the test and control line, respectively. The complexes were captured on the test line by the second hybridization between the target DNA and capture probe T. A characteristic red band was then observed on the test line as the result of the accumulation of AuNPs. The excess AuNP–probes were captured on the control line by

hybridization with capture probe C as they migrated further, and a second red band was formed. Excess reagents were entrapped in the absorbent pad. In the absence of target DNA, no red band was observed on the test line.

3.2 Feasible analysis of the universal primer mediated asymmetric amplification strategy

The primers PiO8-F/PiO8-R (Table 1) were used to amplify and detect a 245 bp *P. infestans*-specific repetitive DNA sequence [38]. To verify whether the addition of universal sequence to the 5' end of PiO8-F would affect the efficiency of the PCR process, traditional PCRs were performed using conventional (PiO8-F/PiO8-R) and UAFP (PiO8-U-F/PiO8-R) primer pair, respectively (Table 1). No apparent difference in band intensity was observed between the two primer pair, which demonstrated that the UAFP primer pair had equivalent amplifying efficiency to the corresponding conventional primer pair (Fig. S1, lanes 1, 2). Because the PiO8-U-F had a longer universal sequence (21 nt) at the 5' end than the PiO8-F, the amplified fragment of UAFP primer pair was 21 bp larger than that of the conventional primer pair.

We investigated the feasibility of the universal primer by comparison of amplifications with and without its presence. Two groups of PCR (with and without UPs) were performed with UAFP primer pairs at the concentration of 200 nM and 20 nM, respectively. When the concentration of UAFP primer pairs decreasing from 200 to 20 nM, the intensity of amplified fragments fell markedly to nothing in the absence of UPs (Fig. S2, lanes 1, 2), but the amplified fragments were detected at the concentration of 20 nM with UPs (Fig. S2, Lane 4). The results showed that the UP was well-designed for efficient PCR amplification.

After asymmetric amplification, polyacrylamide gel electrophoresis (PAGE) was

conducted to confirm the presence of the desired ssDNA products, together with the dsDNA products counterpart (Fig. 2). The band below 300 bp indicated the presence of dsDNA products. As the concentration of the UAFP primer pair increased from 20 to 60 nM, the yield of dsDNA products increased, as did the intensity of the bands in the electrophoresis. The band, located between 500 bp and 600 bp, represented the ssDNA products. As the UAFP primer pair increased in concentration, the intensity of this band also increased, reaching a maximum intensity at 30 nM. Above this level band intensity declined, consequently, 30 nM PiO8-U-F/PiO8-R and 300 nM universal primer were chosen for the following PCR reactions.

3.3 The sensitivity of the proposed gold nanoparticle-based lateral flow biosensor

Different concentrations of genomic DNA from *P. infestans* were tested to evaluate the sensitivity of the proposed biosensor. Figure 3A presents typical photographic images of the sensor in the presence of target DNA concentrations of 100 pg μL^{-1}, 10 pg μL^{-1}, 1 pg μL^{-1}, 0.1 pg μL^{-1} and 10 fg μL^{-1}. Reproducibility of the approach was confirmed by testing each sample three times. There was an obvious and continuous decrease in the intensity of the test line as the concentration of the target DNA decreased. When the target template was lower than 0.1 pg μL^{-1}, no distinct red band was observed on the test line, so the detection limit of this biosensor was estimated to be approximately 0.1 pg μL^{-1}. The result of the electrophoresis was consistent with that of the lateral flow biosensor (Fig. 3B). The peak area in the cartogram represents the intensity of the test line of each strip (Fig. 3C). The resulting calibration plot shows a linear relationship between peak area with the concentration of target DNA (R^2=0.96).

The sensitivity of the proposed biosensor is higher than previously reported nucleic

acid-based pathogen detection platforms [39-42]. The high sensitivity can be attributed to a number of factors. Firstly, the UP-APCR has shown strong and reliable ability for target fragment amplification, so target sequences were sufficient for detection, which improves the sensitivity of lateral flow biosensor. Secondly, this amplification process produces ssDNA targets, which can be acted upon directly at room temperature without the requirement for further heating or denaturation at the visual detection step. Thirdly, AuNPs used as colorimetric labels on our lateral flow biosensor, are stable and have an intense red color that is easily detected by naked eye, which help the biosensor to achieve better performance. These characteristics enable the method to have high sensitivity.

3.4 The specificity of the proposed gold nanoparticle-based lateral flow biosensor

Specificity is another important quality index in practical applications for the identification of infectious plant pathogens. The specificity of the proposed biosensor was tested using the closely related *Phytophthora* species and other potato fungal pathogens (Table S1). Genomic DNA of *P. infestans*, *P. capsici*, *P. sojae*, *P. nicotianae*, *Alternaria solani* and *Rhizoctonia solani* with an isometric concentration of 10 pg μL^{-1} were analyzed using this biosensor. As shown in Fig. 4A, two clear red bands were observed at the test and control line only for *P. infestans*, whereas only one red band at the control line was observed for the control sample and for the five other pathogens assessed. Fig. 4B showed that no amplified products were observed in the assays of any of the non - *P. infestans* pathogens (lanes 1-5) or the control, but a distinct band was observed in the assay with the *P. infestans* (lane 6). The cartogram in Fig. 4C was consistent with the result of lateral flow biosensor and the gel electrophoresis. These results demonstrate that the proposed biosensor shows very

high specificity.

