Electronic Supplementary Information for

Portable and Amplicon Contamination Prevention Cartridge for DNA Amplification Coupled to Lateral Flow Detection

Rui Wang,[a] Fang Zhang,[a] Jian Wu,[a*] Liu Wang,[a] Yuan Chen,[a] Xiaofu Wang [b] and Junfeng Xu [b]

[a] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China.
[b] Institute of Agriculture Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China.

* (J.Wu) E-mail: wujian69@zju.edu.cn.

Cartridge Fabrication

Three types of cartridges were designed in this paper. For the two horizontal types, they comprise three components, as shown in figure S1 and figure S2, including a reaction tube, an LFD container on its top and an end cap. The wall thickness is 1mm. After assembled of the three components, it can form a completely sealed structure. For the LFD container, its real size is 50 mm x 2 mm x 1mm. The reaction tube is cylindrical. Its inner diameter is 3 mm and the height is 15 mm.

Additionally, as shown in figure S1, in the non-oil sealed horizontal type, the diameter of interconnecting orifice between the LFD container and the reaction tube is 1.5 mm. It can prevent water evaporation during DNA amplification. Conversely, as shown in figure S2, for the oil sealed horizontal type, the cross-section size of interconnecting orifice is as big as possible. The purpose is to let the reaction mixture flow to the LFD strip sufficiently during the operation of reversed shaking.

Besides, there is another type of disposable cartridge called “injector” cartridge. As shown in figure S3, it contains three parts including a reaction tube, a syringe outer tube wall and a syringe piston. After assembled, the cartridge can also be adequately sealed. As shown in figure S3, there is a small bulge portion on the syringe piston. It is a binding site for LFD strip. With this design, LFD strip can keep hanging over the reaction solution before and during DNA amplification. Then, after DNA amplification is completed, the LFD strip will be immersed into the solution under a gentle press on the syringe piston.

Furthermore, the three types of cartridges were produced with 3D printing technology. The components were low-cost and made of photosensitive resin. After printing, the components were polished with acetone vapor. In this assay, the highest printing precision, 0.1 mm, was used. High-volume printing of these components was executed with only an hour needed, which greatly reduced time costs.

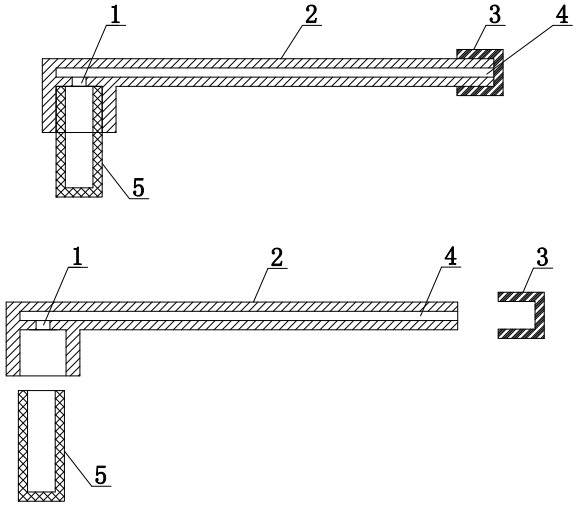

Figure S1 The assembly and exploded view of the non-oil sealed LFD cartridge. Number 1, interconnecting orifice; 2, detection container; 3, end cap; 4, Cavity for LFD strip; 5, reaction tube. The diameter of the interconnecting hole is 1.5 mm. The reaction tube inner diameter and height

are respectively 3 mm and 15 mm.

