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Ultrasensitive fluorescent biosensor for detecting CaMV 35S promoter with proximity extension mediated multiple cascade strand displacement amplification and CRISPR/Cpf 1

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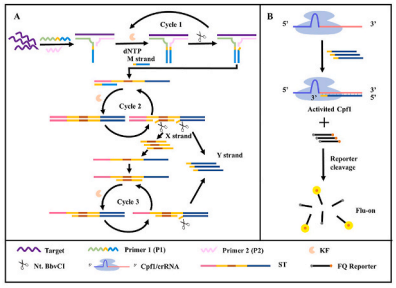
HIGHLIGHTS

- A novel sensing strategy (PE-MC/SDA-CRISPR/Cpf1) was constructed for the detection of genetically modified organisms.
- The combination of multiple cascade SDA and CRISPR/Cas12a achieved effective signal amplification.
- Three-way junction, bases mismatch and terminal modification greatly reduced the background noise.
- The high sensitivity was realized with the limit of detection down to 14.4 fM.
- This work held great application potential in food safety and provided a universal platform for other nucleic acid assays.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel fluorescent biosensor was proposed for detecting the CaMV 35S promoter in genetically modified organisms (GMOs). It was based on a proximity extension mediated multiple cascade strand displacement amplification connected with CRISPR/Cpf 1 (termed PE-MC/SDA-CRISPR/Cpf1). In this protocol, the CaMV 35S was recognized by proximity reaction in the presence of two adjacent primer probes. The proximity extension further triggered the multiple cascade strand displacement amplification (MC/SDA), generating a mass of ssDNA. The products compelled the *trans*-cleavage activity of CRISPR/Cpf 1, so as to cleave nearby ssDNA-FQ reporters and generate a strong fluorescent signal. The ingenious three-link combination design allowed the CaMV 35S a low background interference. And the MC/SDA combined with CRISPR/Cpf 1 dramatically improved the

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detection sensitivity. Under optimized conditions, the detection linear range of ultrasensitive fluorescent biosensor for CaMV 35S was from 50 fM to10 pM and 10 pM–500 pM, along with the limit of detection (LOD) down to 14.4 fM. The sensing platform also had excellent performance in the assay of selectivity and real samples. Therefore, the method earned great application potential for transgenic crops.

1. Introduction

At present, transgenic technology has gained widespread application in many fields such as agriculture, medicine, life science research and so on. Genetically modified organisms (GMOs) increase the yield of crops, improve their strong stress resistance and high nutritional value [1,2], etc. Despite the huge benefits GMOs produced, they are still controversial in terms of human health and ecological security [3–5]. In order to protect the right of consumers, many countries and organizations have strengthened their management of the cultivation and trade of GMOs and stipulated that GMO products must be labeled when they exceed a certain amount of detection [6,7]. Especially in some European countries, the public acceptance of GMOs is very low, and the relevant laws and regulations are more stringent [8]. Therefore, rapid, efficient and specific detection of GMOs is of great necessity and urgency.

The detection technology of GMOs is mainly referred to two aspects: the protein level and nucleic acid level. Protein-level detection includes two-dimensional electrophoresis [9], western hybridization [10], enzyme-linked immunosorbent assay (ELISA) [11], and dipstick strip method [12]. Although these methods have the virtues of low cost, high specificity and rapidity, there are still some limitations. When determining the expressed protein of its target gene, high temperature will affect the transcription and expression process, resulting in false positives and poor sensitivity [13,14]. Fortunately, the nucleic acid-based detection method has attracted wide attention due to its high stability and versatility [15]. It is also known as a direct detection method to identify the genetically modified components by detecting specific DNA fragments of samples. CaMV 35S promoter is a characteristic element of the transgenic process of cauliflower Mosaic virus, which has been found in almost all GMOs. Therefore, many researchers are keen to detect the promoter to identify transgenic ingredients [16]. In the widely reported methods, polymerase chain reaction (PCR) is the conventional nucleic acid-based detection method, which is considered as a fundamental tool due to its high sensitivity, reliability, and relative accuracy. But it is suffered from the sophisticated operation and high equipment costs [17,18]. To solve these problems, many innovative nucleic acid isothermal amplification methods have emerged. Compared with PCR technology, the greatest advantage and feature of isothermal amplification technology for nucleic acid examination is that it does not depend on the change of temperature. Moreover, it is free of the expensive and bulky instrument, and easily realizes the possibility of handy detection. Based on these characteristics, this kind of technology is available on a variety of occasions [19]. The common isothermal amplification techniques include recombinant polymerase amplification (RPA) [20–23], strand displacement amplification (SDA) [24–26], hybridization chain reaction (HCR) [27–30], catalytic hairpin assembly technology (CHA) [31–33], exponential amplification reaction (EXPAR) [34,35], rolling loop amplification reaction (RCA) [36,37], DNA walker [38] and loop-mediated isothermal amplification reaction (LAMP) [39]. Among these ways, SDA is a powerful tool to magnify the signal of nucleic acid. For typical SDA reactions, target-mediated hybridization events initiate primer sequences to extend along the pre-designed template. At the same time, the pre-hybridization target or another generated DNA fragment can be replaced to trigger subsequent cycles of the SDA reaction [40]. In addition, the cascade amplification strategy also exhibits great advantages and potential, which are inflected in the fact that the products of upstream reactions act as triggers for downstream reactions, and these modules are well-integrated. Compared with the exponential amplification strategy, cascade amplification can not only produce higher signal intensity, but also keep lower background signal [41,42].