The primer pair (PiO8-U-F/R) was designed to amplify and detect a *P. infestans*–specific repetitive DNA sequence. This highly repetitive element in *P. infestans* had proved to be 100 times more sensitive than internal transcribed spacer (ITS) detection [43]. Repetitive DNA sequences are abundant in most eukaryotic genomes, which are essential for genome function [44,45]. They have been widely used for molecular taxonomy and sensitive detection or identification of plant and animal pathogens [46-51]. In our method, the high specificity of the PiO8-U-F/R primer pair ensures that our biosensor is specific to *P. infestans.*

3.5 Evaluation of the gold nanoparticle-based lateral flow biosensor using infected plant samples

To evaluate the application of the biosensor, total DNA was extracted directly from *P. infestans*-infected potato tissues and healthy potato tissues and then subjected to asymmetric amplification and biosensor assay. As shown in Fig. 5A and C, two clear red bands were observed at the test line and control line with the *P. infestans*-infected samples, whereas only one red band at the control line was observed with the healthy sample and the control. The results were also validated by electrophoresis and showed high consistency (Fig. 5B and D). These results verified the potential field use of the proposed biosensor.

Nowadays, immunological methods and nucleic acid-based methods have both been applied to identify plant pathogens. Nucleic acid-based methods, especially PCR, show advantages over immunological methods due to their greater sensitivity, specificity and rapidity [52,53]. Furthermore, advancements in the development of biosensor systems for pathogen detection have greatly enhanced the sensitivity, specificity and simplicity of nucleic

acid-based assays [54,55]. So developing convenient, cost-effective and specific diagnosis tools for plant pathogen monitoring applications becomes possible. In consideration of the superiorities of PCR and lateral flow biosensor simultaneously, we have demonstrated a rapid approach for detecting *P. infestans* using UP-APCR with gold nanoparticle-based lateral flow biosensor. This biosensor presents several advantages. First, the UP-APCR based lateral flow biosensor presented here is sensitive, which is attributed to the target amplification of UP-APCR and sensitive gold nanoparticle-based lateral flow biosensor. Second, since the UP-APCR products are primarily single-stranded DNA, avoiding the necessary for any denaturation or purification, the detection procedure becomes much simpler than traditional analytic methods. The test procedure including amplification and visualization can be completed in less than 1.5 h. Third, the design of primers and probes in our method is easy to perform. Furthermore, this approach requires neither complicated operations nor special apparatus. Based on these advantages, we believe that our method has enormous potential for application in the early prediction and surveillance of plant disease and in assisting the implementation of management practices to reduce the risk of epidemics.

4. Conclusions

A gold nanoparticle-based biosensor based on UP-APCR was successfully constructed for sensitive, rapid and visual detection of *P. infestans*. The assay takes advantage of the high amplification efficiency of the UP-APCR and the portable gold nanoparticle-based lateral flow biosensor. As a result, the low detection limit of 0.1 pg μL^{-1} genomic DNA from *P. infestans* was achieved with high specificity within 1.5 h. In addition, we demonstrated the ability of this biosensor to successfully detect *P. infestans* in infected plant samples. With its

rapidity, simplicity, sensitivity and specificity, the method we proposed has great potential for field application.

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Figure captions

Figure 1. Schematic illustration of gold nanoparticle-based lateral flow biosensor for identification of plant pathogens.

Figure 2. Polyacrylamide gel electrophoresis (PAGE) analysis identified the presence of double-stranded (dsDNA) and single-stranded (ssDNA) amplification products. The concentration of universal primers (UPs) was maintained constant at 300 nM while the concentration of universal primer – added primer pairs (PiO8-U-F/R) was varied from 20-60 nM shown above lanes 1-5. Lane M: 2000 bp DNA marker; Lane C: negative control.

Figure 3. Sensitivity assay of the gold nanoparticle-based lateral flow biosensor. (A) Photographic images (left) and corresponding optical responses (right) of the gold nanoparticle-based lateral flow biosensor with different concentrations of *Phytophthora infestans* genetic DNA. Strips 1−5 correspond to concentrations of 100 pg μL^{-1}, 10 pg μL^{-1}, 1 pg μL^{-1}, 0.1 pg μL^{-1} and 10 fg μL^{-1}; strip C is a negative control. (1) − (3) represent the triplicate test results of different concentration of target. (B) 1% agarose gel electrophoresis of the amplification products. Lanes 1–5 are 100 pg μL^{-1}, 10 pg μL^{-1}, 1 pg μL^{-1}, 0.1 pg μL^{-1} and 10 fg μL^{-1}, respectively. Lane M: 2000 bp DNA marker; Lane C: negative control. (C) Histogram presenting the corresponding peak areas of the test line. The inset was calibration curve for this detection method and R^2 value was 0.96.

Figure 4. Specificity evaluation of the gold nanoparticle-based lateral flow biosensor. (A) Photographic images (left) and corresponding optical responses (right) of the gold nanoparticle-based lateral flow biosensor for the specificity assay. Strips 1−6 correspond to the test results for *Phytophthora capsici*, *P. sojae*, *P. nicotianae*, *Alternaria solani, Rhizoctonia solani* and *P. infestans*; strip C is test results for the negative control. (1) − (3) represent the test was repeated for three times. (B) 1% agarose gel electrophoresis of the amplification products with different targets. Lanes 1–6 are *Phytophthora capsici*, *P. sojae*, *P. nicotianae*, *Alternaria solani, Rhizoctonia solani* and *P. infestans*, respectively; Lane M: 2000 bp DNA marker; Lane C: negative control. (C) Histogram presenting the corresponding peak areas of the test line.