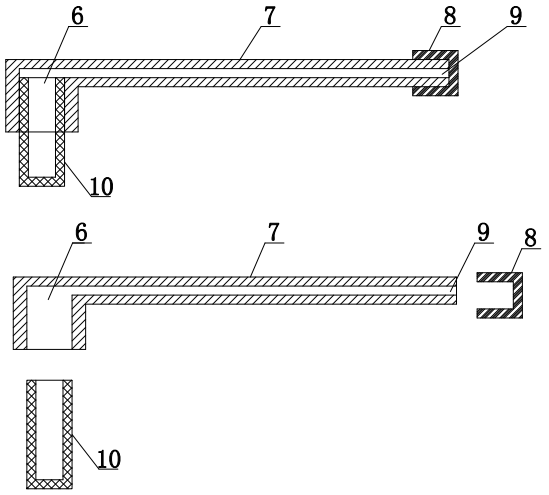

Figure S2 The assembly and exploded view of horizontal type cartridge. Number 6, interconnecting hole; 7, detection container; 8, end cap; 9, LFD container; 10, reaction tube. The reaction tube inner diameter and height are respectively 3 mm and 15 mm.

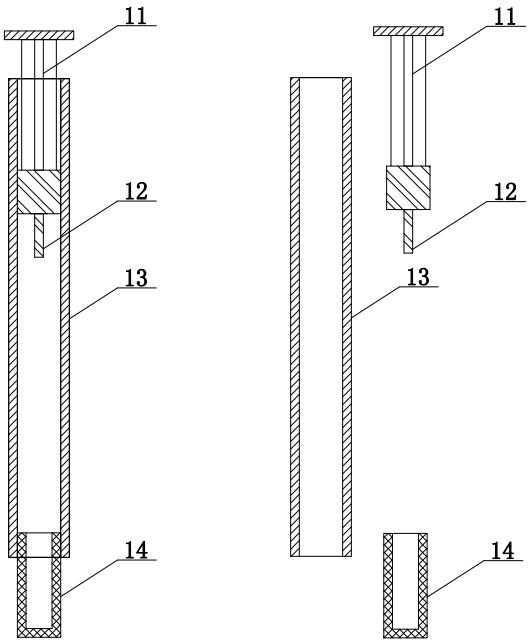

Figure S3 The assembly and exploded view of "injector" cartridge. Number 11, syringe piston; 12, small bulge part of the syringe piston; 13, syringe outer tube wall; 14, reaction tube. The reaction tube inner diameter and height are respectively 3 mm and 15 mm.

DNA Template preparation

The material of genetically modified (GM) rice Huahui 1 was provided by Zhejiang Academy of Agricultural Sciences (Hangzhou, China) and the non-transgenic (O. sativa) Minghui 63 was bought in the market (Hangzhou, China). In order to achieve different doping rates of practical samples, the two kinds of rice seeds were ground into fine powder respectively. Then the two piles of powder were mixed thoroughly as following mixing levels for DNA extraction.

Mixing level	Huahui 1 powder	Minghui 63 powder
0.01%	0.005 g	49.995 g
0.1%	0.05 g	49.95 g
0.5%	0.25 g	49.75 g
1%	0.5 g	49.5 g
100%	50 g	0 g

Figure S4 Preparation of different doping rates of practical samples

DNA extraction

The template DNA was extracted by cetyltrimethylammonium bromide (CTAB) method and purified with our home-made silica coated magnetic particles.[1, 2] Specifically, 500 mg ground rice seeds were placed in a 2 mL centrifuge tube. Then 1 mL lysis buffer (100 mM Tris-HCl, 25 mM EDTA, 1.4 M NaCl, 2% CTAB) and 10 μL proteinase K were added into the tube. The mixture was heating at 65 ºC for 30 min to accelerate cell lysis. After this, the mixture was centrifuged at 12000 g for 15 min and the supernatant was transferred to a new tube. Then added 100 μL silica coated magnetic particles and 300 μLbinding buffer (4 M NaCl, 20% PEG 8000) to the new tube and incubated for 10 min. Next, magnetic particles-DNA conjugates were collected with magnetic separation technology. Followed by, the conjugates were washed two times with 700 μL 70 % (v/v) ethanol solution. Lastly, DNA was eluted from magnetic beads with 50 μL TE buffer. Every real sample was extracted in three replicates. Pure GM rice (100%) powder was prepared in order to indirectly detect the template copy number of practical samples.

