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Label-Free Detection of Transgenic Crops Using an Isothermal Amplification Reporting CRISPR/Cas12 Assay

Xiaoying Zhu^a, Hao Yang^a, Mian Wang^a, Minghu Wu^a, Mohammad Rizwan Khan^b, Aimin Luo^{a*}, Sha Deng^a, Rosa Busquets^c, Guiping He^a, and Ruijie Deng^{*a}

Authors

^aCollege of Biomass Science and Engineering, Healthy Food Evaluation Research Center and Key Laboratory of Food Science and Technology of Ministry of Education of Sichuan Province, Sichuan

^bDepartment of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia;

^cSchool of Life Sciences, Pharmacy and Chemistry, Kingston University London, Kingston upon Thames, Penrhyn road, KT1 2EE, United Kingdom.

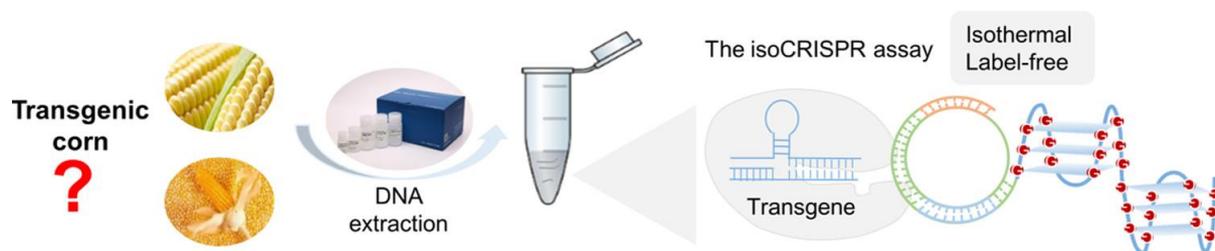
Corresponding Authors

Email: luoam@scu.edu.cn; drj17@scu.edu.cn

Abstract

Current tools for detecting transgenic crops, such as polymerase chain reaction (PCR), require professional equipment and complex operation. Herein, we introduce a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system to analyze transgenes by designing an isothermal amplification to serve as the amplified reporter, allowing an isothermal and label-free detection of transgenic crops. The use of Cas12a allowed direct and specific recognition of transgenes. To enhance the sensitivity of the assay, we used rolling circle amplification (RCA) to monitor the recognition of transgenes by designing the RCA primer as the cleavage substrate of Cas12a. The presence of transgenes can be detected by monitoring

the G-quadruplex in RCA amplicon using a G-quadruplex binding dye, N-methyl mesoporphyrin IX (NMM). We termed the assay as isoCRISPR and showed that the assay allowed distinguishing transgenic corn cultivars (“Bt11” and “MON89034”) from nontransgenic corn cultivars (“yellow”, “shenyu”, “xianyu”, and “jingke”). The isoCRISPR assay will enrich the toolbox for transgenic crop identification and broaden the application of CRISPR/Cas in food authenticity and safety.



Keywords: transgenic crops CRISPR/Cas12 isothermal amplification G-quadruplex food authenticity

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops in the world. With the rapid development of transgenic technology, transgenic corn cultivars with multiple features, such as insect resistance, herbicide tolerance, and product-quality improvement, have been developed. (1) For instance, the transgenic corn cultivar “Bt11” is a strain with both insect resistance and herbicide tolerance. (2) Transgenic corn cultivar “MON89034” is a solitary insect-resistant maize with resistance to lepidopteron. (3) Many governments and international organizations require the detection and labeling of ingredients derived from transgenic crops to enhance the management. (4,5) Consequently, it is highly needed to set up a reliable and accurate identification method for transgenic crops.

Nucleic acid detection techniques are recognized as fundamental tools for detecting transgenic crops. At present, the main nucleic acid detection techniques of transgenic crops are gene chip, (6,7) polymerase chain reaction (PCR), (8,9) and loop-mediated isothermal amplification (LAMP). (10–12) Gene chip can be highly multiplexed; however, it suffers from high cost and complicated experimental processes. The PCR technique is highly sensitive and widely used for determining transgenic crops. Nevertheless, the real-time PCR instrument for qualification of transgenic contents is highly expensive. LAMP can be carried out in test tubes at a constant temperature and is thus promising for rapid and on-site detection of transgenic crops. But LAMP is vulnerable to aerosol pollution and nonspecific amplification, hindering its wide application.

The CRISPR-Cas system, which is composed of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins, has been a promising technology serving for nucleic acid detection. (13–16) Cas12a, as one of the Cas proteins, is an RNA-guided nuclease in the CRISPR-Cas system. It can specifically recognize double-stranded DNA (dsDNA) via hybridization of guide RNA (gRNA). Then, trans-cleavage activity of Cas12a protein is activated, which can cleave single-stranded DNA (ssDNA). When the ssDNA is modified with terminal fluorophore and quencher groups, it can serve as a reporter for detecting target genes using the CRISPR-Cas12a system. (17–19) To achieve fluorescence detection of low-abundance nucleic acids, CRISPR-Cas12a has been combined with nucleic acid amplification including PCR, (20,21) LAMP, (22–24) and recombinase polymerase amplification (RPA). (25–28) These assays have been used to detect viruses, bacteria, and nucleic acid biomarkers (such as microRNA) (29,30) and showed high sensitivity and specificity to detect target RNA/DNA sequences. Up to now, the utility of the CRISPR-Cas system has not been sufficiently explored to detect food authenticity such as the identification of transgenic crops.

