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End-point dual specific detection of nucleic acids using CRISPR/Cas12a based portable biosensor

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ABSTRACT

A CRISPR/Cas12a based portable biosensor (Cas12a-PB) was developed to simultaneously visually detect *CaMV35S* promoter and *Lectin* gene from genetically modified (GM) soybean powders (Roundup Ready@). The Cas12a-PB, mainly made of polymethylmethacrylate (PMMA) and PMMA tape, has a connection structure, three channels and three detection chambers. The CRISPR/Cas12a detection reagents were preloaded in detection chambers and the reaction tube was connected to the connection structure by screw threads. After amplification, the amplicons were gone into three detection chambers by swinging the Cas12a-PB to conduct dual detection. Positive samples would produce green fluorescence while negative samples were black under the irradiation of 490 nm LED light. In this study, the Cas12a-PB successively combined with ordinary PCR, rapid PCR and loop-mediated isothermal amplification (LAMP) to achieve dual detection, which made detection process more convenient and portable. As low as 0.1% transgenic ingredients in soybean powders could be detected and the specificity of Cas12a-PB was confirmed with GM maize powders (MON810, GA21), GM soybean powders (DP305423), non-GM peanut and rice as targets. In the end, an amplification chamber combining with Cas12a-PB on a PMMA chip was further designed to eliminate the use of reaction tube and mineral oil, which made operation simpler. The established Cas12a-PB would provide a new reliable solution for multiple targets detection in clinic diagnostics, food safety, etc.

1. Introduction

Nucleic acids detection plays an important role in food safety, disease diagnosis and environmental monitoring (Kong et al., 2017; Roy et al., 2017; Zhou et al., 2018). Nucleic acids amplification technologies (NAAT) based detection methods are the most widely used to conduct nucleic acids assays because of their ability to amplify low copy nucleic acids into million-fold copies even from complex matrices (Dou et al., 2014, 2019b). Meanwhile, multiplex nucleic acids detection is becoming more and more important (Dou et al., 2017a, 2017b), especially in clinical diagnosis (Elnifro et al., 2000).

To date, multiple PCR amplicons can be detected by many methods, like electrophoresis (Lohmann et al., 1992; Ng et al., 2001), TaqMan probes (Weller et al., 2000; Geyer et al., 2012), melting curve analysis (Giglio et al., 2003; Sakalar and Abasiyanik, 2012), molecular beacon (Vet et al., 1999; Qian et al., 2018), lateral flow strip (Soo et al., 2006; Blazkova et al., 2011; Xu et al., 2014; Jauset-Rubio et al., 2018), etc. Most of these detection methods need rather sophisticated instruments. The detection with lateral flow strip may produce contamination of amplicon due to the opening of reaction vessel (Ang et al., 2012). Besides, for isothermal amplification reactions, it is also required to develop some simple and portable multiplex detection methods, like loop-mediated isothermal amplification (LAMP) (Li and Macdonald, 2015; Dou et al., 2019a).

CRISPR/Cas systems are gradually employed as novel biosensing platforms for ultrasensitive and specific nucleic acids detection by using the "collateral cleavage" activity of Cas protein (Gootenberg et al., 2017; Chen et al., 2018; Li et al., 2019a). And our group has developed a visual method coupled with the CRISPR/Cas12a system to specifically detect single DNA target (Qian et al., 2019; Wu et al., 2020a). In the CRISPR/Cas12a system, the guider RNA (gRNA) specifically combines with

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Abbreviations used

LAMP	Loop-mediated isothermal amplification
PCR	Polymerase chain reaction
GM	Genetically modified
CaMV35S	Cauliflower mosaic virus 35S
dNTP	Deoxyribonucleoside triphosphate
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeat

the target nucleic acids to form a ternary complex (Cas12a protein, gRNA and target nucleic acids). Then, the Cas12a protein would produce single-stranded DNase activity to cut the single-stranded DNA florescent probe and the florescent signal thus could be monitored (Chen et al., 2018). So far, very few articles have been reported to conduct multiplex nucleic acids detection using CRISPR/Cas systems (Bruch et al., 2019; Li et al., 2019b). And the established detection methods are relatively complicated and time-consuming, which limits the practical application for in-field and on-site detection (Gootenberg et al., 2018; Wang et al., 2019). Genetically modified (GM) crops are widely planted in the world and GM labeling is required in many countries. *CaMV35S* promoter is one of most used elements in GM soybean (Becker and Ulrich, 2018). To monitor the compliance of labeling regulation, it is important to establish a rapid, sensitive and specific detection method for *CaMV35S* promoter of GM soybean.

In this paper, a CRISPR/Cas12a based portable biosensor (Cas12a-PB), mainly made of polymethylmethacrylate (PMMA) and PMMA tape, was constructed for visually detecting the *CaMV35S* promoter and *Lectin* gene from GM soybean powders simultaneously. The *Lectin* gene was employed as internal control. As low as 0.1% transgenic ingredients in soybean powders could be detected by using Cas12a-PB coupled with ordinary PCR. Considering the reaction velocity and practicality, the Cas12a-PB was also employed to combine with rapid PCR and LAMP reaction to conduct duplex detection, whose detection results were comparable to results of ordinary PCR. In contrast to other established methods, the problems of amplicons contamination and non-specific amplification could be effectively resolved. The proposed biosensor would provide a new solution for rapid and specific on-site detection for multiple targets and promote the application of CRISPR/Cas systems into the areas of multiplex nucleic acids detection.