In recent years, clustered regularly interspaced short palindromic repeats (CRISPR) and their associated proteins (Cas), entitled CRISPR/Cas systems, are found to constitute an immune defense mechanism of microorganisms [43–45], which have been revealed its great potential in biomolecular detection, particularly in nucleic acid detection [46]. And Cas9, Cas13a and Cas12a are the most frequently used Cas proteins in detection. Cas9, the most common Cas effector protein, is often viewed as a gene-editing tool in combination with CRISPR technology [47,48], which offers unprecedented opportunities for basic research in the life sciences and the treatment of diseases. Another application is in the detection field. It works through specifically recognizing and cleaving dsDNA under the guidance of sgRNA by targeting a short specific sequence called Protospacer Adjacent Motif (PAM) [49]. Then the result of final detection can be viewed in two ways including the digest of the input target to initiate detection or cutting the output sequence to be terminal readout. CRISPR/Cas13a is only equipped with ssRNA-targeting activity, whose target ssRNA needs to function with non-G-PFS sites as the auxiliary (A, U, or C) [50]. When crRNA binds to the target, the accessory cleavaging activity of CRISPR/Cas13a is motivated and non-specifically to cleave the ssRNA reporter in the system [51]. CRISPR/Cas12a system aka CRISPR/Cpf1, composed of crRNA and Cas12a effector protein. In the presence of targets, target DNA and crRNA are closely complementary and paired to unlock the activity of Cas enzyme to ssDNA signal molecules, thereby releasing intensive fluorescence signals [52]. In comparison with Cas9, Cpf1 is in no need of tracrRNA, and the off-target efficiency is lower to match ssDNA without the PAM domain [53,54]. Different from Cas13a, there is great recognition capacity of Cpf1 for dsDNA and ssDNA. Meanwhile, the fluorescence reporter molecules are single-stranded DNA, which is more stable than RNA and will not be degraded by RNA enzymes in the environment, resulting in lower false positives and more convenient operation [55]. Aiming to analyze low-abundance nucleic acids, CRISPR/Cpf1 is often utilized in conjunction with the nucleic acid amplification method mentioned above, reaching the detection limit at a femtomolar level [56].

In view of the above analysis, we proposed an ultra-sensitive biosensor for CaMV 35S detection based on multiple cascade strand displacement amplification reactions and CRISPR/Cpf1 (PE-MC/SDA-CRISPR/Cpf1). First, we introduced template 1 (P1) and extended primers (P2). In the presence of CaMV 35S, the three nucleic acid chains were assembled into a stable three-stranded structure via proximity binding. There was an Nt. BbvCI digestion site designed on P1, and the action of KF polymerase and nicking enzyme Nt. BbvCI triggered the first cyclic amplification, producing the intermediate named M. The amplified chains were used as primers to bind to template 2 (ST) containing two Nt. BbvCI sites and stimulated the cascade amplification of SDA. Accordingly, massive ssDNA activators were produced, which were complementary paired with crRNA to unlock the accessory activity of CRISPR/Cpf1, arbitrarily cutting FQ fluorescence reporter molecules, and then collecting and processing the fluorescence signal. Consequently, the outstanding linear fit and a low LOD were obtained. In addition, the biosensor had a good response in the detection of real transgenic samples, indicating that the proposed biosensor achieved ultra-sensitive detection of GMOs.