To fully exploit the capacity of the CRISPR-Cas system for transgenic crop detection, we used Cas12a/gRNA to directly detect gene markers of transgenic corn. Particularly, to improve the sensitivity of the CRISPR-Cas system, we explored an isothermal amplification strategy, rolling circle amplification (RCA), (31,32) to serve as the reporter of recognition events occurred based on CRISPR-Cas12a. To eliminate the use of the chemically modified nucleic acid probe that is costly, we further designed a G-quadruplex sequence in the RCA template, and the presence of transgenic corn can be monitored using a G-quadruplex binding dye. We termed the assay as isoCRISPR, and it allowed label-free and isothermal amplification detection of transgenic corn. The assay has been used to discriminate transgenic corn cultivars (“Bt11” and “MON89034”) from nontransgenic corn cultivars (“yellow”, “shenyu”, “xianyu”, and “jingke”). The isoCRISPR assay has broadened the use of the CRISPR-Cas system for food authenticity and is promising for on-site detection of transgenic crops.

Experimental section

Materials

Transgenic corns “Bt11” and “MON89034” were both provided by the Testing and Evaluation Center of Chinese Academy of Inspection and Quarantine (Beijing, China). Nontransgenic corn kernels “yellow”, “shenyu”, “xianyu”, and “jingke” were all provided by the Sichuan Academy of Agricultural Sciences (Chengdu, China).

All oligonucleotide sequences in Tables S1–S3 were purchased and purified by Sangon Biotech (Shanghai, China). LbCas12a (cpf1) (cat. no. 32108-01) was obtained from Tolo Biotech (Shanghai, China). T7 RNA polymerase (cat. no. EP0111), T4 polynucleotide kinase (PNK) (cat. no. EK0032), ATP (10 mM) (cat. no. 00802750), T4 DNA ligase (cat. no. 00768771), phi29 DNA polymerase (cat. no. EP0094), and dNTPs (cat. no. 2127062) were obtained from Thermo Fisher Scientific (Waltham). DNase I (cat. no. M0303S) and rNTPs (cat. no. N0466S)

were purchased from New England Biolabs (NEB) (Ipswich, MA). Ezup column deep processed product genomic DNA extraction kit (cat. no. B518264), N-methyl mesoporphyrin IX (NMM) (cat. no. A600936), and DNA marker (cat. no. B600303) were purchased from Sangon Biotech (Shanghai, China). Agarose, 50× TAE buffer, 6× Loading buffer, and 10 000× Gelred dye were purchased from Beijing DingGuo Biotechnology Co., Ltd. (Beijing, China).

DNA Extraction and Sequencing

All DNA extraction procedures were conducted using a Ezup Food Genomic DNA Extraction Kit according to the manufacturer's instructions. "Bt11", "yellow", "shenyu", "xianyu", and "jingke" were sequenced after PCR amplification using primers Bt11-F (CCAGATCTGAGTCGACCTGC) and Bt11-R (ACAACCGCCATTTTGCCGAA), and "MON89034", "yellow", "shenyu", "xianyu", and "jingke" were sequenced using primers MON89034-F (AATATTTAAAAATGGAAGTAATACT) and MON89034-R (TTGTTTCGGTGTTCATCT).

gRNA Transcription

The gRNA synthesis reaction was carried out with a volume of 25 μL mixtures containing 4 μL of phi29 DNA polymerase buffer, 4 μL of DNA template strands (10 μM), 4 μL of promoter strand (10 μM), and 13 μL of nuclease-free H₂O. Then, the mixture was denatured at 90 °C for 5 min and incubated at room temperature for 30 min. Then, 0.4 μL of phi29 DNA polymerase (10 U/ μL) and 1 μL of dNTP mix (10 mM each for dATP, dGTP, dCTP, and dTTP) were added and incubated at 30 °C for 30 min. And, 1 μL of T7 RNA polymerase (20 U/ μL), 8 μL of T7 RNA polymerase buffer, and 1 μL of rNTP mix (25 mM each for ATP, GTP, CTP, and TTP) were incubated at 37 °C for 8 h. After transcription, 1 μL of DNase I and 4 μL of DNase I buffer were added to remove DNA templates at 37 °C for 6 h, following an inactivation at 65

°C for 10 min. Finally, after measuring the concentration using the microplate reader Synergy H1 (BioTek), the obtained gRNA was stored at -80 °C or used immediately.

Padlock Probe Phosphorylation

The phosphorylation reaction was carried out with a volume of 40 μ L mixtures, including 4 μ L of the padlock probe (100 μ M), 4 μ L of T4 polynucleotide kinase buffer, 4 μ L of ATP (10 mM), 1 μ L of PNK (10 U/ μ L), and 27 μ L of nuclease-free H₂O, which were mixed and reacted at 37 °C for 2 h.

Detection of “Bt11” dsDNA

First, 1 μ L of LbCas12a (1 μ M), 2 μ L of LbCas12a buffer, 1 μ L of Bt11 gRNA (2 μ M), and 13 μ L of nuclease-free H₂O were incubated at room temperature for 10 min to allow Bt11 gRNA to bind with LbCas12a protein. Then, 2 μ L of the primer (1 μ M) and 1 μ L of 4 μ M Bt11 dsDNA (the control group used H₂O instead) were added and incubated at 37 °C for 1 h to cleave the primer of RCA. And, 2 μ L of the phosphorylated padlock probe (Cir-59) (1 μ M), 2 μ L of T4 DNA ligase buffer, and 5.5 μ L of nuclease-free H₂O were annealed at 65 °C for 5 min to inactivate Cas12 protein. T4 DNA ligase (0.5 μ L) was added, and the Cir-59 was ligated to the cyclized padlock at room temperature for 1 h. phi29 DNA polymerase buffer (2 μ L), 2 μ L of dNTPs (10 mM), 0.3 μ L of phi29 DNA polymerase, and 5.7 μ L of H₂O were added to initiate RCA amplification (37 °C, 30 min). After RCA amplification, 4 μ L of NMM (20 mM) was added. Fluorescence spectra were recorded at an excitation of 399 nm and emission ranging from 550 to 630 nm using the microplate reader Synergy H1 (BioTek).