2. Materials and methods

2.1. Materials

Certified GM soybean (Roundup Ready$^{@}$) powders with different mass fractions of transgenic ingredients (10%, 1%, 0.1% and 0%) and GM maize powders (MON810, GA21) were purchased from Monsanto Co. (St. Louis, MO, USA). GM soybean powders (DP305423) were purchased from DuPont Pioneer Hi-bred International, Inc., MA, USA. Non-GM peanut and rice were obtained from local supermarket in Hangzhou, China. DNA extraction from 200 mg of samples was carried out using Tiangen Plant Genome Extraction Kit (TIANGEN BIOTECH CO., LTD., Beijing, China). The LAMP primers for *CaMV35S* promoter and *Lectin* gene were prepared according to the Industry Standards of Exit-Entry Inspection and Quarantine of the People's Republic of China (SN/T 3767.2–2014). The F3 and B3 primers for LAMP reaction were respectively employed as forward and reverse primers of PCR. Sequences of primers can be seen in Table S1, Supporting Information. All of the primers were synthesized by Sangon (Shanghai, China).

2.2. Dual ordinary PCR assay

Dual ordinary PCR assay was carried out in a 25 μL reaction mixture. It contained 1 x buffer for Taq HS DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China), 0.2 mM dNTP each (Sangon, Shanghai, China), primer mixtures (0.6 μM 35S-F, 0.6 μM 35S-R, 0.2 μM Lectin-F, 0.2 μM Lectin-R), 0.625 U Taq DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China) and 4 μL DNA templates. The amplification protocol was 95 °C for 5 min, 40 cycles at 95 °C for 30 s and 60 °C for 30 s. 25 μL mineral oil was added above the reaction mixtures. Dual real-time PCR, which contained 4 μM of SYTO 9 dyes (Thermo Fisher Scientific Inc., Waltham, MA, USA), was conducted in a QuantStudio^{TM}3 Real Time PCR system (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.3. Dual rapid PCR assay

Dual rapid PCR was carried out in a previous designed water bath system which employed two water baths to control reaction temperatures (Wang et al., 2018). The total reaction volume was 25 μL, which contained 1 x buffer for Taq HS DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China), 0.2 mM dNTP each (Sangon, Shanghai, China), primer mixtures (1.2 μM 35S-F, 1.2 μM 35S-R, 0.4 μM Lectin-F, 0.4 μM Lectin-R), 6.25 U Taq HS DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China) and 4 μL DNA templates. 25 μL mineral oil was added above the reaction mixtures. The amplification protocol was 95 °C water bath for 40 s, 40 cycles at 96 °C water bath for 10 s and 60 °C water bath for 10 s.

2.4. Dual LAMP assay

Dual LAMP reaction mixtures contained 1 x Thermopol Reaction buffer (New England Biolabs Inc., Ipswich, MA, USA) for *Bst* DNA polymerase, 0.8 M betaine (Sigma-Aldrich Co. LLC. St Louis, MO, USA), 0.35 mM dNTP each (Sangon, Shanghai, China), 2 mM $MgSO_4$ (New England Biolabs Inc., Ipswich, MA, USA), primer mixtures (1.6 μM 35S-FIP, 1.6 μM 35S-BIP, 0.2 μM 35S-F3, 0.2 μM 35S-B3, 0.4 μM 35S-LF, 0.4 μM 35S-LB, 0.8 μM Lectin-FIP, 0.8 μM Lectin-BIP, 0.1 μM Lectin-F3, 0.1 μM Lectin-F3, 0.2 μM Lectin-LF, 0.2 μM Lectin-LB), 16 U *Bst* DNA polymerase, large fragment (New England Biolabs Inc., Ipswich, MA, USA) and 4 μL DNA templates. LAMP reaction was performed at 63 °C for 30 min and 25 μL mineral oil was added above the reaction mixtures. The real-time LAMP reaction, which contained 2 μM of SYTO 9 dyes (Thermo Fisher Scientific Inc., Waltham, MA, USA), was conducted in a QuantStudio^{TM}3 Real Time PCR system (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.5. CRISPR/Cas12a detection system

The CRISPR/Cas12a detection system mainly contains Cas12a protein, gRNA, single-stranded DNA-FQ florescent probe, RNase inhibitor and buffer. The single-stranded probe would be cut by Cas12a protein to produce fluorescence signal after ternary complex (Cas12a protein, gRNA and target DNA) is formed. According to previous optimized results (Wu et al., 2020a), the reaction volume of CRISPR/Cas12a detection system was 20 μL, which contained 1 x NEB buffer 2.1 (New England Biolabs Inc., Ipswich, MA, USA), 2.5 μM custom single-stranded DNA-FQ florescent probe (5'6-FAM-TTATT-3'BHQ1, Sangon, Shanghai, China), 600 nM gRNA (SunYa Biotechnology Co., Ltd, Hangzhou, China), 10 U RNase inhibitor (Takara Biotechnology Co., Ltd., Dalian, China), 200 nM Cas12a protein (New England Biolabs Inc., Ipswich, MA, USA). The fluorescence signal of reaction solution was recorded every 30 s by using QuantStudio^{TM}3 Real Time PCR System. The gRNA was designed according to the gRNA structural requirements for activating Cas12a protein (Zetsche et al., 2015; Chen et al., 2018). Sequences of gRNA-CaMV35S and gRNA-Lectin can be seen in Table S2, Supporting

Information.

