Electronic Supplementary Information for

On-point Detection of GM Rice in 20 Minutes with Pullulan as CPA Acceleration Additive

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Pulullan structure

The structure of pullulan consists units of three α-1,4-linked glucose molecules, which are polymerized via -1,6- linkages, as shown in figure S1. The alternation of 1,4 and 1,6 bonds makes pullulan possess the feature of structural flexibility and enhanced solubility. The considerable hydroxyl groups in pullulan molecular enable it to easily establish hydrogen bonds with other substance molecules.[1]

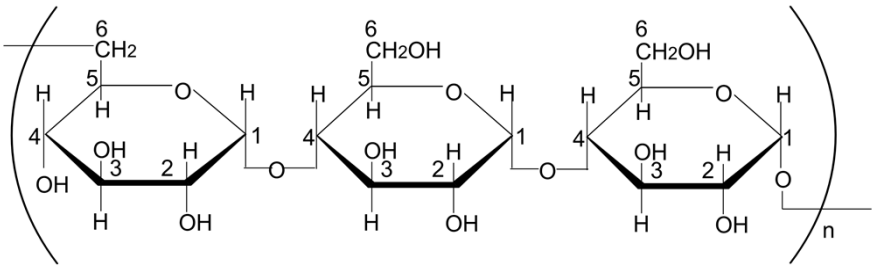

Figure S1 Structure of pullulan

Pullulan solution

The storage concentration of pullulan is 10 % (w/v). Because of the viscosity of high pullulan concentration, the centrifuge tube containing 0.1 g pullulan and 1 mL H_2O was shocked three times in an oscillator (Ultrasonic Instrument Co., Ltd., Kunshan, China). Every concussion and interval continued 30 minutes in order to accelerate the dissolution rate and avoid generating lots of heat simultaneously. The prepared pullulan solution was stored in refrigerator at 4℃.

Template preparation

The material of GM rice (O. sativa) Huahui 1 was provided by Huazhong Agricultural University (Wuhan, China) and the non-transgenic (O. sativa) Minghui 63, was collected by our laboratory. The seeds of rice were ground into fine powder and mixed thoroughly for DNA extraction. Here, the sample was prepared into a mixing level of 0.5 % by adding 0.25 g GM rice powder into 49.75 g non-GM rice powder to a total mixture of 50 g. At the same time, pure GM rice powder was prepared in order to indirectly detect the template copy number of practical samples.

For DNA extraction, the template DNA was extracted by cetyltrimethylammonium bromide (CTAB) method and purified with our home-made silica coated magnetic particles (previous work of our group).[2,3] Three practical samples and three pure transgenic samples of 50 mg were extracted at the same time with exactly the same operation. Hence, it can be considered that the quantity of T-*Nos* in practical samples is 0.5 percent of that in pure transgenic samples. After DNA extraction, the three pure samples were estimated with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Before reading OD values (absorption peaks at 260 nm and 280 nm), 3 μL DNA solution and 27 μL TE buffer were added into the same hole on ELISA plates. Every sample was set into three repeats. As shown in figure 2, all ratios of $A_{260/280}$ were between 1.8 and 2.0 and the average A_{260} value of pure transgenic sample was 0.566. Every A_{260} value equals to 50 ng/μL dsDNA in buffer solution. After a series of calculation and transformation, the final practical template was 750 copies for CPA reaction in this assay.

	Sample 1			Sample 2			Sample 3		
Wells	D12	A10	A11	E12	B10	B11	F12	C10	C11
A_{230}	0.13215	0.111066	0.108254	0.30162	0.025394	0.230006	0.306758	0.207149	0.183013
A_{260}	0.414119	0.45008	0.465234	0.6699	0.637428	0.633649	0.768374	0.515804	0.540575
A_{280}	0.20942	0.233043	0.239102	0.37422	0.345708	0.332481	0.406327	0.273846	0.277687
$A_{260/280}$	1.977457	1.931317	1.945755	1.790123	1.843834	1.90582	1.891024	1.883349	1.946706

Figure S2 OD values of pure transgenic samples. Every sample was set into three repeats.

CPA primers

Specific primers for CPA were designed to target the T-*Nos* gene from the GenBank database (accession no. AJ007624.1, AJ007623.1), which have a set of six specific primers, recognizing eight distinct regions on T-*Nos* sequence. As shown in figure 2, they are respectively two displacement primers (NOSF3 and NOSB3); two detector primers (NOSD5F and NOSDR5B); cross primer NOSPF (NOSPF (f) and NOSPF (b)) and cross primer NOSPR (NOSPR (f) and NOSPR (b)).[3] In addition, NOSDF5F and NOSDR5B are respectively labeled with fluorescein isothiocyanate (FITC) and biotin at 5' end. All of the primers and modified groups used were synthesized from Sangon. Shanghai, China.