Gel Electrophoretic Analysis

The gel-loading solution was prepared by mixing 1 μ L of the gel-loading buffer with 5 μ L of oligonucleotide mixtures. Next, 5 μ L of the solution was loaded into the prepared gel (3%

agarose, 1× TAE buffer, and 1× Gelred dye). Gel electrophoresis was carried out in 1× TAE at 150 V for 30 min and visualized via the Gel Doc XR + system (Bio-Rad).

Detection of Transgenic Corn

Ezup column deep processed product genomic DNA extraction kit was used to extract DNA from the transgenic corn powder (“Bt11” and “MON89034”) and nontransgenic corn kernels (“yellow”, “shenyu”, “xianyu”, and “jingke”). The extracted DNA concentration was diluted to 600 ng/μL, and 4 μL of extracted DNA was used to determine the contents of transgenic corn using the isoCRISPR assay.

Results and Discussion

Working Principle of the isoCRISPR Assay

Direct and specific identification of transgene was achieved by the use of CRISPR/Cas12 and a post-amplification process using RCA (Figure 1). Gene sequences with a protospacer-adjacent motif (PAM) of Cas12a, specific for transgenic corn, were used as the target dsDNA of the isoCRISPR assay. The total DNA was extracted from different corn cultivars. When gRNA was bound with the target dsDNA, the activated Cas12a-gRNA ribonucleoprotein complex can cleave nontargeted ssDNA via the collateral trans-cleavage activity. In the isoCRISPR assay, the primer of RCA was used as the cleavage substrate of Cas12a/gRNA. The primer can hybridize with the padlock probes, extend using phi29 DNA polymerase, and produce abundant copies of padlock probes. Particularly, the padlock probe was anchored with a G-quadruplex sequence, and thus the RCA amplicon can be detected using G-quadruplex-specific dye, NMM. In the presence of target dsDNA, however, the primer was cleaved and could not hybridize with the padlock probe, thus ending the RCA process. Therefore,

CRISPR/Cas12 can directly recognize different transgenes, and RCA serves as the amplified reporter of CRISPR/Cas12 to improve the sensitivity for detecting transgenic corn.

We used fluorescence and electrophoresis analysis to investigate each step of the isoCRISPR assay. NMM dye was used to label the RCA amplicon via binding with G-quadruplex. The addition of G-quadruplex dramatically increased the fluorescence of NMM (Figure S1 in the Supporting Information).

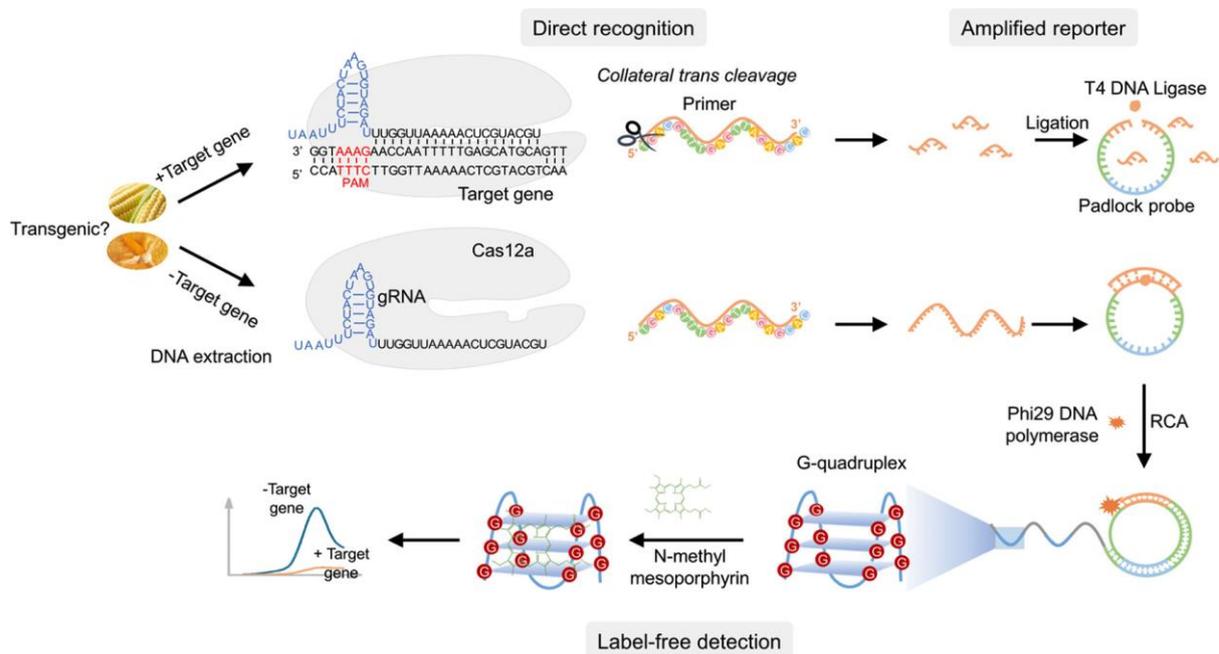


Figure 1. Working principle of the isoCRISPR assay for the detection of transgenic corn. Cas12a recognizes the target gene from transgenic corn by guide RNA hybridization. Activated Cas12a cleaves the primer, hindering the occurrence of RCA. G-quadruplex sequence in the RCA product can be detected by the specific dye NMM.

Figure 2A illustrates that the absence of either phi29 DNA polymerase or T4 DNA ligase did not lead to significant fluorescence enhancement. In the presence of the two enzymes, Cas12a,

padlock probe, primer, and target dsDNA led to a low fluorescence (black line). Further removal of target dsDNA dramatically increased the fluorescence of NMM, indicating the success of the RCA process. The electrophoresis result is shown in Figure 2B. The simultaneous presence of the padlock probe and the primer contributed a band with an increased molecular weight (lane 4), indicating the hybridization of padlock probes and primer. The addition of the phi29 DNA polymerase and T4 DNA ligase resulted in a band located in the loading well. The band was supposed to be RCA products that were too long and thus cannot enter into the gel. RCA products were dramatically reduced in the presence of target dsDNA (lane 2). The result indicated that target dsDNA activated Cas12a and led to the cleavage of the primer of RCA, in turn reducing the RCA products.