2.6. Design and fabrication of Cas12a-PB

The components of Cas12a-PB, which are shown in Fig. 1a, were designed using the SOLIDWORKS®2014. The overall width and length of Cas12a-PB are respectively 33 mm and 70 mm. It consists of a PMMA plate, PMMA tape and a cylindrical internal screw pipe. The patterns on PMMA plate contain a circular hole, three channels and three detection chambers. The circular hole is connected with three detection chambers through three channels. The channels and detection chambers on both sides are vertically symmetric with the channel in the middle as the axis of symmetry. Besides, the three channels converge at the edge of the circular hole and thus the reaction mixtures can flow into three detection chambers as evenly as possible. The diameters of circular hole and detection chamber are 9 mm and 4 mm, respectively. The distances from the center of the circular hole to the left and upper edge of the pattern are respectively 14 mm and 17 mm. The width of channel is 0.5 mm. The lengths of upper and lower channels are 36 mm while the length of intermediate channel is 41 mm. The angels between upper or lower channel and the intermediate channel are around 8 degrees. These patterns were directly carved on a 5 mm thick PMMA plate by laser engraver (WER 4060, VOLLERUN Laser Technology Co., Ltd, Liaocheng City, China). The depths of channels and detection chambers were respectively 2 mm and 4 mm. A cylindrical internal screw pipe with 8.5 mm diameter and 5.8 mm length, which was made by 3D printing, was stuck with the inner wall of circular hole using AB glue (EX-507, CHANDOR ADHESIVE SCIENCE &TECHNOLOGY CO., LTD, Fujian, China) to form the connection structure of Cas12a-PB. The upper surface of the PMMA plate would be sealed by 1 mm thick PMMA tape (Maoye Industrial Tape Products Co., Ltd, Dongguan, China) whose surface was hydrophobic. Then, the Cas12a-PB was completed and the connection structure of Cas12a-PB could tightly connect with the reaction tube by screw threads. The Cas12a-PB was light weight (14.4 g), mobile (70 x 33 x 6 mm) and suitable for field deployment.

2.7. Dual visual detection by using Cas12a-PB

The CRISPR/Cas12a detection reagents for *CaMV35S* promoter and *Lectin* gene were added into the detection chambers on both sides. And the CRISPR/Cas12a detection reagents without gRNA were added into the middle detection chamber as negative control. Then, a PMMA tape was employed to seal the upper surface of PMMA plate. The amplification reaction mixtures and templates were added into the reaction tube with 25 μL mineral oil added above them. Subsequently, the connection structure of Cas12a-PB connected with the reaction tube by screw threads to conduct detection. After amplification, the reaction tube was inverted and shook to make the reaction solutions enter into connection structure. Then, by swinging the Cas12a-PB, the solutions were further thrown into three detection chambers to mix with the corresponded CRISPR/Cas12a detection reagents. After 37 °C for 5 min, a blue LED light (490 nm, Shenzhen Kobe Trading Co., Ltd, Shenzhen, China) was employed to irradiate the detection chambers. Positive samples would produce green fluorescence while negative samples were black (Fig. 1b). A simple and portable device, which was made by 3D printing, was used to observe the detection results. In the end, to further eliminate the use of mineral oil and reaction tube, an amplification chamber with smaller volume was designed to combine with Cas12a-PB by a 2 mm thick PMMA chip.

3. Results and discussion

3.1. Liquids flow test of Cas12a-PB

Since the reaction solutions in reaction tube had to enter into three detection chambers to conduct detection, it was necessary to ensure the reaction solutions could be distributed evenly among three detection chambers. In this work, a red liquid, which was employed to mimic the reaction solution, was firstly added into the reaction tube with 25 μL mineral oil on top of it (Fig. 2a). Then, three operators (two males and one female) were respectively employed to swing the Cas12a-PB to make the red liquids enter into detection chambers (Fig. 2b). As can be seen in Fig. 2c, the red liquids could be evenly distributed in the left and right detection chambers, but the liquids in middle detection chamber were slightly more than other two detection chambers. It was mainly determined by the connection directions of three channels. The middle channel was relative smoother for liquids flow because it was consistent with the direction of the force. Therefore, the middle detection chamber was employed as negative control while other two detection chambers were employed to conduct detection in the study.

3.2. Dual ordinary PCR coupled with Cas12a-PB

To judge whether the established dual PCR system could simultaneously detect *CaMV35S* promoter and *Lectin* gene, dual real-time PCR was firstly used to assay the GM soybean (Roundup Ready®) powders with different transgenic contents (10%, 1%, 0.1% and 0%) and the amplicons were analyzed by agarose gel electrophoresis. As a result, the