Figure 5. Visual detection of *Phytophthora infestans* from infected potato tissues. (A) Photographic images (left) and corresponding optical responses (right) of the gold nanoparticle-based lateral flow biosensor. Strips 1−7 correspond to the test results for *P. infestans*-infected leaf samples; Strip 8: healthy leaf sample; strip C: negative control. (B) 1% agarose gel electrophoresis of the amplification products with different leaf samples. Lanes 1–7 are *P. infestans*-infected leaf samples; Lane 8: healthy leaf sample; Lane M: 2000 bp DNA marker; Lane C: negative control. (C) Photographic images (left) and corresponding optical responses (right) of the gold nanoparticle-based lateral flow biosensor. Strips 1−5 correspond to the test results for *P. infestans*-infected stem samples; Strip 6: healthy stem sample; strip C: negative control. (D) 1% agarose gel electrophoresis of the amplification products with

different stem samples. Lanes 1–5 are *P. infestans*-infected stem samples; Lane 6: healthy stem sample; Lane M: 2000 bp DNA marker; Lane C: negative control.

Table 1 Oligonucleotide Sequences

Oligonucleotide	Sequence(5'-3')
PiO8-F	AAGATGATGTTGGATGATTG
PiO8-U-F	*CCAATCCAATCCAATCCCCCC*AAGATGATGTTGGATGATTG
PiO8-R	TGCCTGATTTCTACCTTCT
Universal primer	CCAATCCAATCCAATCCCCCC
Thiolated detection probe	GGGATTGGATTGGATTGGTTTTTTTTTTTTTT-C6-SH
Biotinylated capture probe C	CCAATCCAATCCAATCCCTTTTTTTTTTTT-Biotin
Biotinylated capture probe T	Biotin-TTTTTTTTTTTTTGCCTGATTTCTACCTTCT