2. Experimental sections

2.1. Reagents and instruments

All oligonucleotide sequences used in this experiment were purchased from Sangon Biotech (Shanghai, China) and purified by high-performance liquid chromatography. The detailed information was seen in Table S1. AsCpf 1 (1 μg μL^{-1}, 50 μg), Klenow Fragment (5000 U mL^{-1}), 10 × NEB buffer 2.0, Nt. BbvCI (10 000 U mL^{-1}), 10 × Cutsmart buffer and HiScribe T7 Quick High Yield RNA Synthesis were all obtained from New England Biotechnology Co., Ltd (Beijing, China). The Plant Genomic DNA Kit was available from TianGen Biotech (Beijing) CO., Ltd. The mixture of deoxy-ribonucleoside triphosphate (dNTP, 25 mM), DEPC treated water, double distilled water, 5 × TBE solution, N, N, N′, N′-Tetramethylethylenediamine (TEMED), 30% acrylamide and bis-acrylamide solution (29:1), 10% ammonium persulphate (APS), 4S GelRed, 6 × loading buffer and DNA Marker (25–500 bp) were ordered from Sangon Biotech (Shanghai, China). Denaturation and annealing of nucleic acid sequences were carried out on the PCR apparatus. The fluorescence spectra were detected at excitation wavelengths of 500 nm and measuring the emission at 556 nm for HEX on an LS-55 Spectro photo fluorometer (P. E. America). During the process of measurement, the slits for excitation and emission were set at 12 nm and 9 nm, respectively. The gel was visualized by the Azure Biosystems C150 (America).

2.2. Synthesis of crRNA

CrRNA was synthesized using the method previously reported by our research group [57]. Firstly, 2 μL of the template (100 μM), 2 μL of 100 μM T7 promoter and 16 μL of ddH_2O were added into a 200 μL PCR tube for denaturation at 95 °C for 5 min and cooled down to room temperature slowly. Then 2 μL of RNA polymerase buffer, 10 μL of NTP mix (100 μM), and 2 μL of T7 polymerase were dropped and kept at 37 °C for 16 h. Afterwards, 3 μL of DNase I buffer and 2 μL of DNase I were added and placed at 37 °C for 2 h to dissolve the DNA template. Finally, RNA was purified by the miRcutergets miRNA Isolation Kit and dissolved in DEPC water. The concentration of crRNA was measured by NanoDrop 2000C and then stored at −20 °C for subsequent experiments. The final crRNA was identified by gel electrophoresis analysis.

2.3. Procedure for CaMV 35S detection

The first step was the formation of tristranded bodies. The reaction system (10 μL) included 2 μL of 10 × NEB buffer 2.0, 2 μL of different concentrations of target CaMV 35S, 2 μL of primer 1 (P1, 100 nM) and 2 μL of primer 2 (P2, 100 nM). Then the mixture was annealed at 95 °C for 5 min, slowly decreased to 46 °C and held for 10 min to form a stable three-chain structure, followed by the synthesis of a large number of activators, 1 U Klenow Fragment (KF), 2 U Nt. BbvCI, 2 μL of Cutsmart buffer (10 ×), 2 μL of SDA template (ST, 200 nM), 1 μL of dNTP (25 mM), as well as a certain amount of RNase-free water were put to achieve a total volume of 20 μL, lightly shaken and held at 37 °C for 120 min.

Next, a signal generation experiment was prepared for the volume of 20 μL including 4 μL of the product, 2 μL of NEB Buffer 2.1 (10 ×), 2 μL 0.02 μg Cpf 1 and 2 μL crRNA (10 μM) as well as 1 μL ssDNA reporter were successively added and supplemented with RNase-free water. All of these were mixed evenly and maintained at 37 °C for 40 min. Then 80 μL ddH_2O was mixed with the reaction liquid above and transferred the mixture immediately to a micro colorimetric dish for fluorescence signal detection under excitation light of 500 nM and emission light of 556 nM.

2.4. Assay of real samples

Sample leaves were treated with liquid nitrogen and ground into powder under freezing conditions, the plant genomic DNA kit was taken to extract wild-type and transgenic plant genomes, which were used as objects for CaMV 35S fluorescence detection and stored at −20 °C before use. 2 μL of wild-type plant genome was used to replace synthetic CaMV 35S as the control group, and transgenic plant genome DNA was used to replace CaMV 35S as the experimental group. The other operating steps were the same as the experimental group in 2.3.