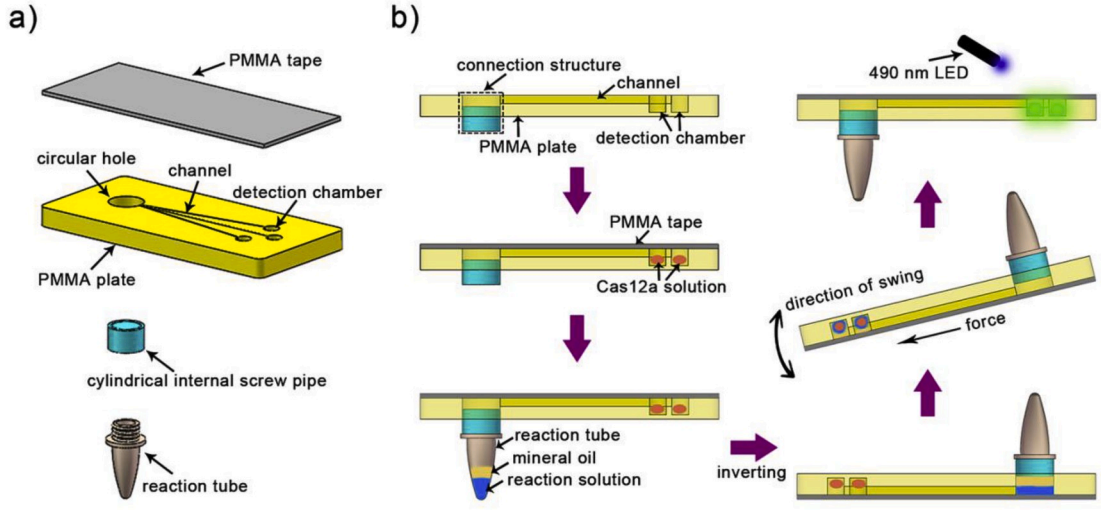

Fig. 1. a) The components of Cas12a-PB. **b)** The whole operation process using Cas12a-PB.

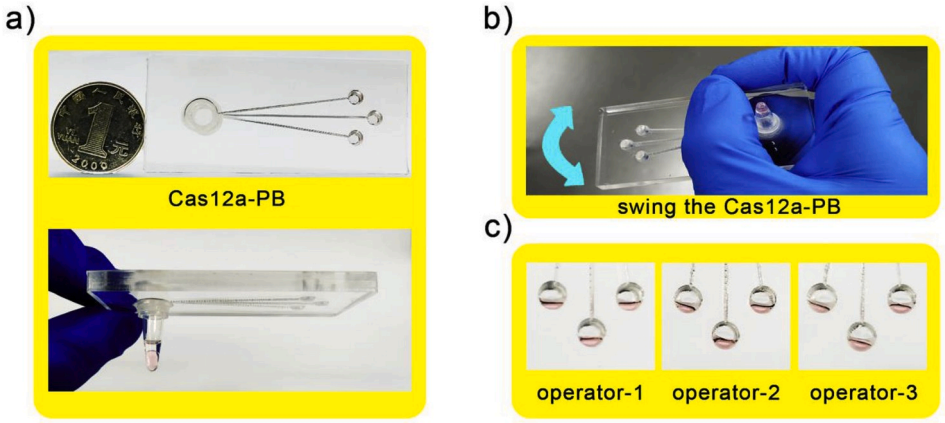

Fig. 2. a) The actual picture of Cas12a-PB and a red liquid was employed to mimic the reaction solution. **b)** The illustration of swinging the Cas12a-PB. **c)** The liquid distribution results in three detection chambers by three operators. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

amplification curves were all similar and the gel image indicated that 0.1% GM soybean powders could be detected (Fig. 3a). To achieve end-point visual detection, the CRISPR/Cas12a detection system was employed to respectively detect *CaMV35S* promoter and *Lectin* gene from dual PCR amplified products. Results indicated the fluorescence curves were all able to reach the plateau in 20 min using 10% GM soybean powder and the results could be identified by the naked eye in 5 min under 490 nm LED light (Fig. S1, Supporting Information).

Subsequently, dual ordinary PCR coupled with Cas12a-PB was used to conduct dual detection on a metal thermal block (LongGene Scientific Instruments Co., Ltd, Hangzhou, China). Because the activity of Cas12a protein will be significantly reduced over 60 °C, the temperature of detection chamber was tested using a type-K insulated thermocouple (PN: 5TC-TT-K-36-36, Omega Engineering, Norwalk). According to the real-time temperature curve (Fig. 3b), the temperature of detection chamber was always below 30 °C when performing PCR. Besides, to make detection process more convenient, a portable 3D printing instrument coupled with the mobile phone was employed to observe the detection results (Fig. 3c). It contained a mobile power, 490 nm LED light, 520 nm filter, card slot and observation port. The Cas12a-PB was put into the card slot and the camera of mobile phone was placed above the observation port. Then, detection results could be presented on the screen of mobile phone.

When conducting dual ordinary PCR with Cas12a-PB, 20 μL of CRISPR/Cas12a detection reagents for *CaMV35S* promoter and *Lectin* gene were respectively added into the upper and lower detection chambers while 20 μL of CRISPR/Cas12a detection reagents without gRNA were added into the middle chamber as negative control. As low as 0.1% GM soybean powder could be visually detected (Fig. 3d), which was consistent with the results of gel image (Fig. 3a). The fluorescence