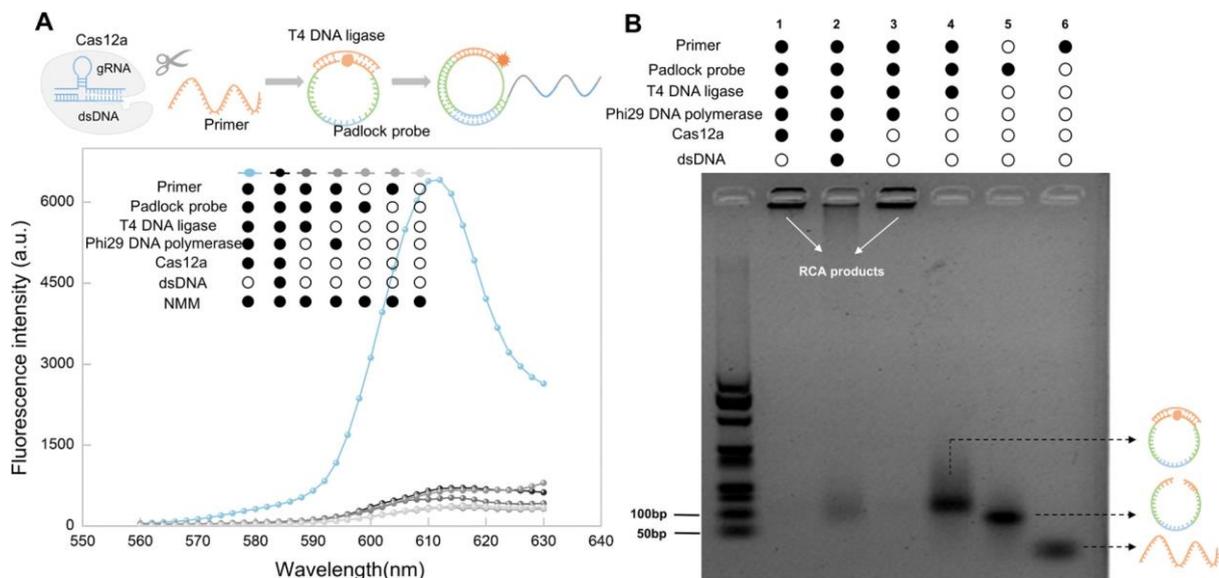


Figure 2. Investigation of the mechanism of the isoCRISPR assay. (A) Fluorescence analysis and (B) electrophoresis analysis of each step of the isoCRISPR assay.

gRNA Site Screening

Ribonucleoprotein complexes formed by binding of different gRNAs yielded varied degrees of cleavage ability of Cas12a protein after recognizing the target dsDNA. Therefore, gRNA site screening was the most critical step in the isoCRISPR assay. We designed 10 gRNAs

targeting different sites of specific insertion sequences of transgenic corn “Bt11” (located from 52 to 671 nucleotide) and “MON89034” (located from 5 to 397 nucleotide) to study the cleavage ability of Cas12a after binding to the corresponding transcribed gRNAs (Figure 3). The chosen sites (~24 nt) all owned rich T nucleotides to serve as PAM domain for Cas12a. We used a ssDNA reporter with a terminal modification of fluorophores and quenchers to estimate the response of the isoCRISPR assay toward the chosen target sites. The S/N ratio was defined as the ratio of fluorescence intensity in the presence of target DNA to that in the absence of target DNA. For detecting “Bt11”, the S/N ratio ranged from 1.02 to 7.84, and for detecting “MON89034”, the S/N ratio ranged from 1.01 to 7.27. The result further confirmed that the binding sites of target genes posed significant effects on the activation of the CRISPR-Cas12 system. The target bias may result from the complex structure of gene sequences and sequence bias of CRISPR-Cas12. The 10th gRNA yielded the highest S/N ratio for detecting “Bt11” (7.84), and the 1st gRNA contributed a S/N ratio of 7.27 to maximize the response of the isoCRISPR assay toward “MON89034”. The two gRNAs were chosen to detect the two transgenic corn cultivars.