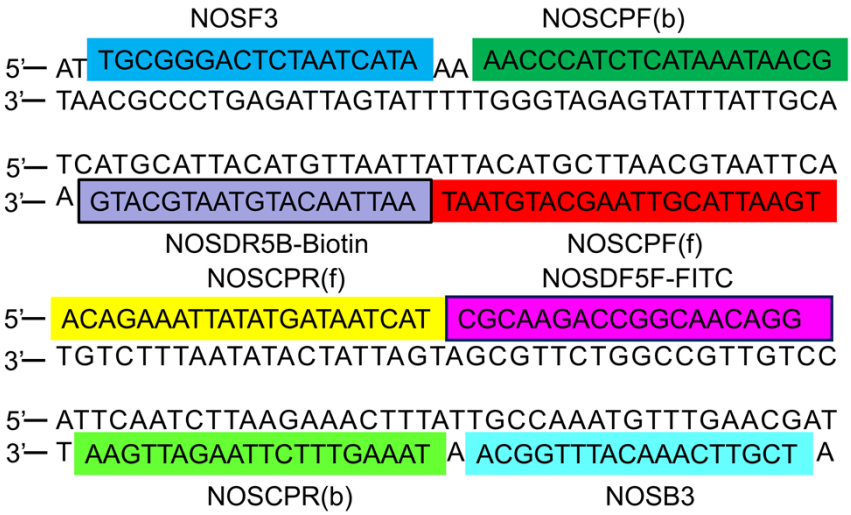

Figure S3 Primers for CPA targeting T-*Nos* gene

CPA reaction system and operation

CPA reactions were carried out in 25 μL reaction mixtures containing 0.1 μM NOSF3 and NOSB3, 1 μM NOSCPF, 0.8 μM NOSCPR, 0.3 μM NOSDF5F and 0.5 μM NOSDR5B, a 0.4 mM concentration of each dNTP, 3 mM $MgCl_2$ (Sigma, St Louis, MO, USA), 6 U Gsp Fast DNA polymerase, GspF Buffer 1* (Ustar Biotech Co., Ltd., Hangzhou, China)，five different concentration gradients of pullulan（Aladdin industrial Co.，Shanghai, China.）respectively at the level of 0.5 %，1 %，2 %，4 %，5 %（w/v）and 1 μL DNA template.

To detect the optimal concentration of pullulan in CPA reaction, five concentration gradients of pullulan mentioned above were determined in this assay with pullulan-free system as control.

The assay was executed in three repeats. CPA reaction was carried out at 63 ℃ for 60 minutes in a simple heat block (MSC-100 Thermo shaker, Hangzhou Allsheng Instruments CO. Ltd., Hangzhou, China). As shown in figure 2, the 1 % (w/v) concentration of pullulan resulted in the maximum quantities of reaction products with the least time.

LFD principle

After nucleic acid amplification, positive products will carry biotin and FITC simultaneously because of the labeled primers and then combine with colloidal gold-labeled FITC-antibody on PVC card to form antigen-antibody conjugates. With siphon force, the conjugates move forward to combine with capture antibodies, and a red color (Test line) appears at the same time. The structure of triple-labeled complex is like "sandwich". While, the no binding colloidal gold-labeled antibodies go on moving and combine with goat anti-mouse IgG and generate a red color (Control line). If no positive products, it will move directly pass the test line and be trapped at the control line by goat anti-mouse IgG and a red color develops there.[4]

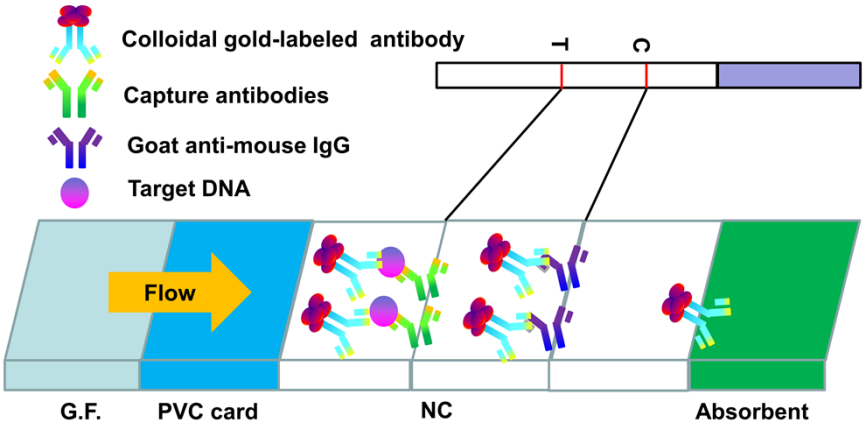

Figure S4 Operational principle of lateral-flow dipstick (LFD)