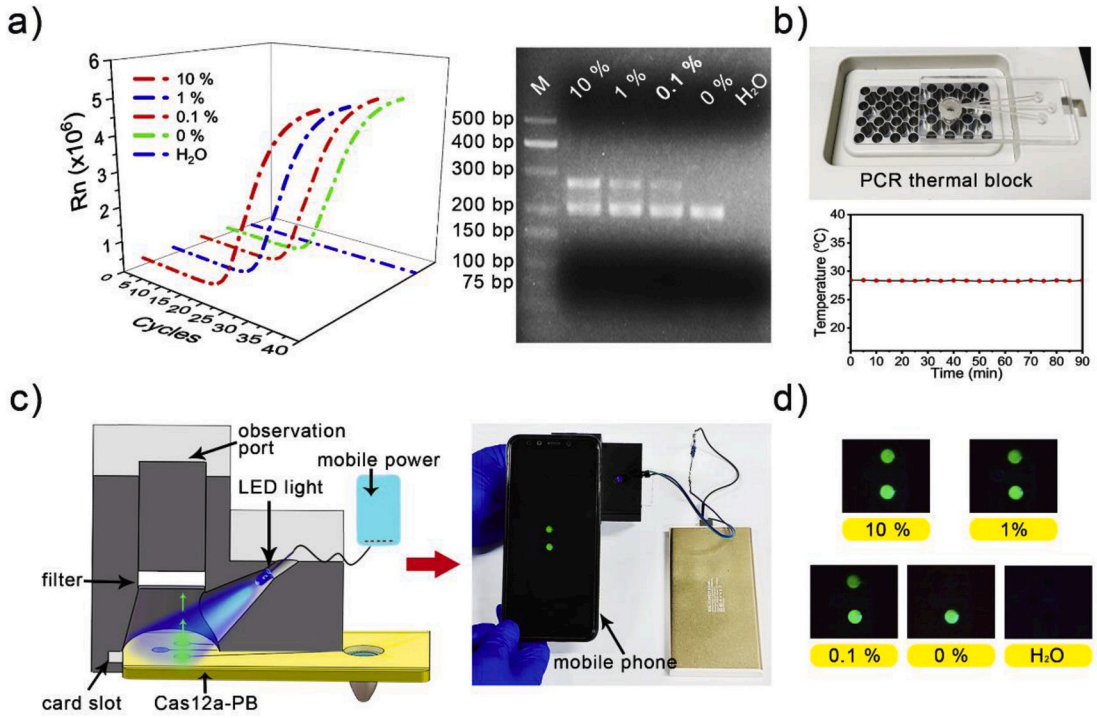

Fig. 3. a) The amplification curves of dual ordinary PCR and the gel image of amplified products. **b)** Performing dual ordinary PCR coupled with Cas12a-PB and the real-time temperature curve of detection chamber. **c)** Visual detection using 3D printing instrument and mobile phone. **d)** Sensitivity of dual ordinary PCR coupled with Cas12a-PB. 10%, 1%, 0.1% and 0% represented the transgenic contents in soybean powders.

intensity of upper detection chamber gradually decreased when the contents of *CaMV35S* promoter in the samples gradually decreased, but the positive (green) and negative (black) results could still be clearly identified by the naked eye. It illustrated the Cas12a-PB had good sensitivity.

3.3. Specificity of Cas12a-PB

Next, to further verify the specificity of Cas12a-PB, GM maize powders (MON810, GA21), GM soybean powders (DP305423), non-GM peanut and rice were employed as targets. The dual ordinary PCR coupled with Cas12a-PB was employed to conduct detection. As can be seen in Fig. 4a, with GM maize (MON810) as target, the upper detection chamber produced green fluorescence while the lower detection chamber was black, which indicated GM maize (MON810) contained *CaMV35S* promoter and did not contain soybean *Lectin* gene. For GM soybean (DP305423), the lower detection chamber produced green fluorescence and the upper detection chamber was black, which illustrated the GM soybean (DP305423) contained soybean *Lectin* gene and did not contain *CaMV35S* promoter. And the upper and lower detection chambers were both black when detecting other samples, which meant they did not contain both *CaMV35S* promoter and soybean *Lectin* gene. These detection results were consistent with the results of agarose gel electrophoresis. Therefore, the Cas12a-PB coupled with dual PCR could display high specificity.

3.4. Dual rapid PCR coupled with Cas12a-PB

Considering the reaction temperature changed slowly by thermal block, a developed water bath system which included a servo motor and two water baths was employed to conduct dual rapid PCR (Wang et al., 2018; Wu et al., 2020b). The reaction temperatures were controlled by swinging back and forth between two water baths, which could significantly increase the rate of temperature change. In this work, the temperatures of two water baths were set at 96 °C and 60 °C, respectively. The temperature of reaction tube was tested by the thermocouple. After optimizing the time spent in two water baths, 96 °C for 10 s and 60 °C for 10 s, which could guarantee the highest temperature of reaction tube reached around 91 °C while the lowest temperature of reaction tube reached around 61 °C, were employed to conduct dual rapid PCR assay (Fig. S2, Supporting Information). Besides, the concentrations of polymerase and primers were respectively increased by 10 times and 2 times. The temperature of detection chamber of Cas12a-PB was also tested during rapid PCR. Although the two water baths temperatures respectively exceeded 90 °C and 60 °C, the temperature of detection chamber was always below 45 °C without inactivating the Cas12a protein (Fig. 4b). It was mainly because PMMA had a very low thermal conductivity (0.19 W/(m·K)) and the distance between the reaction region and detection region was relatively large (about 35 mm). Subsequently, GM soybean powders (10%, 1%, 0.1% and 0%) were detected by using dual rapid PCR. As can be seen in Fig. 4c, the gel image of dual rapid PCR was comparable to the results of Cas12a-PB, which could detect as low as 0.1% GM soybean powders. The whole detection process could be finished within 30 min by using dual rapid PCR coupled with Cas12a-PB.