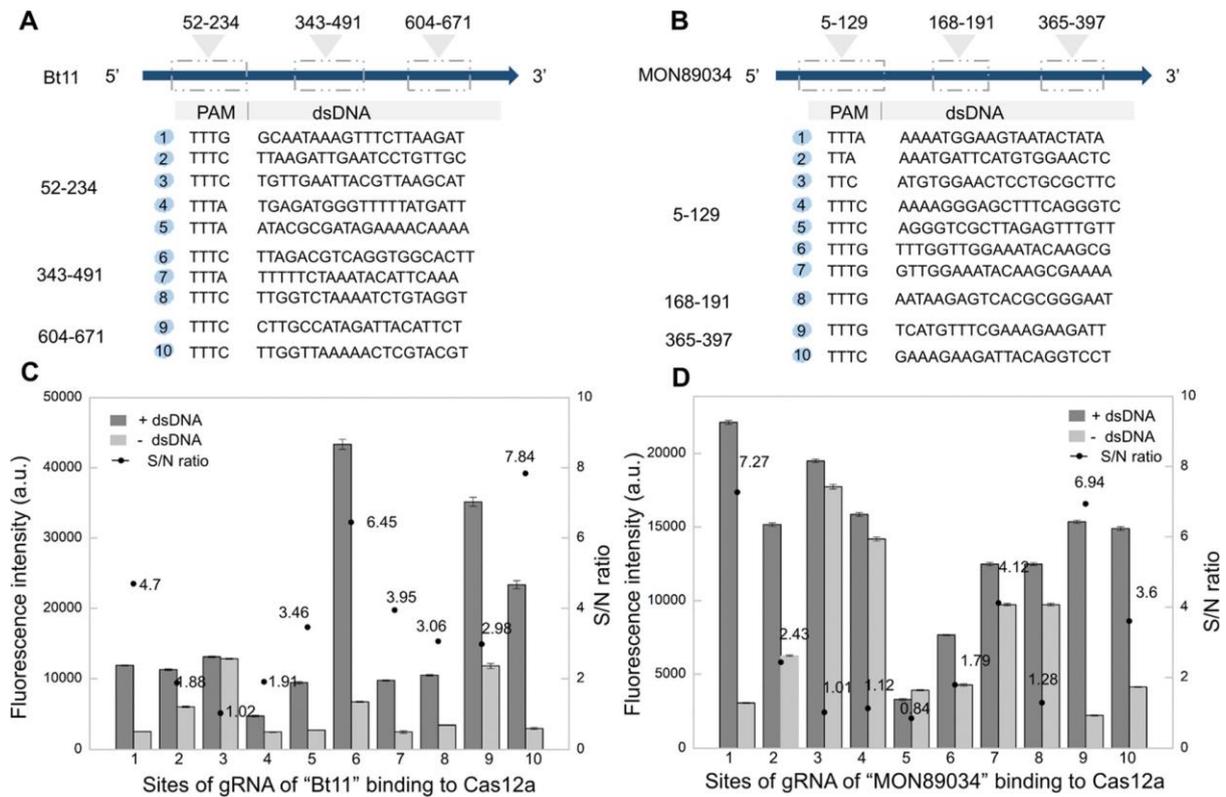


Figure 3. Screening of gRNA sites in transgenic corn “Bt11” and “MON89034”. (A) Sequences of “Bt11” gene sites. (B) Sequences of “MON89034” gene sites. Fluorescence spectra and the ratios of signal to background (S/N ratio) of the isoCRISPR assay using gRNAs binding to different (C) “Bt11” gene sites and (D) “MON89034” gene sites.

Optimization of Experimental Conditions

The primers served as two roles, the cleavage substrate of Cas12a and the initiator of the RCA process, thus posing significant effect on the detection performance of the isoCRISPR assay. When the length of the primers changed from 24 to 14 nt, the fluorescence of the isoCRISPR assay remained at a low level in the presence of target dsDNA (Figure 4A). The result indicated that Cas12a activated via target dsDNA can sufficiently cleave the primers falling in 14–24 nt. However, in the absence of target dsDNA, the fluorescence intensity dramatically reduced when the primer was shortened to be 14 nt. This may be because the short primer can hardly

hybridize with the padlock probe, thus hindering the RCA reaction. When the length of the primer was 20 nt, the fluorescence value of background was the highest, and the ratio of background to signal was 9.12 (Figure 4B).

Subsequently, for exploring the optimized time of the RCA reaction, the fluorescence of NMM was measured every 5 min. The ratio of background to signal increased with the time increasing from 0 to 30 min and decreased slightly after 30 min (Figure 4C, D). Thus, RCA was continued for 30 min in the isoCRISPR assay.

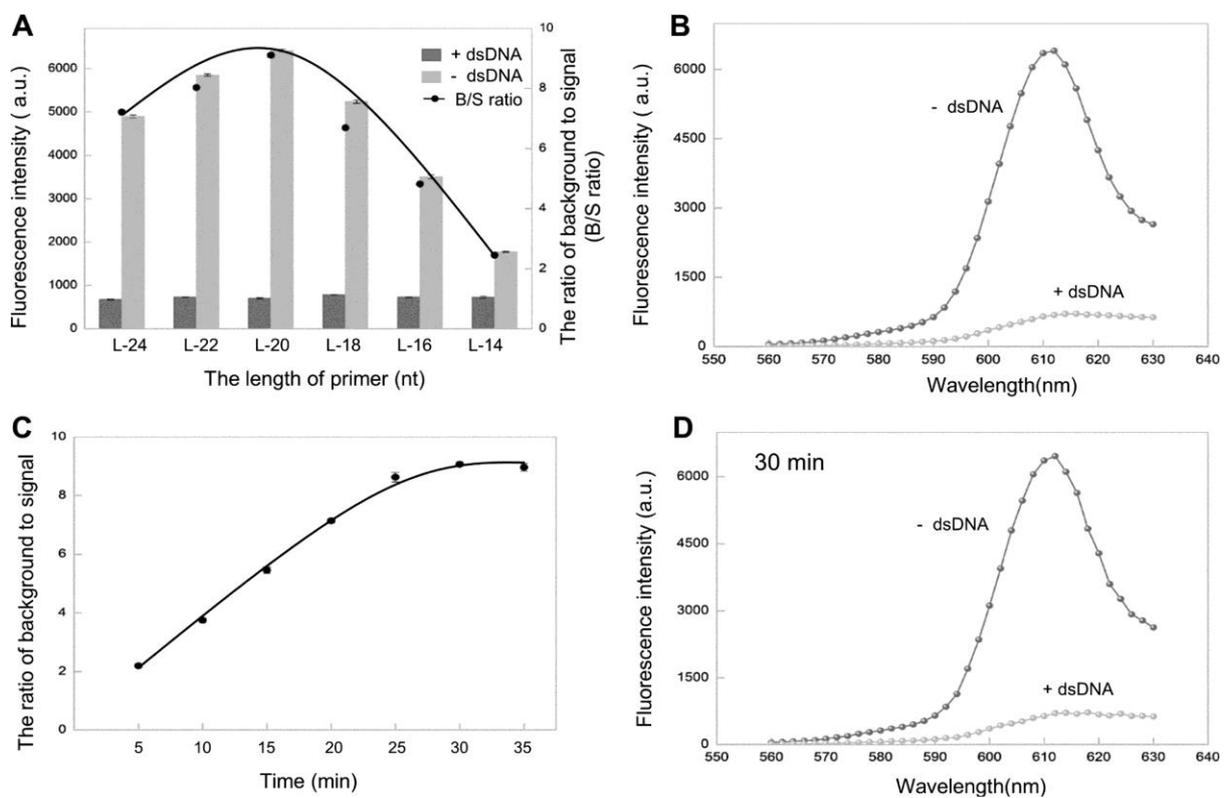


Figure 4. Optimization of experimental conditions. (A) Effect of different lengths of primers on the fluorescence value of the reaction system. (B) Fluorescence spectra of the signal group (+dsDNA) and background group (-dsDNA) when the length of the primer was 20 nt. (C) Effect of RCA reaction time on the ratio of background to signal. (D) Fluorescence spectra of the signal group (+dsDNA) and background group (-dsDNA) when the reaction time of RCA was 30 min.