LFD operation

To further study the acceleration effect of pullulan, LFD method was carried out to simulate real-time detection of CPA products monitored every five minutes. Based on data analysis, the optimal pullulan condition of 1 % (w/v) was directly selected in this assay with pullulan-free system as control. In order to reduce individual differences, reaction system was prepared largely and dispensed into a plurality of the centrifuge tubes. To ensure the simultaneity of DNA amplification, tubes of CPA reaction were carried out at 63 ℃ in a thermo block at the same time. Then, a group of three replicates were taken out every five minutes and cooled simultaneously. Meanwhile, complete SYTO 9-based CPA reaction for the same systems were executed as real-time control of LFD method.

After amplification, 8 μL of the hybridized product was mixed with 100 μL deionized water in a new tube. The commercially prepared LFD strip (LFD, Ustar Biotech Co., Ltd., Hangzhou, China) was then dipped into the mixture for 3 minutes.

LFD and AGE comparison of pullulan-free system

To further confirm the testing results of LFD, samples were processed with agarose gel electrophoresis (AGE) afterwards. In this assay, 10 μL of CPA products were electrophoresed at constant voltage (90 V) on a 3 % (w/v) agarose gel with a 50 bp DNA Ladder (Takara

Biotechnology Co., Ltd., Dalian, China). As shown in figure S5, the sensitivity of LFD method was equal to the limit of detection for the CPA assay followed by AGE. The two methods both displayed positive result at the 25th minute of pullulan-free system, which was nearly ten minutes later than the experimental group and strongly proved the accelerating effort of pullulan.

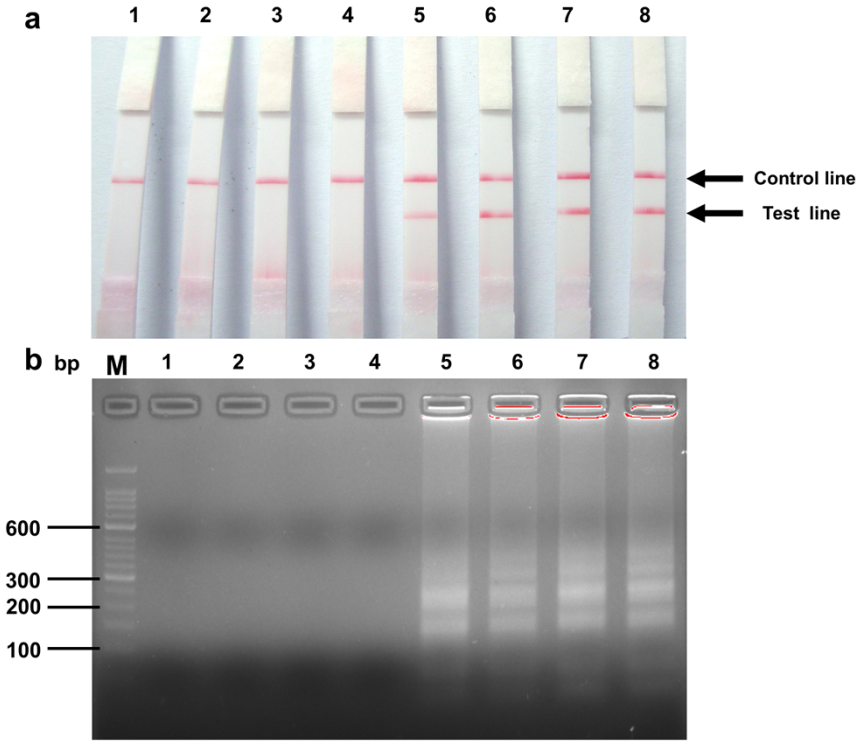

Figure S5 Sensitivity comparison of AGE and LFD assays for T-*Nos* amplification of pullulan-free condition. Lanes from left to right, DNA products amplified for 5, 10, 15, 20, 25, 30, 35, 40 minutes relatively.

References

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4. X. Wang, D. Teng, Q. Guan, F. Tian and J. Wang, *Food Control*, 2013, **29**, 213-220.