3.5. Dual LAMP coupled with Cas12a-PB

However, PCR needs at least two temperature zones, which is still not convenient for field detection. LAMP reaction, one of the frequently used isothermal amplification techniques, works under around 65 °C. Because the LAMP amplicons are cauliflower-like structures with multiple loops and the enzyme lacks 5' to 3' exonuclease activity, it is relatively difficult to identify multiple targets by LAMP reaction. Some researchers employed lateral flow biosensor to conduct end-point multiplex detection, but it generally needed to label multiple primers, which was relative complicated. To verify whether the Cas12a-PB could combine with dual LAMP reaction to conduct multiple targets detection, we first employed single real-time LAMP reaction to respectively detect *CaMV35S* promoter and *Lectin* gene from GM soybean powders (10%, 1%, 0.1% and 0%). As a result, as low as 0.1% GM soybean powders could be detected in 30 min when detecting *CaMV35S* promoter while there was almost no difference in the amplification curves when detecting *Lectin* gene (Fig. 5a). Afterwards, the two single LAMP reactions were integrated to build a dual LAMP amplification system for dual detection. Similar to the results of PCR, the dual LAMP reaction coupled with Cas12a-PB could also detect as low as 0.1% GM soybean powders (Fig. 5b). It illustrated the Cas12a-PB coupled with dual LAMP reaction did not decrease the sensitivity of single LAMP reaction. The

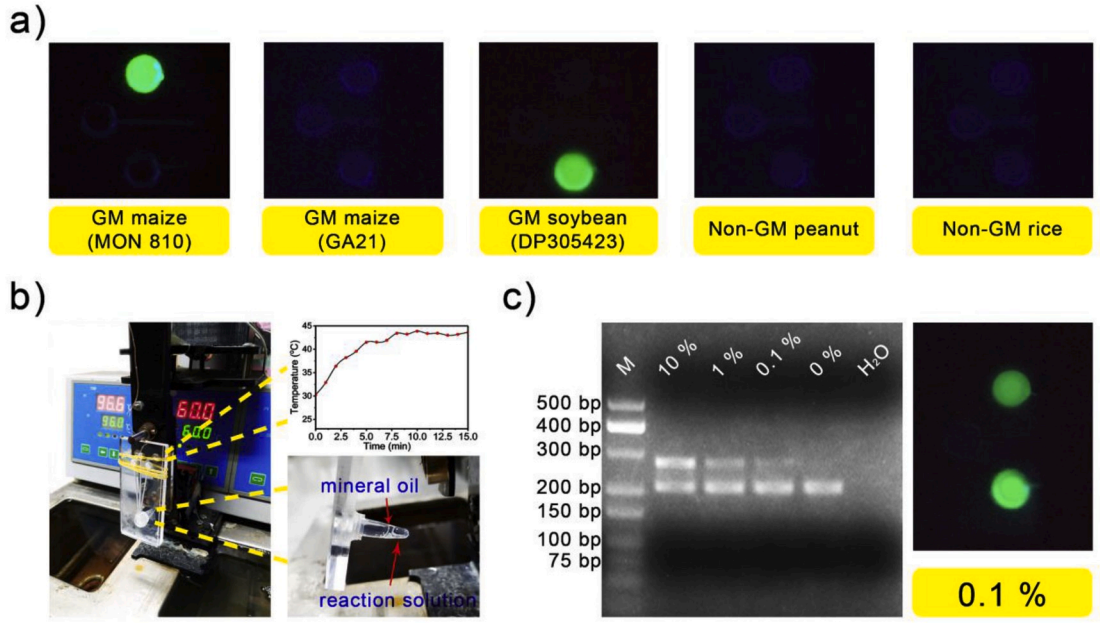

Fig. 4. a) Specificity of dual ordinary PCR coupled with Cas12a-PB. **b)** Dual rapid PCR coupled with Cas12a-PB and the real-time temperature curve of detection chamber. **c)** Gel image of dual rapid PCR amplified products and visual results of dual rapid PCR coupled with Cas12a-PB when detecting 0.1% GM soybean powders.