Quantification Performances

The quantitative performance of the isoCRISPR assay was verified using a series of target dsDNA solutions with different concentrations. As shown in Figure 5A, when the concentration of target dsDNA decreased, the fluorescence intensity of the signal gradually increased and finally tended to be stable. This showed that the decrease of the concentration of target dsDNA led to the decrease of the collateral trans-cleavage ability of the Cas12a-gRNA complex, and the primer could only be partially cleaved, which led to the gradual enhancement of the RCA reaction until its fluorescence intensity was near to that of the background. The linear regression equation between the concentration of target dsDNA and the fluorescence intensity was calculated as $A = -70.87 \times B + 7709.1$ ($R^2 = 0.996$), where A and B represented the fluorescence intensity and the concentration of target dsDNA, respectively (Figure 5B). The detection limit (LOD) of the isoCRISPR assay was estimated to be 45.0 pM.

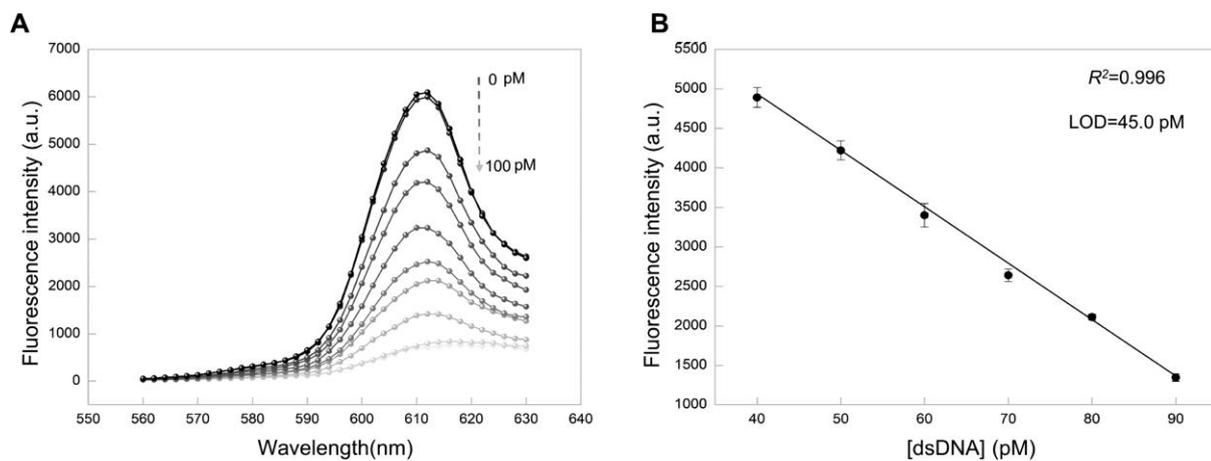


Figure 5. Quantification of target genes using the isoCRISPR assay. (A) Fluorescence spectra of the synthesized “Bt11” gene at different concentrations (0, 20, 40, 50, 60, 70, 80, 90, and 100 pM). (B) Linear relationship between the concentrations of synthetic “Bt11” gene and fluorescence signals of the isoCRISPR assay.

Discrimination between Transgenic and Nontransgenic Corn

Two transgenic corn cultivars (“Bt11” and “MON89034”) and four nontransgenic corn cultivars (“yellow”, “shenyu”, “xianyu”, and “jingke”) were selected to test the capacity of the isoCRISPR assay to identify transgenic corn. The DNA of the corn was extracted using a Ezup column deep processed product genomic DNA extraction kit. The extracted DNA was diluted to a concentration of 600 ng/ μ L, and 4 μ L of DNA extraction was used for further test. The insertion genes of transgenic corn were sequenced (Figures 6A, S1, and S2).

For comparison, we used a CRISPR-Cas12-based assay using the terminal modified DNA reporter. The results in Figure 6B show that, using the assay, there was no significant fluorescence difference between transgenic corn cultivars (“Bt11” and “MON89034”) and nontransgenic corn cultivars (“yellow”, “shenyu”, “xianyu”, and “jingke”). And all of the ratios of background to signal were around 1. Thus, the CRISPR-Cas12-based assay using the terminal modified DNA reporter was not sensitive enough to detect transgenic corn under the protocols. We used the isoCRISPR assay to detect the same samples. In contrast, highly significant differences between two transgenic corn cultivars (“Bt11” and “MON89034”) and four nontransgenic corn cultivars (“yellow”, “shenyu”, “xianyu”, and “jingke”) ($P < 0.0001$) were obtained (Figure 6C). The result indicated that the substitution of a modified ssDNA reporter for isothermal amplification would increase the ability of the CRISPR-Cas-based assay to detect low abundant genes, thus allowing a precise detection of transgenic crops.