Since the pure GM rice samples and practical samples were extracted at the same time with exactly the same operation, the quantity of T-*Nos* in practical samples could be derived from the pure transgenic samples according to doping rates.[2] After DNA extraction, the DNA template of pure samples were detected with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Experimental result showed that the ratios of all $A_{260/280}$ were between 1.8 and 2.0 and the average A_{260}value of pure transgenic sample was 0.566. According to that every A_{260} value equals to 50 ng/μL dsDNA in buffer solution, the 100% pure GM sample template was $1.5x10^5$ copies of T-*Nos* gene. Hence, the practical samples in different doping rates of 0.01%, 0.1%, 0.5%, 1%, whose DNA templates of T-*Nos* gene were respectively 15 copies, 150 copies, 750 copies, 1500 copies.

CPA primers design

Specific primers for CPA were designed to target the T-*Nos* gene. The complete sequence of the T-*Nos* was obtained from the GenBank database (accession no. AJ007624.1, AJ007623.1). A set of six specific primers, recognizing eight distinct regions, were respectively two displacement primers, two detector primers and two cross primers.[3] The primers were designed by Ustar Biotech Co., Ltd. In addition, the two detector primers were labeled with fluorescein isothiocyanate (FITC) and biotin at 5' end respectively. All of the primers and modified groups used were synthesized from Sangon. (Shanghai, China).

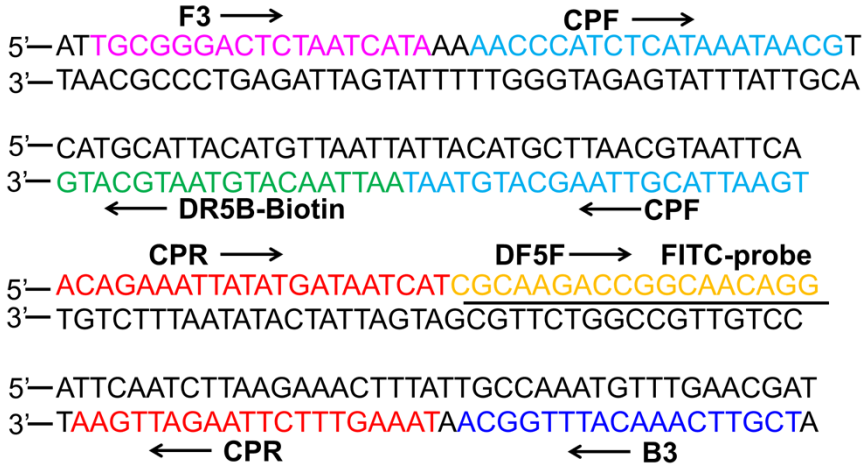

Figure S4 Nucleotide sequence of T-*Nos* gene. The sequences used to design primers F3, B3, CPF, CPR, DF5F and DR5B were shown by different font colors. The FITC-labeled probe and biotin-labeled sequence were shown in underlined yellow font.

Gel electrophoresis of cartridge products

In this assay, the detection results of cartridges were verified with gel electrophoresis. Take "injector cartridge as an example. The identical replicates prepared as following were executed in a thermo block of 63 ºC for an hour with no template as control. Due to the identical template and amplification progress, we identified that the quantity of amplicon products were almost the same in different replicates. After amplification, readouts of some replicates were displayed with a gentle press operation. For the other replicates, gel electrophoresis was carried out for the amplicon products. In gel electrophoresis operation, the agarose gel had been supplemented with Goldview (Sbs Genetech Co., Ltd., Shanghai, China) in preparation. Then, 10 μL amplified mixture was electrophoresed at constant voltage (90 V) on a 3% (w/v) agarose gel. Here, a 50 bp DNA Ladder (Takara Biotechnology Co., Ltd., Dalian, China) was employed for size control of amplicon. The gel was photographed with a ChemiDoc XRS+ System (BioRad, Hercules, CA, USA).