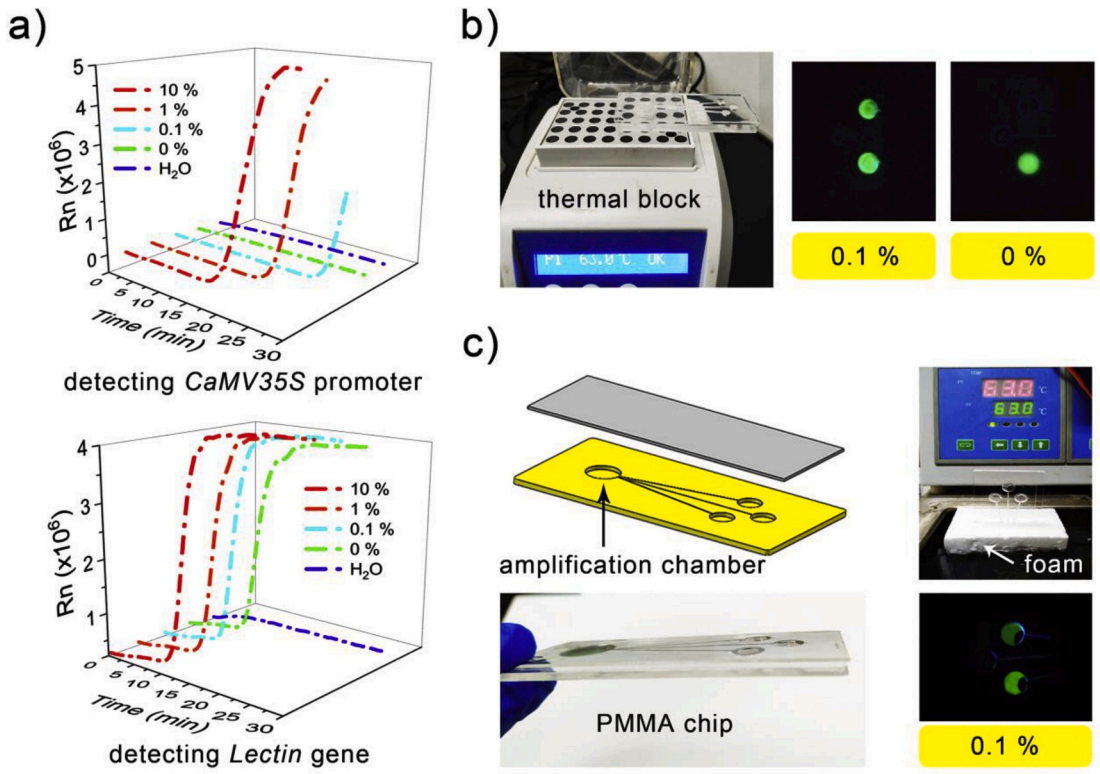

Fig. 5. a) The amplification curves of LAMP reaction for *CaMV35S* promoter and *Lectin* gene. **b)** Performing dual LAMP reaction combined with Cas12a-PB and visual results when detecting 0.1% and 0% GM soybean powders. **c)** Integrating amplification chamber and Cas12a-PB into a PMMA chip and visual results of dual LAMP reaction coupled with PMMA chip when detecting 0.1% GM soybean powders.

specificity of dual LAMP reaction was also tested with GM maize powders (MON810, GA21), GM soybean powders (DP305423), non-GM peanut and rice as targets (Fig. S3, Supporting Information). The detection results were the same with results of previous PCR. By the Cas12a-PB, the primers did not need to be labeled and no expensive instruments were needed. Thus, it would provide a new solution to conduct end-point dual detection for other isothermal amplification reactions.

3.6. Amplification chamber combining with Cas12a-PB by a PMMA chip

When the Cas12a-PB coupled with PCR or LAMP reaction conducted detection, the mineral oil was employed to prevent evaporation of reaction solutions. After amplification, it was necessary to wait for the reaction solution to be separated from the mineral oil in the connection structure of Cas12a-PB before entering detection chambers, which was a little troublesome. Therefore, an amplification chamber was designed to combine with Cas12a-PB by a PMMA chip. The chip is made by a 2 mm thick PMMA plate which was carved by laser engraver (Fig. 5c). The length and width of chip are 85 mm and 40 mm, respectively. An amplification chamber with a diameter of 11 mm and a depth of 1.5 mm replaces the previous connection structure and the diameter of detection chamber is 7 mm. The depths of detection chamber and channel are respectively 1.5 mm and 0.5 mm. With the aid of a piece of foam, the amplification chamber was soaked into a 63 °C water bath to perform LAMP reaction. Compared with reaction tube, the volume of amplification chamber is smaller and the amplification chamber is completely immersed in water, which can effectively prevent solution evaporation without needing mineral oil. As a result, 0.1% GM soybean powders could be visually detected (Fig. 5d). It further simplified the operation process and promoted the application of Cas12a-PB in household or in low-resource settings.

4. Conclusions

A Cas12a-PB, mainly made of PMMA plate and PMMA tape, has been successfully developed. The production process is very simple and the cost is very low. When conducting detection, the connection structure of Cas12a-PB only needs to connect the reaction tube by screw threads and the whole detection process is always isolated from external environment. And no additional centrifugal devices are required. By using the CRISPR/Cas12a detection system, it can provide simple, rapid and naked-eye readout with high sensitivity and specificity. In this work, ordinary PCR, rapid PCR and LAMP reaction successively combined with the Cas12a-PB to simultaneously detect *CaMV35S* promoter and *Lectin* gene from GM soybean powders, which made detection process more and more convenient. All of them can detect as low as 0.1% transgenic contents in soybean powders. Besides, the Cas12a-PB is further integrated into a PMMA chip to make operation simpler. It will promote the application of Cas12a-PB in different NAAT and provide a new reliable solution for multiple targets detection. In the future, the DNA extraction module will be considered to combine with the Cas12a-PB to finally achieve from sampling to results on a portable biosensor platform.

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.

CRediT authorship contribution statement

Hui Wu: Methodology, Investigation, Writing - original draft. **Cheng Qian:** Investigation, Validation. **Cui Wu:** Investigation. **Zhen Wang:** Investigation. **Dacheng Wang:** Investigation. **Zunzhong Ye:** Conceptualization, Writing - review & editing, Supervision. **Jianfeng Ping:**

Conceptualization, Writing - review & editing. **Jian Wu:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Feng Ji:** Conceptualization, Writing - review & editing.