2.5. Gel electrophoresis verification analysis

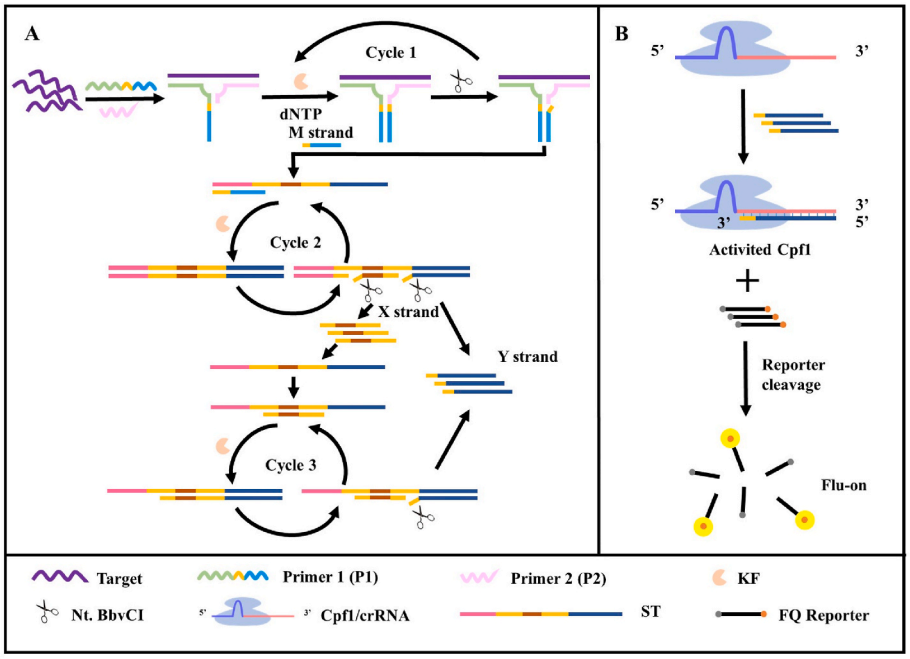
The feasibility of the assay was investigated by 10% polyacrylamide gel electrophoresis (PAGE). The gel was immersed in 1 × TBE buffer and run for 60 min at 120 V. After dyeing 15 min, gel result images were captured on Azure Biosystems C150 (America).

3. Results and discussion

3.1. Detection principle of CaMV 35S

On the basis of intrinsic merits of CRISPR/Cpf1 coupled with SDA reaction, a platform termed as Proximity Extension Mediated Multiple Cascade Strand Displacement Amplification and CRISPR/Cpf 1 (PE-MC/SDA-CRISPR/Cpf1) was devised for detecting trace CaMV 35S promoter. In Scheme 1A, 7 nt complementary sequences is firstly designed for hybridization of two primer probes P1 and P2. In the absence of CaMV 35S, they cannot reliably pair up with each other. In contrast, only the targets can recruit two primers to form a three-way junction through adjacent hybridization and trigger the polymerization of KF. Then, a restriction site (orange zone) introduced to P1 is cut by Nt. BbvCI, so the first cycle reaction is enabled, which brought the dissociation of a short single-stranded intermediate M (5′-TCAGCGCACACCTTGAACTTA-3′) and Y strands. After that, the obtained M chain combines with the SDA template (ST) to promote the next extension nicking reaction, implementing the second cycle reaction under the simultaneous action. The ST (SDA template) is designed with two special domains of nicking endonuclease (Nt. BbvCI). the M chain mentioned above can combine with it as the primer to promote the next extension nicking reaction, implementing the second cycle reaction under the simultaneous action. Consequently, two short ssDNA chains (X and Y strands) are dissociated. The X strands (5′-TCAGCCCTTCC-3′) can further be employed as primers to bind to ST and facilitate the next round reaction, which generated numerous Y strands (5′-CCTTTTTTTTTTTTTTTTTTTTTT-3′) that can act as activators of CRISPR/Cpf1. As seen in Scheme 1B, the crRNA we designed contains a 22 bp poly-A sequence, which can be perfectly paired with Y chains. In this case, the non-specific ssDNase activity of CRISPR/Cpf 1 is exerted to degrade the ambient fluorescence probes, which causes the terminal modification of the fluorophore and the quenching agent of the FQ signal molecule to separate from each other, releasing a strong fluorescence signal.