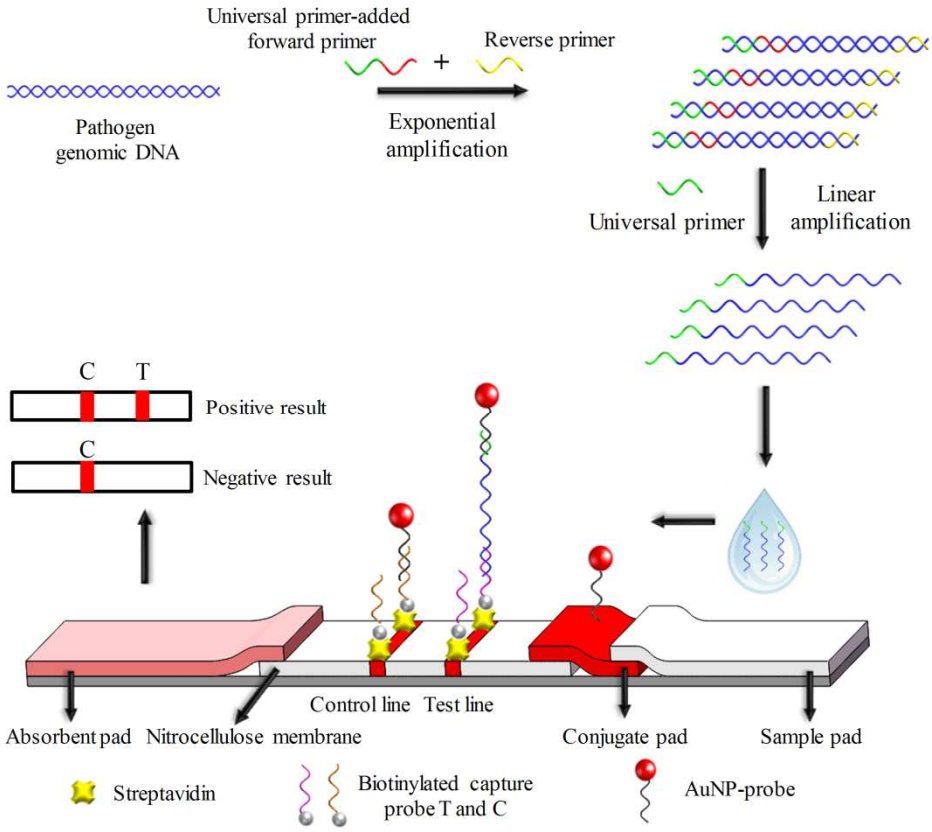


Figure 1

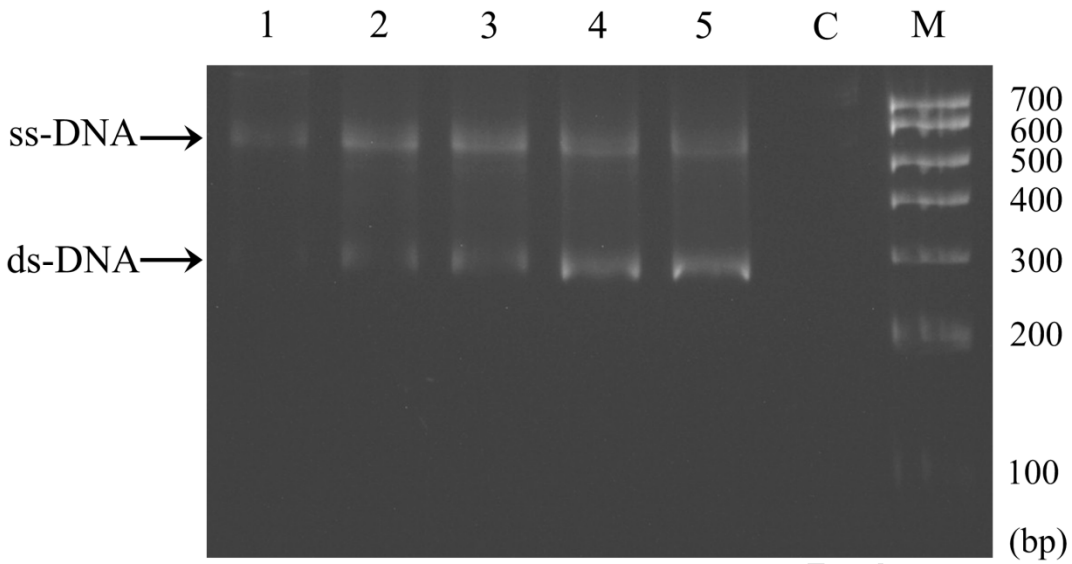


Figure 2