CPA reaction system and operation

CPA reactions were carried out in 25 μL (or 50 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), 1μM SYTO 9 and 1 μL DNA template. CPA reaction was carried out at 63 ºC for 60 minutes in a simple heat block (MSC-100 Thermo shaker, Hangzhou Allsheng Instruments CO. Ltd., Hangzhou, China).

Limitation detection of different types of cartridges

For the initial T-*Nos* template of 750 copies, large quantities of reaction mixture were prepared as above with no template as control. Then, three replicates of 25 μL reaction mixture were added to three horizontal type cartridges and sealed with 15 μL paraffin oil respectively. For the "injector" cartridge, operation was the same with that in horizontal type cartridge. For the non-oil sealed cartridge, three replicates of 50 μL reaction mixture were added to cartridges without

sealing oil. For the other initial templates of 15 copies, 150 copies, 1500 copies, the operations were the same as above. For every initial template, three replicates of 25 μL reaction mixture were added to 200 μL centrifuge tubes with no template as control. Then the cartridges containing reaction mixture were heated in a thermo block of 63 ºC for an hour. The centrifuge tubes were executed SYTO 9-based fluorescent real-time CPA reaction in a MyiQ2 Real Time PCR Detection System (BioRad, Hercules, CA, USA). After amplification, LFD readouts were achieved under simple reversed shake or gentle press. Then, make comparison of the cartridge readout images with fluorescent real-time CPA curves.

Afterwards, false positive contamination was further tested with the melt curve in florescent CPA. As shown in figure S5, the melt curve peak positions of different positive samples were the same and there was no melt curve peak for samples as control. It proved that there was no false positive contamination or non-specific amplification, which further verified the credibility of LFD cartridge detection.

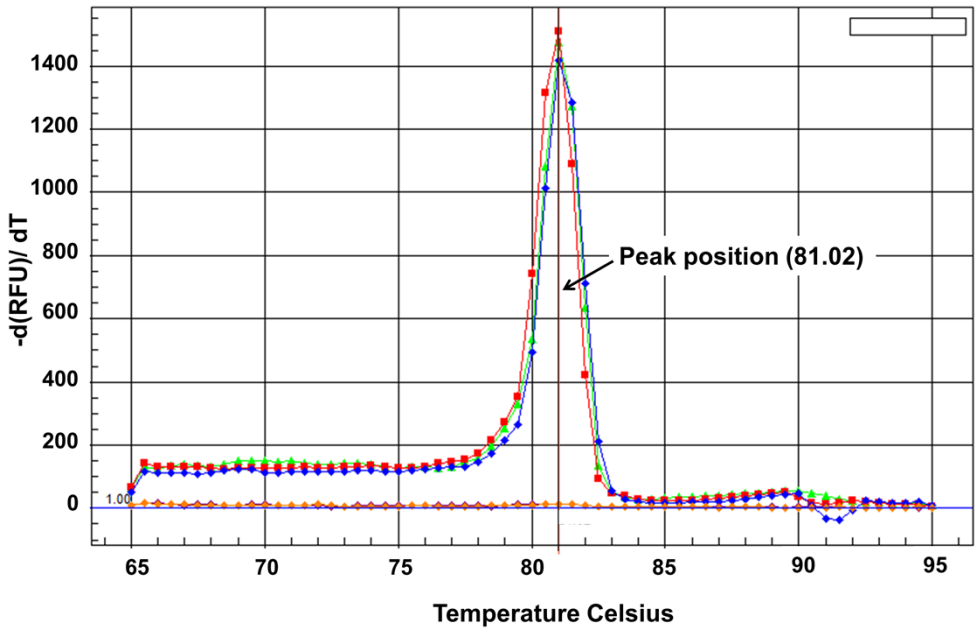

Figure S5 Melt curve of different initial T-*Nos* templates. Red square, 1500 copies; green triangle, 750 copies; blue diamond, 150 copies; orange diamond, 15 copies; purple diamond (overlapped with orange diamond), no template control.