To further lift the detection sensitivity, eliminating the background interference caused by self-extension and nonspecific amplification of the template, we deliberately engineered two extra “T" bases on P2 primer at the three-way junction that were not paired with the other two chains. Only in the presence of the target, an additional raised ring was formed at the nucleotide junction, which enhanced the thermodynamic stability [58,59] and made P1 and P2 more difficult to combine, reducing the background signal. Conversely, P1 could not be well-matched with P2 without targets. Besides, an amino group at the 3′-end of P1 and ST was modified to make their 3’ -tails blunt and be in a stable state, which effectively impeded the self-extension and enzymatic hydrolysis, minimizing the background signal to a great extent. Through the above scheme design, the sensitive and effective detection of CaMV 35S was enabled.



Scheme 1. The principle of Proximity Extension Mediated Multiple Cascade Strand Displacement Amplification and CRISPR/Cpf 1 for CaMV 35S detection.

3.2. Feasibility of the CaMV 35S detection

The fluorescence test and gel electrophoresis were used to verify the feasibility of the methodology we described. As displayed in Fig. 1A, when the concentration of CaMV 35S is 1×10^{-10} M, the fluorescence signal of the experimental group is strong whereas the control group owns a low fluorescent value, illustrating that the biosensor has a strong fluorescence response to the target and CaMV 35S can be effectively detected through this method. To further determine that the activator can only be generated in the presence of the target, a 10% polyacrylamide gel electrophoresis (PAGE) analysis was conducted. Fig. 1B shows that samples subjected to channels successively from left to right are DNA marker, CaMV 35S, P1, P2, the three-chain compound (formed by CaMV 35S, P1 and P2), ST, experimental group and control group. In lane 4, there is a bright band at the top, which proves that the three-chain complex has successfully been made with a relatively larger molecular weight. Meanwhile, a distinct band consistent with lane 4 is found in the experimental group (lane 6). Next, the proximity extension mediated multiple cascade strand displacement amplification is initiated by KF and Nt. BbvCI, so as to amplify the product information. The middle band that appears in lane 6 is the combination of primer and amplification template. On the contrary, these bands are not observed in lane 7. Therefore, the PAGE result suggests that the amplicon is successfully generated in the MC/SDA step. Then the feasibility of the scheme is fully verified by fluorescence experiment and PAGE.

3.3. Optimization of detection conditions

In order to explore ideal working conditions, it is necessary to optimize the two essential factors, the amplification time of SDA and the

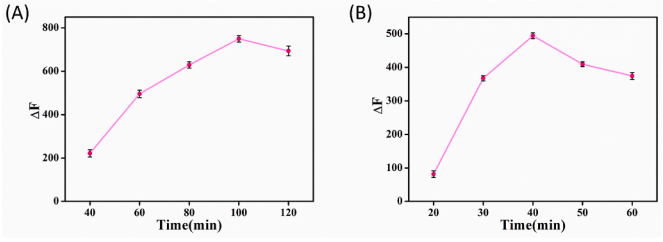


Fig. 2. Optimization for (A) MC/SDA reaction time and (B) CRISPR/Cpf 1 shearing time (Total concentration of 1×10^{-10} M). Error bars were obtained from triplicated independent experiments.

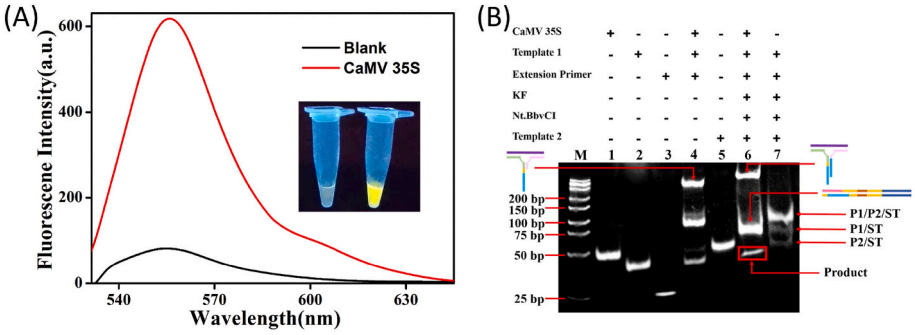


Fig. 1. Feasibility of the proposed method. (A) MC/SDA-mediated fluorescence spectra based on CRISPR/Cpf 1 for CaMV 35S detection; (B) PAGE verification of feasibility. (M: DNA marker. Lane1: CaMV 35S. Lane 2: P1. Lane 3: P2. Lane 4: three-chain structure formed by CaMV 35S–P1–P2. Lane 5: ST. Lane 6: experimental group. Lane 7: control group).