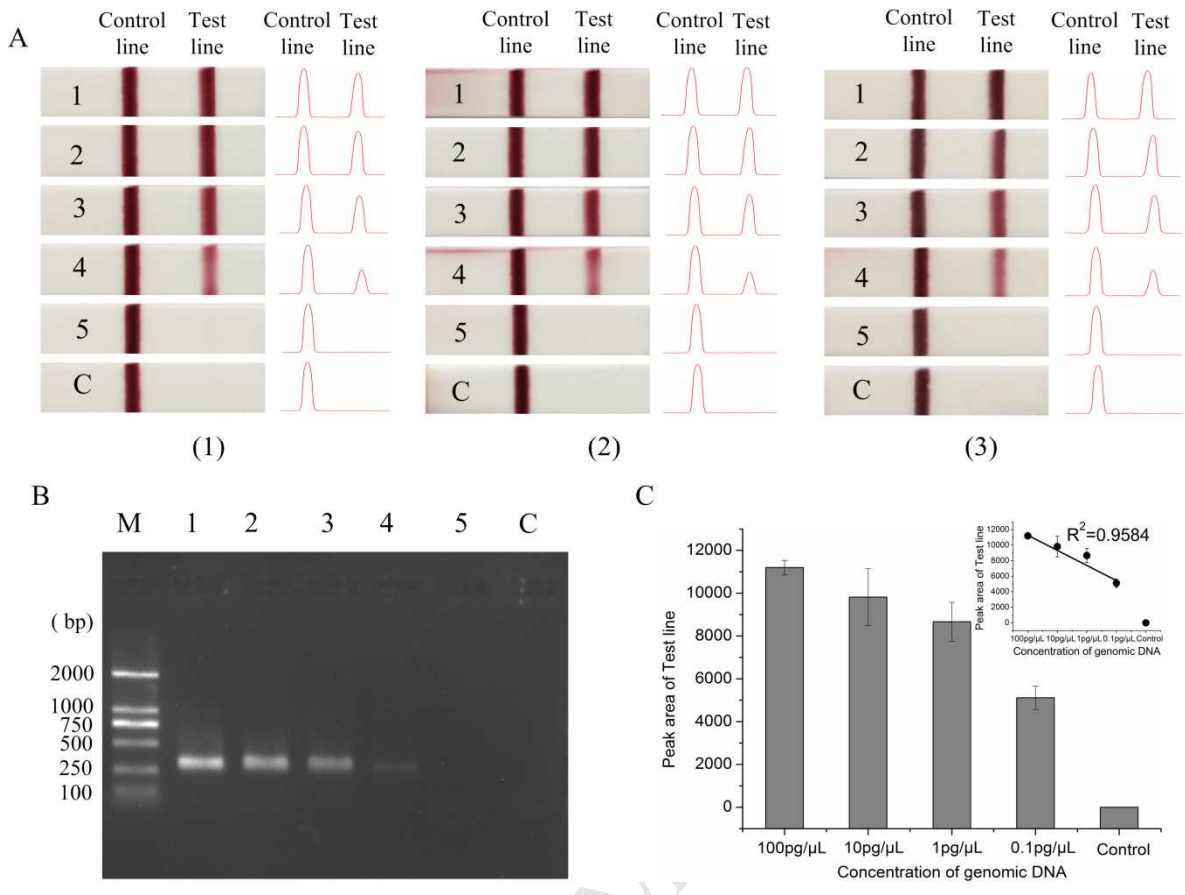


Figure 3

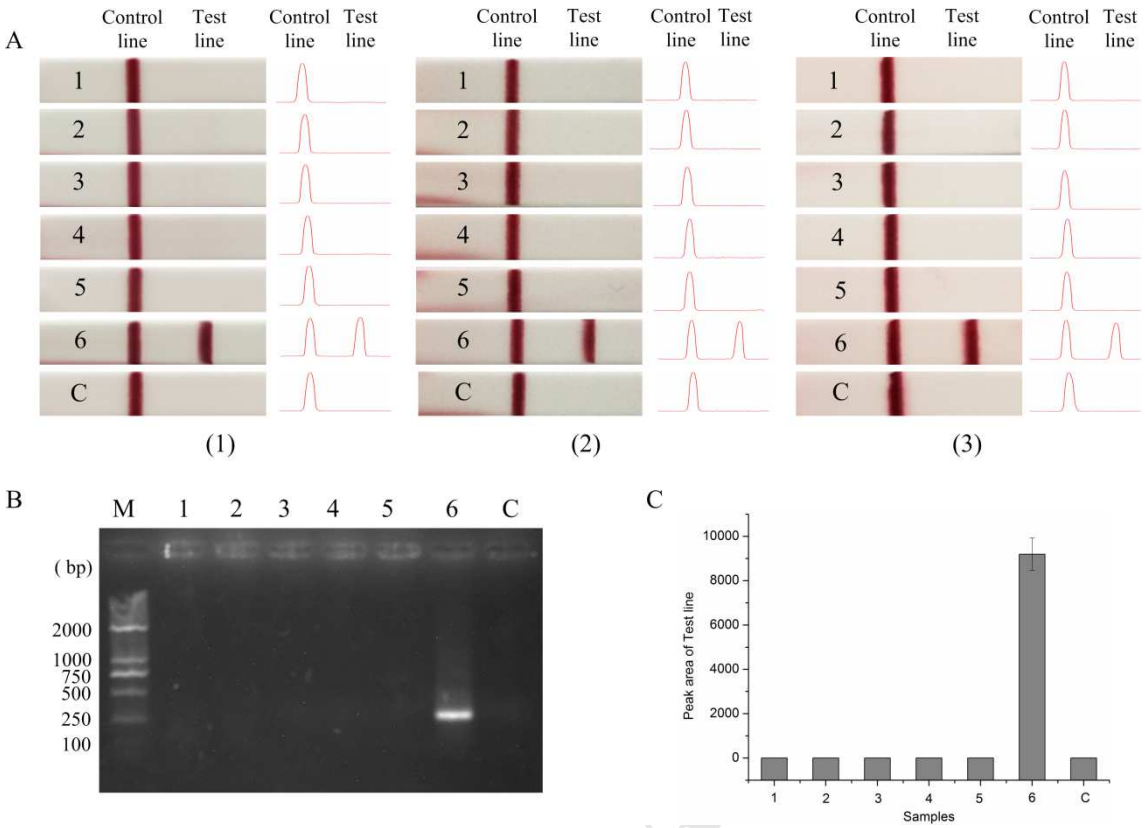


Figure 4