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Appendix A. Supplementary data

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References

Ang, G.Y., Yu, C.Y., Yean, C.Y., 2012. Biosens. Bioelectron. 38, 151–156.
Becker, R., Ulrich, A., 2018. Eur. Food Res. Technol. 244, 1861–1871.
Blazkova, M., Javurkova, B., Fukal, L., Rauch, P., 2011. Biosens. Bioelectron. 26, 2828–2834.
Bruch, R., Urban, G.A., Dincer, C., 2019. Trends Biotechnol. 37, 791–792.
Chen, J.S., Ma, E.B., Harrington, L.B., Da Costa, M., Tian, X.R., Palefsky, J.M., Doudna, J. A., 2018. Science 360, 436–439.
Dou, M., Dominguez, D.C., Li, X., Sanchez, J., Scott, G., 2014. Anal. Chem. 86, 7978–7986.
Dou, M., Macias, N., Shen, F., Bard, J.D., Dominguez, D.C., Li, X., 2019a. EClinicalMedicine 8, 72–77.
Dou, M., Sanchez, J., Tavakoli, H., Gonzalez, J.E., Sun, J., Bard, J.D., Li, X., 2019b. Anal. Chim. Acta 1065, 71–78.
Dou, M., Sanjay, S.T., Dominguez, D.C., Liu, P., Xu, F., Li, X., 2017a. Biosens. Bioelectron. 87, 865–873.
Dou, M., Sanjay, S.T., Dominguez, D.C., Zhan, S., Li, X., 2017b. Chem. Commun. 53, 10886–10889.
Elnifro, E.M., Ashshi, A.M., Cooper, R.J., Klapper, P.E., 2000. Clin. Microbiol. Rev. 13, 559–570.
Geyer, C.N., Reisbig, M.D., Hanson, N.D., 2012. J. Clin. Microbiol. 50, 3722–3725.
Giglio, S., Monis, P.T., Saint, C.P., 2003. Nucleic Acids Res. 31, e136.
Gootenberg, J.S., Abudayyeh, O.O., Kellner, M.J., Joung, J., Collins, J.J., Zhang, F., 2018. Science 360, 439–444.
Gootenberg, J.S., Abudayyeh, O.O., Lee, J.W., Essletzbichler, P., Dy, A.J., Joung, J., Verdine, V., Donghia, N., Daringer, N.M., Freije, C.A., Myhrvold, C., Bhattacharyya, R.P., Livny, J., Regev, A., Koonin, E.V., Hung, D.T., Sabeti, P.C., Collins, J.J., Zhang, F., 2017. Science 356, 438–442.
Jauset-Rubio, M., Tomaso, H., El-Shahawi, M.S., Bashammakh, A.S., Al-Youbi, A.O., O'Sullivan, C.K., 2018. Anal. Chem. 90, 12745–12751.
Kong, J.E., Wei, Q., Tseng, D., Zhang, J., Pan, E., Lewinski, M., Garner, O.B., Ozcan, A., Di Carlo, D., 2017. ACS Nano 11, 2934–2943.
Li, J., Macdonald, J., 2015. Biosens. Bioelectron. 64, 196–211.
Li, Y., Li, S., Wang, J., Liu, G., 2019a. Trends Biotechnol. 37, 730–743.
Li, Y., Liu, L., Liu, G., 2019b. Trends Biotechnol. 37, 792–795.
Lohmann, D., Horsthemke, B., Gillessenkaesbach, G., Stefani, F.H., Hofler, H., 1992. Hum. Genet. 89, 49–53.
Ng, L.K., Martin, I., Alfa, M., Mulvey, M., 2001. Mol. Cell. Probes 15, 209–215.
Qian, C., Wang, R., Wu, C., Wang, L., Ye, Z.Z., Wu, J., Ji, F., 2018. Anal. Chim. Acta 1040, 105–111.
Qian, C., Wang, R., Wu, H., Zhang, F., Wu, J., Wang, L., 2019. Anal. Chem. 91, 11362–11366.
Roy, S., Mohd-Naim, N.F., Safavieh, M., Ahmed, M.U., 2017. ACS Sens. 2, 1713–1720.
Sakalar, E., Abasiyanik, M.F., 2012. Food Chem. 130, 1050–1054.
Soo, P.-C., Horng, Y.-T., Hsueh, P.-R., Shen, B.-J., Wang, J.-Y., Tu, H.-H., Wei, J.-R., Hsieh, S.-C., Huang, C.-C., Lai, H.-C., 2006. J. Microbiol. Methods 66, 440–448.
Vet, J.A.M., Majithia, A.R., Marras, S.A.E., Tyagi, S., Dube, S., Poiesz, B.J., Kramer, F.R., 1999. Proc. Natl. Acad. Sci. U.S.A. 96, 6394–6399.
Wang, R., Zhang, F., Qian, C., Wu, C., Ye, Z.Z., Wang, L., Qian, W.J., Ping, J.F., Wu, J., Ying, Y.B., 2018. Chem. Commun. 54, 1105–1108.
Wang, X.-W., Hu, L.-F., Hao, J., Liao, L.-Q., Chiu, Y.-T., Shi, M., Wang, Y., 2019. Nat. Cell Biol. 21, 522–530.
Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonham, N., Stead, D.E., 2000. Appl. Environ. Microbiol. 66, 2853–2858.
Wu, H., He, J.-s., Zhang, F., Ping, J., Wu, J., 2020a. Anal. Chim. Acta 1096, 130–137.
Wu, H., Qian, C., Wang, R., Wu, C., Wang, Z., Wang, L., Zhang, M., Ye, Z., Zhang, F., He, J.-s., Wu, J., 2020b. Food Contr. 109, 106905.
Xu, Y., Liu, Y., Wu, Y., Xia, X., Liao, Y., Li, Q., 2014. Anal. Chem. 86, 5611–5614.
Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., Volz, S.E., Joung, J., van der Oost, J., Regev, A., Koonin, E.V., Zhang, F., 2015. Cell 163, 759–771.
Zhou, H., Liu, J., Xu, J.-J., Zhang, S.-S., Chen, H.-Y., 2018. Chem. Soc. Rev. 47, 1996–2019.