cleavage time of CRISPR/Cpf1. Firstly, the shearing time was fixed at 40 min to study the amplification time of MC/SDA. Fig. 2A reveals that the ΔF (ΔF is viewed as the difference of fluorescence values between the experimental group and control group) is gradually strengthened with the increase of reaction time beginning at 40 min and reaching its peak at 100 min. Then the fluorescence difference is decreased when amplification time proceeds, which may be due to increased nonspecific amplification over time and results in the augment of background value. Therefore, 100 min is selected as the ideal time for MC/SDA reaction. Next, we keep the amplification time of MC/SDA at 100 min to optimize the cleavage time of CRISPR/Cpf 1. As time extends, the fluorescence signal increases to the maximum at 40 min. However, the fluorescence intensity descends as the cutting time proceeds (Fig. 2B). This is probably because the non-specific shearing of CRISPR/Cpf 1 also goes up, causing the growth of background signal values. Hence, 40 min is marked as proper responsive time.

3.4. Sensitivity of CaMV 35S detection

The analytical performance of the proposed biosensor was evaluated at different concentrations of CaMV 35S under optimal conditions. As depicted in Fig. 3A, there is a positive correlation between the concentration of CaMV 35S and its fluorescence response, showing a stronger fluorescence signal related to a higher target concentration. In Fig. 3B, an obvious linear relationship that can be divided into two sections is seen within the ΔF and the logarithm of CaMV 35S concentration. In the scope of 50 fM-10 pM, the fitted linear regression equation is: $F_1 = 68.05$ lgC +129.08 ($R^2 = 0.999$). In the range from 10 pM to 500 pM, the linear equation is calculated as $F_2 = 318.77$ lgC-137.11 ($R^2 = 0.999$). The final detection limit of this method can theoretically be as low as 14.4 fM according to the 3σ rule. Compared with other methods (Table 1), the presented biosensor attains a lower limit of detection and a wider detection range. The several reasons for excellent performances are as follows: (1) the proximity extension of the three-way junction, the two T bases mismatch of P1 and P2 at the link of three forks as well as the terminal modification of P1 and ST vastly whittles the background value; (2) multiple cascade strand displacement amplification exerts a large amount of single-strand activators and immensely amplifies the signal; (3) the specific recognition of CRISPR/Cpf1 improves the specificity of the target and its unique *trans*-cleavage activity results in a further magnification of the fluorescent data. In view of the above, superior detection performance is obtained.

3.5. Specificity and reproducibility analysis

To further explore the specificity of the scheme, several random ssDNA with basically the same length were selected for anti-interference detection at the concentration of 1×10^{-10} M. The non-target DNA groups execute negligible fluorescence response while the fluorescence signal value gives a sudden rise with the existence of CaMV 35S (Fig. 4A). Fig. 4B reveals the net fluorescence value and the specificity of the scheme is further explained. The above analysis means that a stable triplex be built only with the help of CaMV 35S, which can motivate the following multiple amplification process. And the amplified products further stimulate the *trans*-cleavage activity of CRISPR/Cas12a to emit the fluorescence signal. Briefly, the constructed biosensor can detect the target specifically and has a good anti-interference ability.

Moreover, reproducibility is also an important indicator to characterize the platform performance, we measured the fluorescence spectra of the target at high concentration (1×10^{-10} M) for 5 consecutive times. The obtained RSD is 0.59% (Fig. 5A and Fig. 5B), and a lower concentration (2×10^{-13} M) was also detected in Fig. S2 with the RSD of 2.24%, illustrating the excellent reproducibility of this biosensor.

Table 1
Comparison between PE-MC/SDA-CRISPR/Cpf1 and other CaMV 35S detection methods.