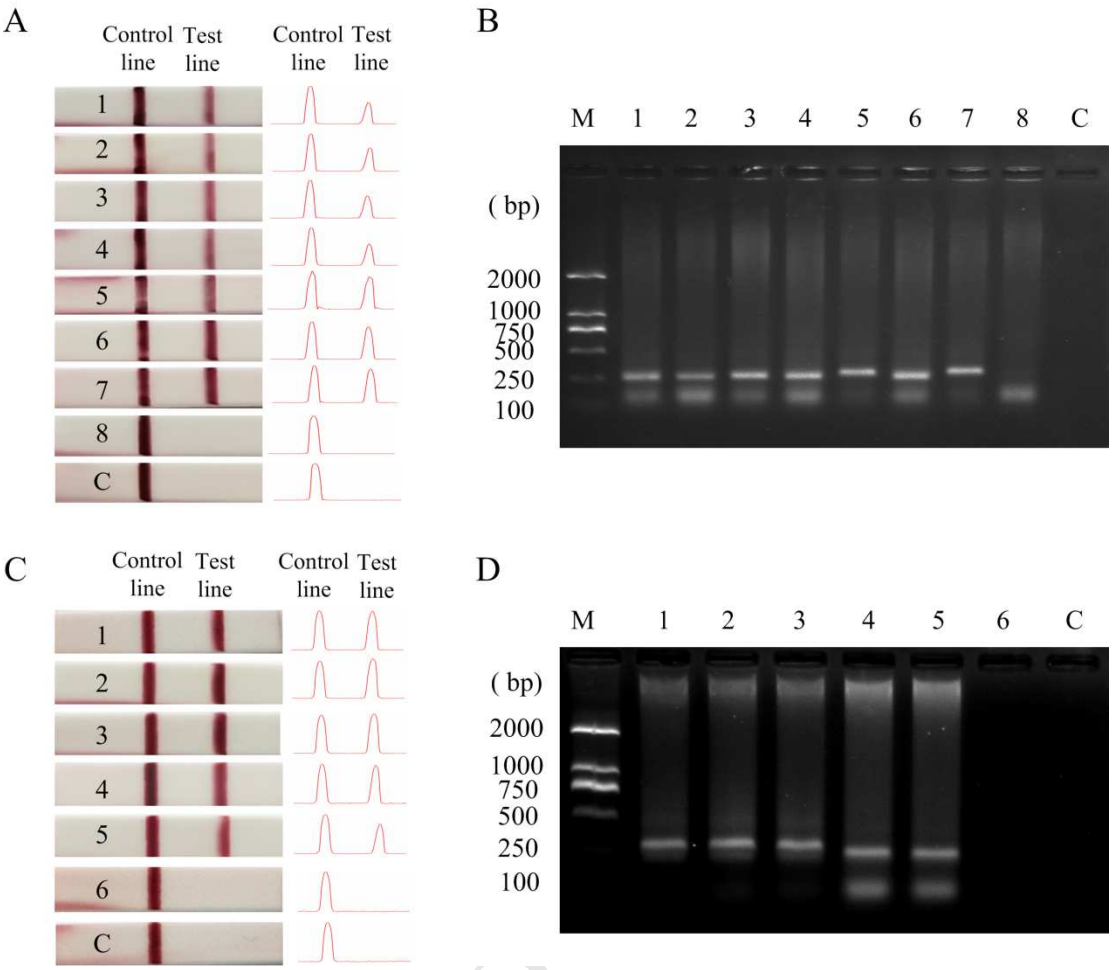
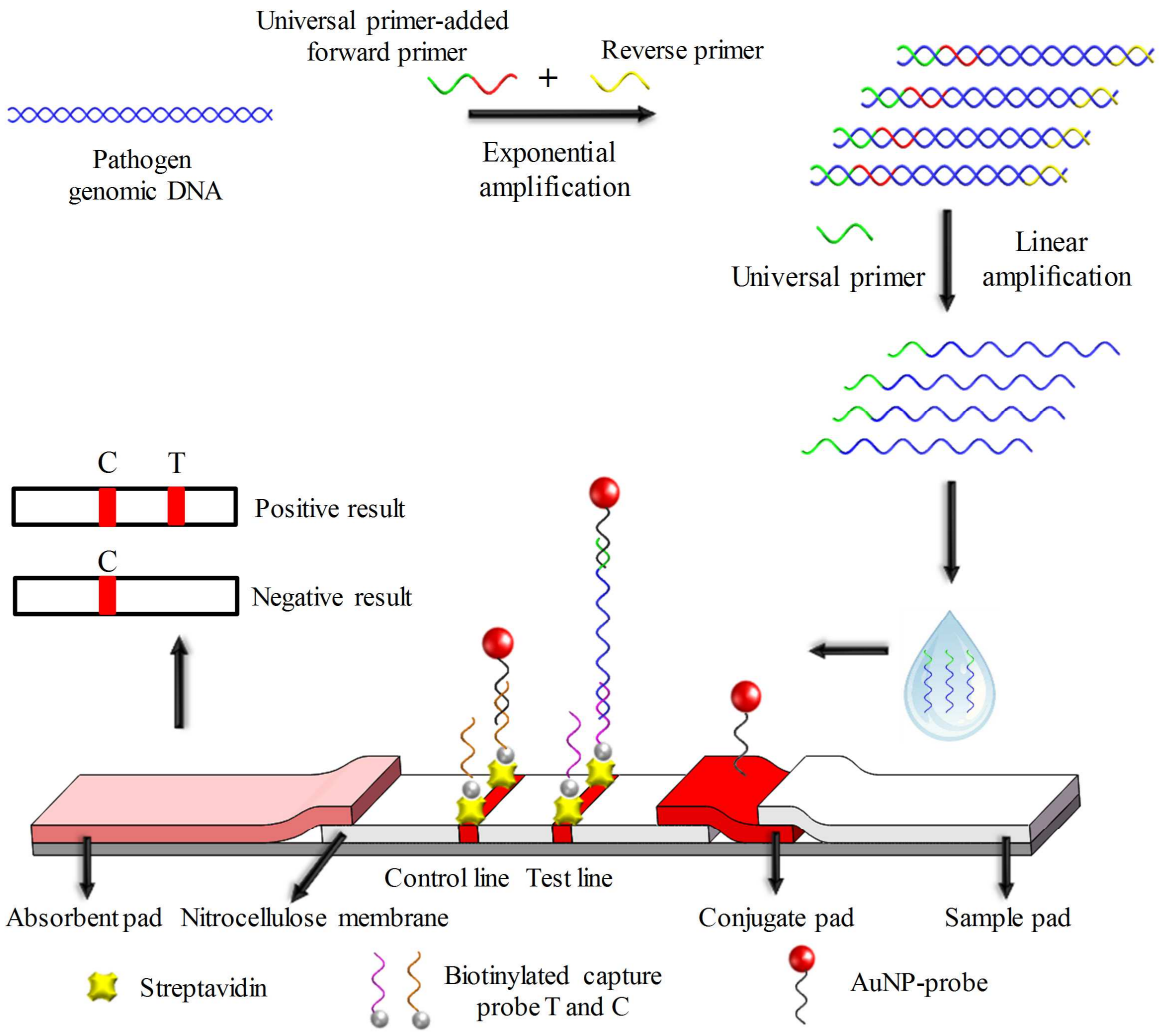
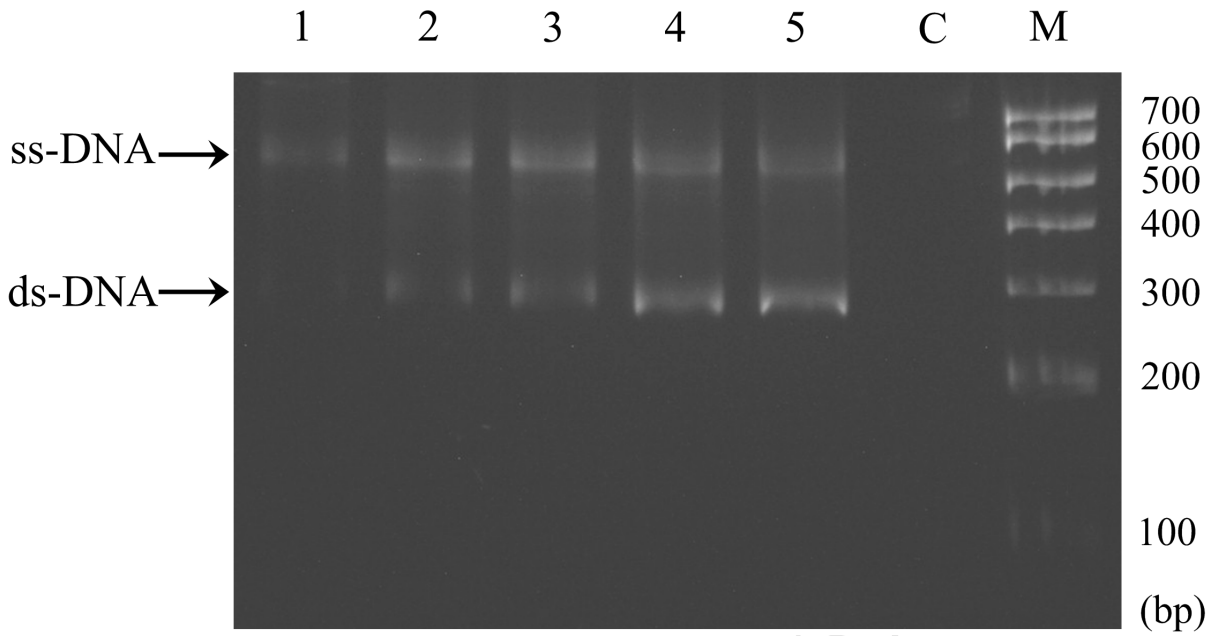