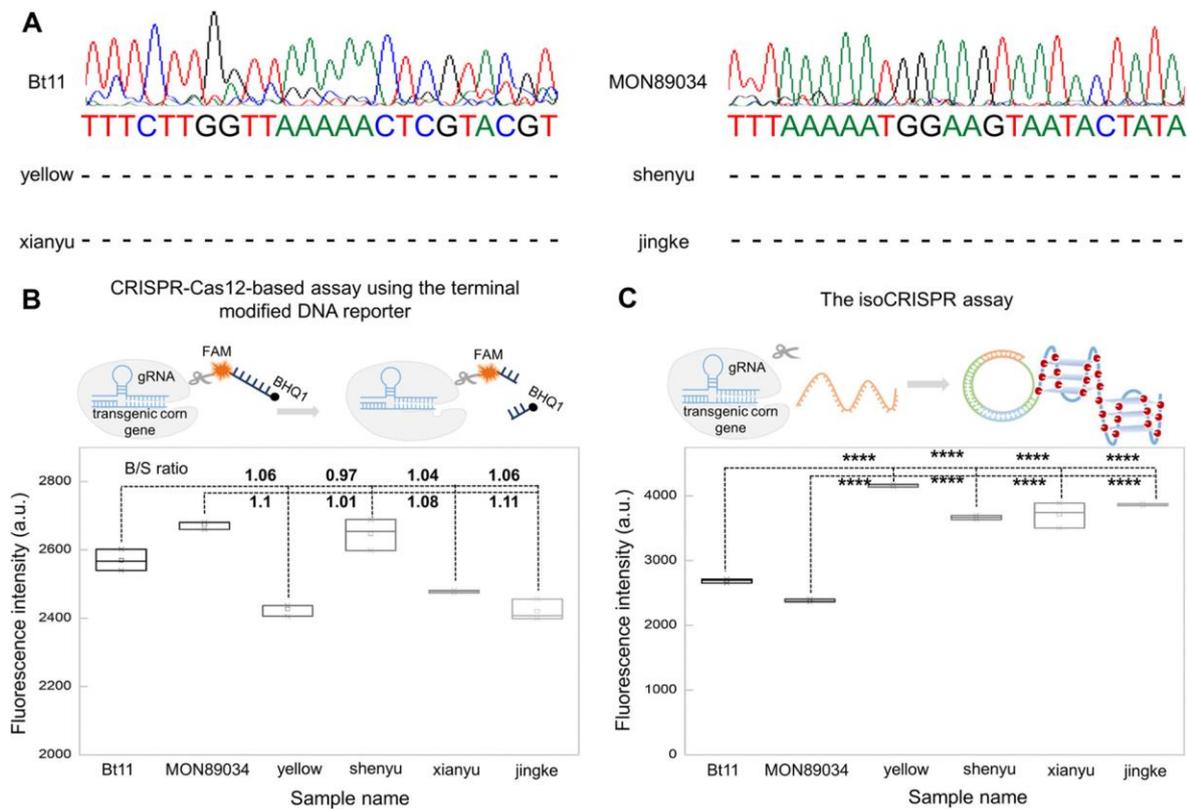


Figure 6. Discrimination between transgenic corn and nontransgenic corn. (A) Sequencing results of the insertion genes of “Bt11” and “MON89034” recognized by the CRISPR/Cas12a-based assay. “Yellow”, “shenyu”, “xianyu”, and “jingke” had no corresponding DNA sequences. (B) Fluorescence intensity of the CRISPR-Cas12-based assay using the terminal modified DNA reporter toward “Bt11”, “MON89034” and “yellow”, “shenyu”, “xianyu”, “jingke”. (C) Fluorescence intensity of the isoCRISPR assay toward “Bt11”, “MON89034” and “yellow”, “shenyu”, “xianyu”, “jingke”. Statistical significance data were obtained by two-tailed unpaired Student’s *t*-test: **** $P < 0.0001$.

Conclusions

In summary, we introduced the CRISPR/Cas system to detect transgenic crops via designing an isothermal amplification to serve as the amplified reporter. The assay termed as isoCRISPR presents several features: (1) specific and rapid: high-degree specificity for the direct

recognition of transgenes using the CRISPR/Cas12a system; (2) isothermal operation: the implementation of the assay does not need complex thermal cycling devices; (3) single-tube test: all detection processes are carried out in one test tube, eliminating the separation process, which is suitable for the field detection of transgenic corn; (4) a potential general platform: this assay has been successfully applied to the determination of transgenic corn cultivars “Bt11” and “MON89034”, and different gene sequences can be targeted by simply changing the gRNAs. Therefore, the isoCRISPR assay offers broad prospects for on-site rapid detection of various food contamination (transgenic products, foodborne viruses, pathogenic bacteria, etc.), facilitating the guarantee of food safety and food authenticity.

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Author Contributions

R.D., X.Z., H.Y., and A.L. designed the experiments, X.Z., H.Y., M.W., and S.D. performed the experiments, M.W., M.K., R.B., and G.H. analyzed the data, R.D. X.Z., and A.L. wrote the manuscript, and all authors reviewed the manuscript.

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Supporting Information

Label-free Detection of Transgenic Crops Using Isothermal Amplification Reporting CRISPR/Cas12 Assay

Xiaoying Zhu^a, Hao Yang^a, Mian Wang^a, Minghu Wu^a, Mohammad Rizwan Khan^b, Aimin Luo^{a*}, Sha
Deng^a, Rosa Busquets^c, Guiping He^a, and Ruijie Deng^{*a}

Authors

^aCollege of Biomass Science and Engineering, Healthy Food Evaluation Research Center and Key
Laboratory of Food Science and Technology of Ministry of Education of Sichuan Province, Sichuan

^bDepartment of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia;

* Correspondence: luoam@scu.edu.cn; drj17@scu.edu.cn

Table S1. Oligonucleotide sequences used for detecting “Bt11”