Method	Platform	Linear range	LOD	Ref.
LAMP and CRISPR/Cpf1	Fluorescence	0.2–800 nM	0.07 nM	[60]
Dual-emission cluster of Ag atoms	Fluorescence	10–1000 nM	1.5 nM	[61]
QDs and MWCNTs@GONRs	Fluorescence	1.2–900 nM	0.35 nM	[62]
Exfoliated Graphene Oxide and Gold NanoUrchins	Electrochemistry	40-1100 fM	13.0 fM	[63]
CHA and nanocomposites	SPR	0.5–500 nM	12 pM	[64]
Exonuclease III and hemin/G-quadruplex DNAzyme amplification	Colorimetry	0.5–500 nM	0.23 nM	[65]
SiO_2@CdTe quantum dots core-shell nanoparticle	PEC	0.1 pM-0.5 nM	0.05 pM	[66]
Ag/TiO_2/N-GNR	PEC	0.01–500 nM	3 pM	[67]
QDs-DNA nano-probe	PRET	12–300 nM	4.03 nM	[68]
Phosphorescent quantum dots (QDs)	Fluorescence	10–200 nM	0.48 nM	[69]
Multiple Cascade SDA and CRISPR/Cpf1	Fluorescence	50 fM-10 pM 10–500 pM	14.4 fM	This work

3.6. Detection in real samples

The practical application of this sensor is our ultimate goal. For examining its reliability and adaptability, we finally determined to extract the genomic DNA of wild-type and transgenic poplar for a real sample test. The obtained wild-type DNA and transgenic DNA

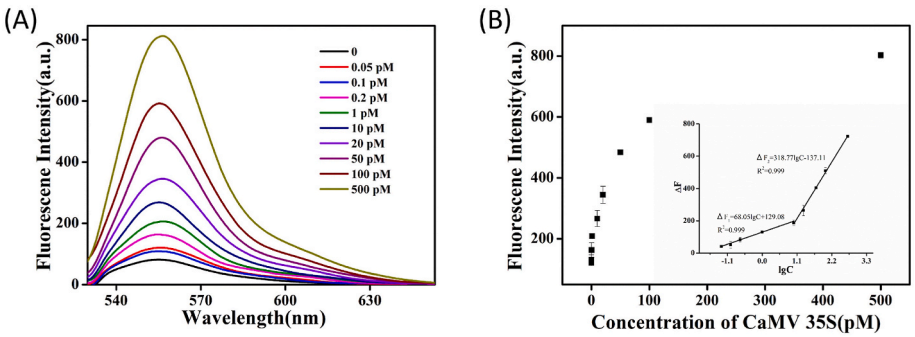


Fig. 3. (A) Fluorescence spectra of CaMV 35S at different concentrations (500 pM, 100 pM, 50 pM, 20 pM, 10 pM, 1 pM, 0.2 pM, 0.1 pM, 0.05 pM and 0 (blank)). (B) Fitting curves of fluorescence intensity at 556 nM and logarithms of fluorescence intensity and detection concentration at different concentrations of CaMV 35S. Error bars were obtained from triplicated independent experiments.

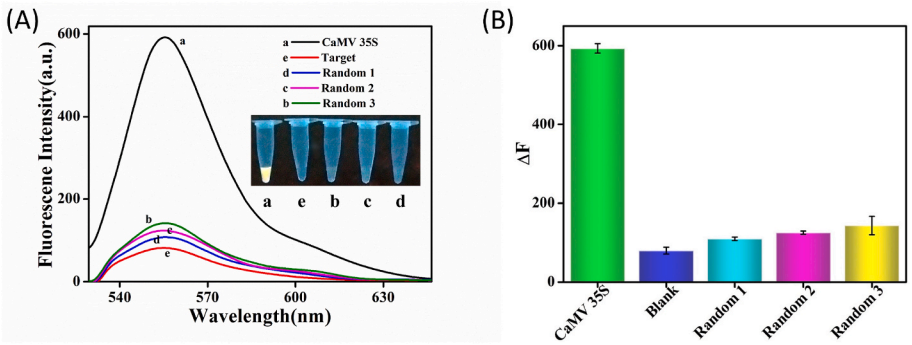


Fig. 4. (A) Fluorescence spectra of different DNA. (a: CaMV 35S; b: Blank; c: Random 1; d: Random 2; e: Random 3. Total concentration of 1×10^{-10} M) (B) and their corresponding fluorescence signal values. Error bars were obtained from triplicated independent experiments.