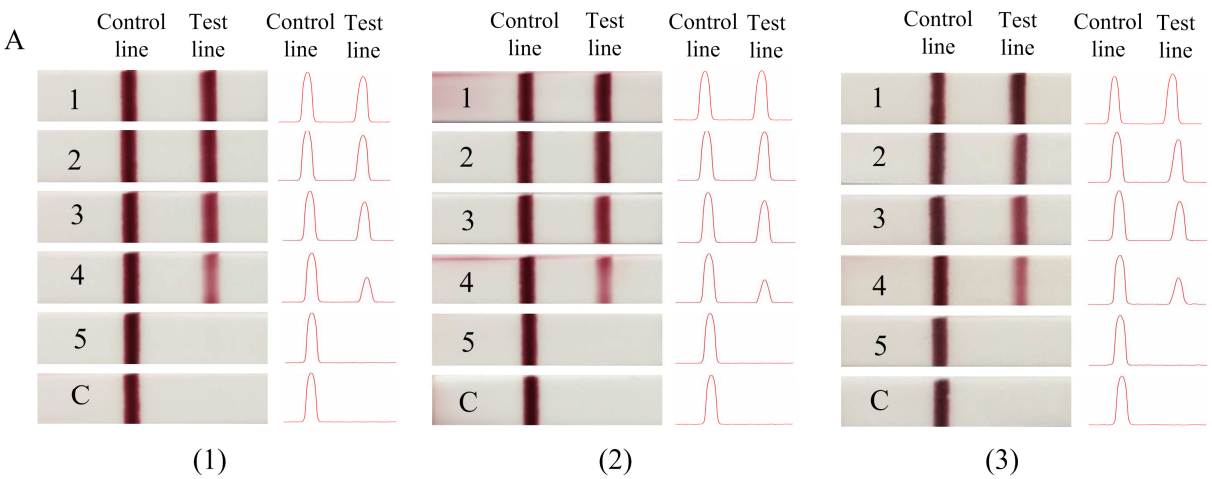
Figure 5

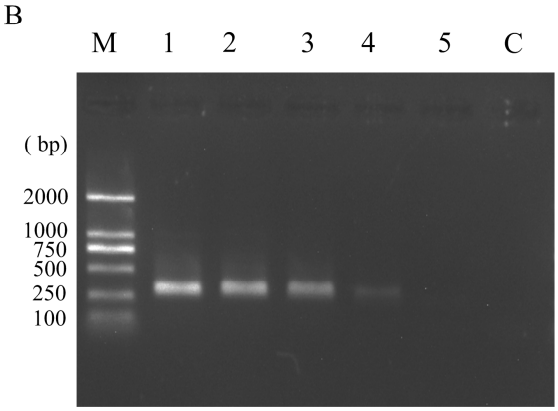
Table 1 Oligonucleotide Sequences

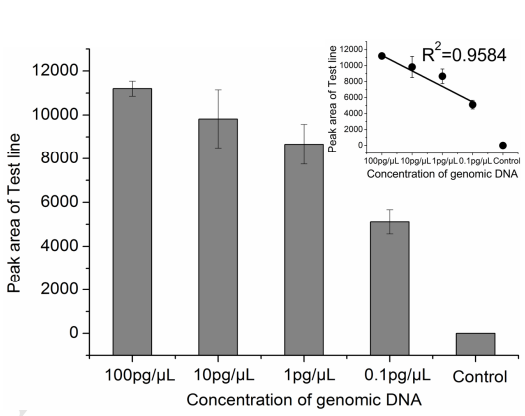
Oligonucleotide	Sequence(5'-3')
PiO8-F	AAGATGATGTTGGATGATTG
PiO8-U-F	*CCAATCCAATCCAATCCCCCC*AAGATGATGTTGGATGATTG
PiO8-R	TGCCTGATTTCTACCTTCT
Universal primer	CCAATCCAATCCAATCCCCCC
Thiolated detection probe	GGGATTGGATTGGATTGGTTTTTTTTTTTTTT-C6-SH
Biotinylated capture probe C	CCAATCCAATCCAATCCCTTTTTTTTTTTT-Biotin
Biotinylated capture probe T	Biotin-TTTTTTTTTTTTTGCCTGATTTCTACCTTCT