LFD principle

After nucleic acid amplification, positive products will carry biotin and FITC simultaneously because of the labeled primers. There are two kinds of colloidal gold-labeled antibodies located on PVC card. Only one kind of the gold-labeled antibodies can combine with FITC while the other can only connect with the antibody located on the control line. Besides this, on the test line, it is also labeled antibody that can combine with biotin. With siphon effect, positive products will move forward and combine with gold-labeled FITC-antibody to form antigen-antibody conjugates. Then the conjugates go on moving and combine with antibody labeled on the test line through biotin-antibody combination. A red color appears on the test line simultaneously (Test line).

Similarly, the other kind of antibody on the PVC card will move forward under siphon force and combine with antibody labeled on the control line to generate a red color (Control line). If no positive products, the antibody will move directly pass the test line and be trapped at the control line by corresponding antibodies and a red color develops there.[4]

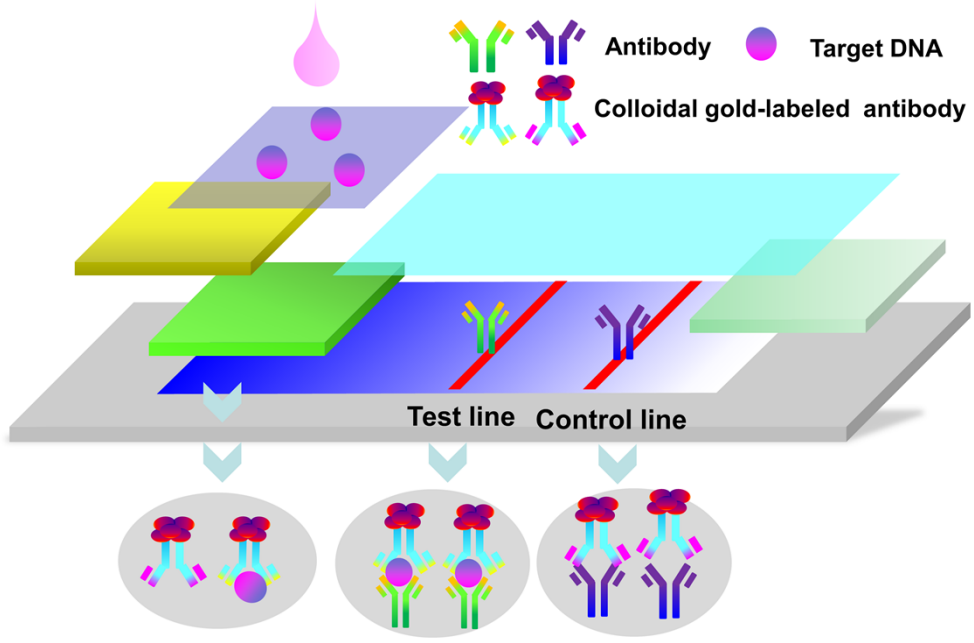

Figure S6 Principle of lateral-flow dipstick (LFD)

References

1. F. Zhang, J. Wu, R. Wang, L. Wang and Y. Ying, *Chemical Communications*, 2014, **50**, 8416-8419.
2. R. Wang, J. Wu, F. Zhang, L. Wang and F. Ji, *Analytical Methods*, 2014, **6**, 9198-9201.
3. F. Zhang, L. Wang, K. Fan, J. Wu and Y. Ying, *Analytical and Bioanalytical Chemistry*, 2014, **406**, 3069-3078.
4. X. Wang, D. Teng, Q. Guan, F. Tian and J. Wang, *Food Control*, 2013, **29**, 213-220.