Oligonucleotide	Sequences (5' to 3')
L-Bt11 gRNA1	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATGCAATAAAG TTTCTTAAGAT
L-Bt11 gRNA2	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTAAAGATTG AATCCTGTTGC
L-Bt11 gRNA3	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTGTTGAATTA CGTTAAGCAT
L-Bt11 gRNA4	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTGAGATGGG TTTTTATGATT
L-Bt11 gRNA5	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATATACGCGATA GAAAACAAAA
L-Bt11 gRNA6	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTAGACGTCA GGTGGCACTT
L-Bt11 gRNA7	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTTTTTCTAAAT ACATTCAAA
L-Bt11 gRNA8	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTGGTCTAAAA TCTGTAGGT
L-Bt11 gRNA9	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATCTTGCCATAGA TTACATTCT
L-Bt11 gRNA10	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTGGTTAAAAAC TCGTACGT

Table S2. Oligonucleotide sequences used for detecting “MON89034”

Oligonucleotide	Sequences (5' to 3')
L-MON89034 gRNA1	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATAA AATGGAAGTAATACTATA
L-MON89034 gRNA2	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATAA ATGATTCATGTGGAAGCTC
L-MON89034 gRNA3	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATATG TGGAAGCTCCTGCGCTTC
L-MON89034 gRNA4	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATAA AAGGGAGCTTTCAGGGTC
L-MON89034 gRNA5	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATAG GGTCGCTTAGAGTTTGTT
L-MON89034 gRNA6	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTTT GGTTGGAAATACAAGCG
L-MON89034 gRNA7	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATGT TGGAATACAAGCGAAAA
L-MON89034 gRNA8	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATAAT AAGAGTCACGCGGGAAT
L-MON89034 gRNA9	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTC ATGTTTCGAAAGAAGATT
L-MON89034 gRNA10	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATGA AAGAAGATTACAGGTCCT

Table S3. Oligonucleotide sequences serving in RCA reaction

Oligonucleotide	Sequences (5' to 3')
Padlock probes	TCAAACGTCAGCAGAACAGCGTCCTGATCGACCCACCCACCCACCCA TGGTGCTCTAAC
L-24	GCTGACGTTTGAGTTAGAGCACCA
L-22	CTGACGTTTGAGTTAGAGCACC
L-20	TGACGTTTGAGTTAGAGCAC
L-18	GACGTTTGAGTTAGAGCA
L-16	ACGTTTGAGTTAGAGC
L-14	CGTTTGAGTTAGAG

Figure S1. Turn on the fluorescence of NMM dye using G-quadruplex sequence. The concentrations of NMM dye and G-quadruplex sequence were 1 μM and 0.5 μM , respectively. The excitation wavelength was 399 nm.

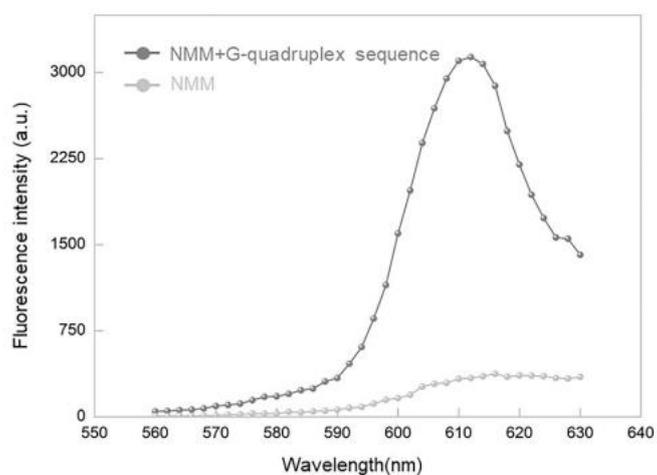


Figure S2. Herbicide-tolerant Pat gene sequence of transgenic corn “Bt11”.

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CCAGTTAGGCCAGTTACCCAGATCTGAGTCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGAT 100
GATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTATGATTAGAGTCCCG 200
CAATTATACATTTAATACGCGATAGAAAAACAAAATATAGCGCGCAAACCTAGGATAAATTATCGCGCGCGGTGCATCTATGTTACTAGATCTGGCCTCG 300
TGATACGCCCTATTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGAAATGTCGCGGAAACCCCTATTTGTT 400
ATTTTTCTAAATACATTCAAATATGTATCCGCTCATGGAGGGATTCTTGATTTTTGGTGGAGACCACTTCTGGTCTAAAATCTGTAGGTGTTAGCCTC 500
TAGTATTATTGAAAATGGTCGCTCATGGCTATTTTCATCAAAAATGGGGTGTGTGGCCATTTATCATCGACCAGAGGCTCGTACACCTCACCCACAT 600
ATGTTTCCTTGCCATAGATTACATTCTTGGATTTCTGGTGGAAACCACTTCTGGTAAAAACTCGTACGTGTTAGCCTTCGGTATTATTGAAAATGGTC 700
ATTCATGGCTATTTTCGGCAAAATGGCGGTGTGTGGCCATTTGA 747
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Figure S3. Gene sequence of transgenic “MON89034”.

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AATATTTAAAAATGGAAGTAATACTATATATAAATGATTCATGTGGAACCTCTGCGCTTCTTTTGAAGTTTCAAAGGGAGCTTTCAGGGTCGCTTAGA 100
GTTTGTGTTGGTTGAAAATACAAGCGAAAAGAGAGCTAATGAGGGGACATCCATATTTCTATGGTGTGTAATAAGAGTCACGCGGAATAAGATGAAC 200
ACCGAAACAATTTTTTTGTAGCTACGTGGTTCCAAAAATCGAGTAGACGGTGTGCTCCACCTCATACTACTTCAACCTCAAACACACATCCTTACG 300
CGCCCGCCGGTGTCTCCGTCTCAAGTTCTCAACATACCTACACATGTAACCACTACCGGTCTTTGTCATGTTTCGAAAAGAGATTACAGGTCCTCTA 400
GAAGAGAGGACGCGGGTGGCGAGAAAGCTGGGGAAGAAAAAGGCTAGTACATATGATTGGTCTGTGAACCTGTGAGGTGGGTAGGTAGGTAGGTGAGAGA 500
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