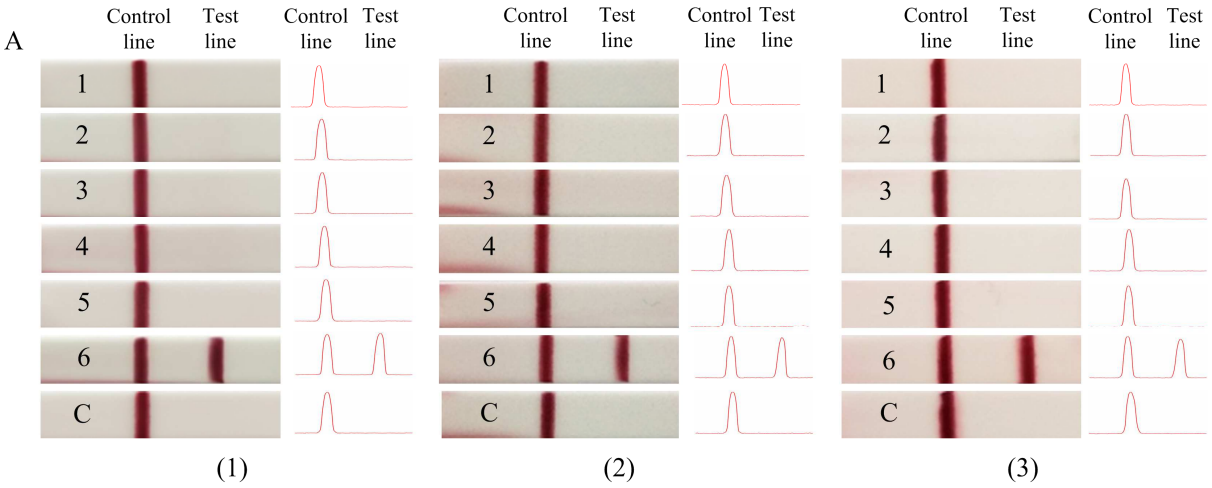


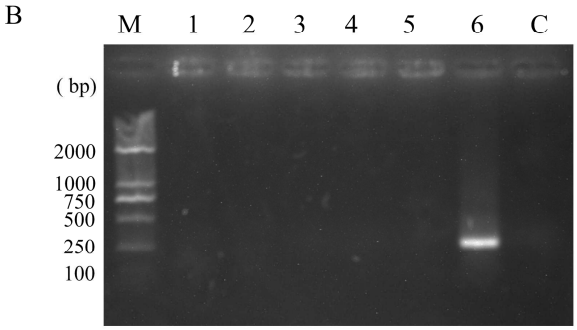


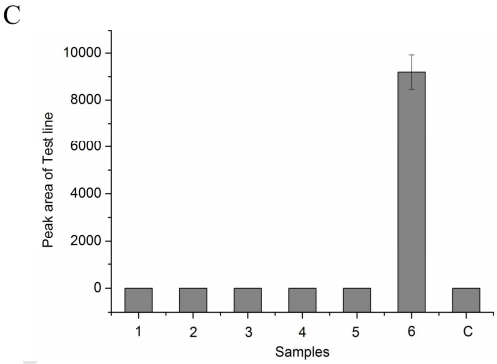












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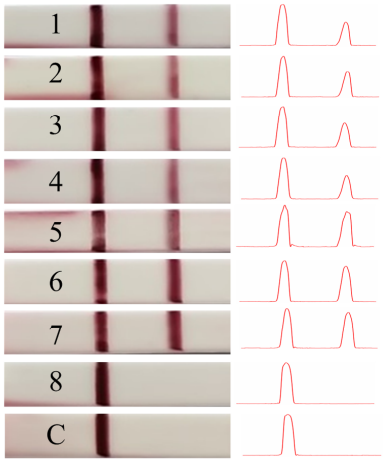

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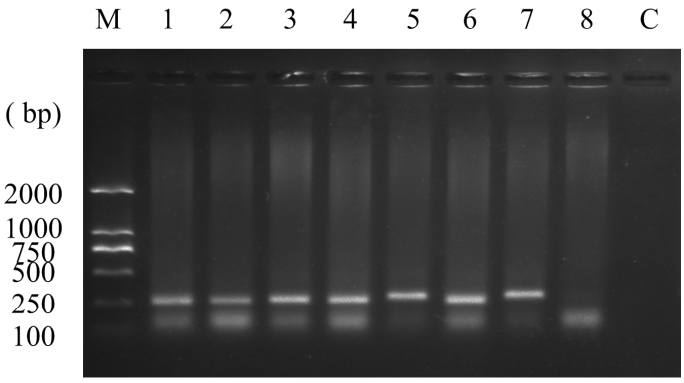

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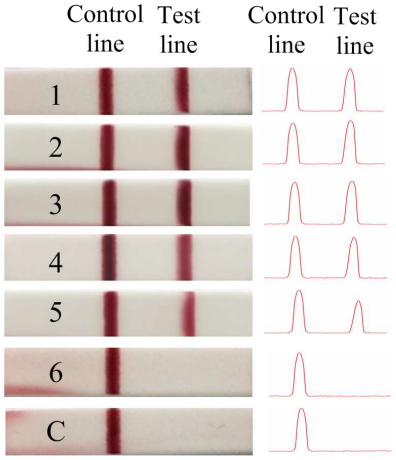

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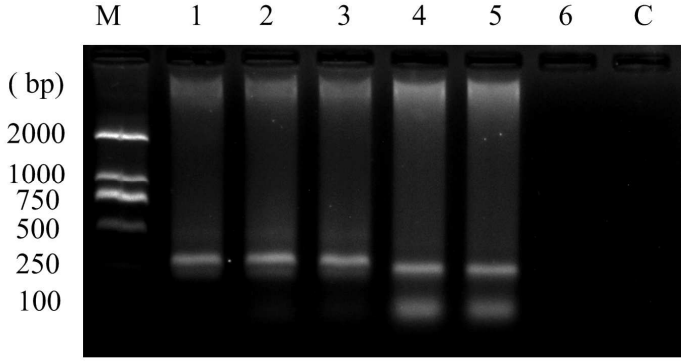

Highlights:

- A gold nanoparticle-based lateral flow biosensor for visual detection of *Phytophthora infestans* has been developed.
- The results of polyacrylamide gel electrophoresis confirmed the presence of the desired single-stranded DNA products.
- The specificity of the biosensor was confirmed by detecting three other *Phytophthora* species and two pathogenic fungi.
- The biosensor was used to detect infected leaf and stem samples.