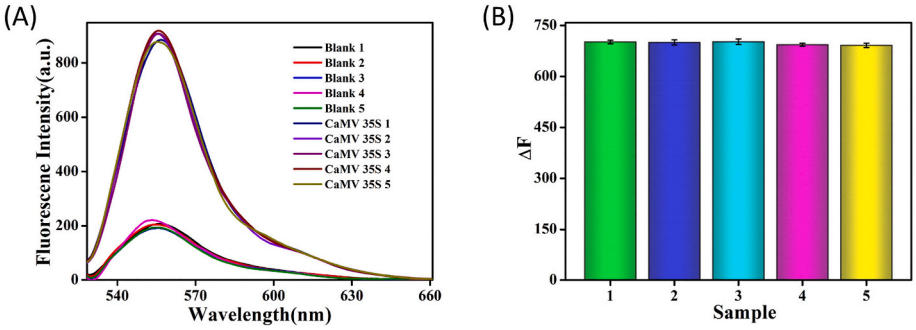


Fig. 5. (A) Fluorescence spectra of reproducibility for blank and experimental groups (Total concentration of 5×10^{-10} M) (B) and their corresponding fluorescence signal values. Error bars were obtained from triplicated independent experiments.

concentrations were all diluted to 400 ng μL^{-1}, which was used as the final concentrations. As exhibited in Fig. 6A and Fig. 6B, the fluorescence signal of the transgenic experimental group is distinctly higher than that of the wild-type control group, and there is little difference in the fluorescence signal between the control and the blank, which shows that the biosensor has satisfactory detection capability for real samples.

4. Conclusions

In conclusion, a novel fluorescent biosensor based on PE-MC/SDA-CRISPR/Cpf1 was established to achieve ultra-sensitive detection of transgenic landmark promoter CaMV 35S. Several innovations were summarized below. Firstly, the contribution of the target to proximity extension, the base mismatch of P1 and P2 at the junction of the complex together with the terminal modification of P1 and ST greatly weakened the background signal. Secondly, three cycles of multiple cascade strand displacement amplification induced a large amount of single-strand activator and immensely amplified the signal. Thirdly, the specific recognition of CRISPR/Cpf1 vastly improved the specificity of the target and its unique *trans*-acting deoxyribonuclease activity led to the fluorescent data with a further magnification. As a result, the method worked with a satisfactory linear detection range from 50 fM to 10 pM and 10 pM–500 pM and a low detection limit down to 14.4 fM. Compared to most PCR-based and fluorescence-based techniques, distinct edges in sensitivity, selectivity and practical utility were also created, eminently discriminating genetically modified ingredients. It was worth noting that the method also had great application prospects in the field of food safety. More importantly, due to its simplicity, the fungibility of each nucleic acid sequence, the programmability of crRNA and enhancement of Cpf1, our strategy can be regarded as a general sensing platform serving a wider range of nucleic acid detection.

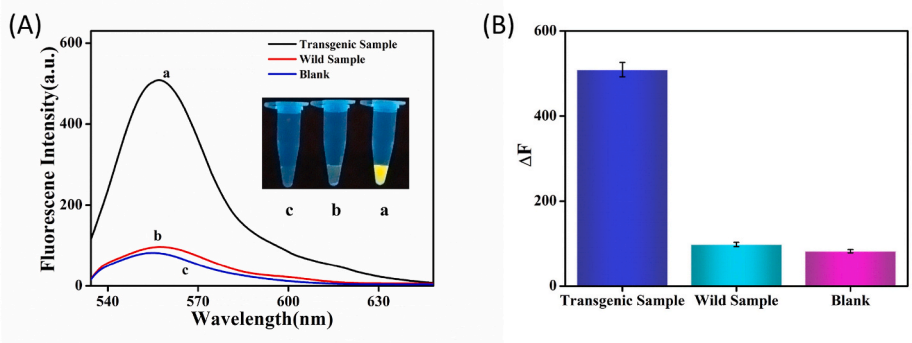


Fig. 6. (A) Fluorescence spectra of transgenic samples (curve a), wild-type samples (curve b), and blank group (curve c) (B) and their corresponding fluorescence signal values. Error bars were obtained from triplicated independent experiments.

CRediT authorship contribution statement

Yin Liu: Methodology, Experiments, Formal analysis, Writing – original draft, Writing – review & editing. **Shiying Zhou:** Methodology, Experiments, Formal analysis, Validation. **Human Sun:** Methodology. **Jiangbo Dong:** Validation. **Liyuan Deng:** Experiments. **Na Qi:** Project administration. **Yongzhong Wang:** Writing – review & editing. **Danqun Huo:** Guidance, Fund support, Instruments and Eequipment for experiments. **Changjun Hou:** Writing – review & editing, Writing guidance, Project administration, Funding acquisition, All authors who have made substantial contributions to the work are listed in the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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