Electronic Supplementary Information for

A portable pH-inspired electrochemical detection of DNA amplification

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Plant Material

Seeds of GM rice (O. sativa) Huahui 1 were provided by Huazhong Agricultural University, Wuhan, China.

DNA extraction

As described in our previous work, genomic DNA of GM rice was extracted with a cetyltrimethylammonium bromide (CTAB)-based protocol and purified with our home-made silica coated magnetic particles.[1] 500 mg ground rice seeds were placed in a 1.5-mL Eppendorf tube and

suspended in 1 mL preheated lysis buffer (100 mM Tris-HCl, 25 mM EDTA, 1.4 M NaCl, 2% CTAB, pH 8.0) at 65 °C for 30 min with occasionally shaking. Then, 10 μL 20 g L^{-1} proteinase K was added and after another 30 min at 65 °C the mixture was centrifuged at 12000 g for 15 min. The rude cell lysates were then transferred to a fresh 1.5-mL Eppendorf tube, in which subsequently added 100 μL 10 mg mL^{-1} of silica coated magnetic particles suspension and 300 μL binding buffer (4 M NaCl, 20% PEG 8000). After 4 min incubation at room temperature, the magnetic particles-DNA conjugates were collected magnetically with a magnetic separation stand. Discarded the supernatant, washed the conjugates with 700 μL 70 % (v/v) ethanol solution, and dried the conjugates at room temperature. After that, DNA was eluted from magnetic particles by the addition of 50 μL H_2O and incubation at room temperature for 5 min, followed by magnetic separation. The eluent containing the genomic DNA was removed, retained and used as the template for subsequent amplification. DNA concentrations were estimated with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

CPA primers

Specific primers for CPA were designed targeting the T-Nos gene from the GenBank database (accession no. AJ007624.1, AJ007623.1). Sequences of the primers were as followed:

Table S1 Primers for CPA targeting T-Nos gene.

Primer	Sequence
NOSF3	tgcgggactctaatcata
NOSB3	TCGTTCAAACATTTGGCA
NOSCPF	TGAATTACGTTAAGCATGTAATaacccatctcataaataacg
NOSCPR	acagaaattatatgataatcatTAAAGTTTCTTAAGATTGAA
NOSDF5F	cgcaagaccggcaacagg
NOSDR5B	AATTAACATGTAATGCATG

All of the primers used were synthesized from Sangon, Shanghai, China.

pH-CPA reaction

pH-CPA reactions were carried out in 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, 1 M betaine (Sigma, St Louis, MO, USA), 3 mM $MgCl_2$ (Sigma, St Louis, MO, USA), 0.1% Triton X-100, 7.5 mM $(NH_4)_2SO_4$, 3 mM KOH, 47 mM KCl, 6 U Gsp Fast DNA polymerase (Ustar Biotech Co., Ltd., Hangzhou, China), and 1 μL DNA template. All of these reactions were sealed with 50 μL mineral oil for real-time and end-point detection.

For reactions with different concentrations of KOH, the total concentration of K^+ was adjusted to

50 mM by KCl. For example, in the reaction with 3 mM KOH, 47 mM KCl would be added. In this study, CPA reaction was carried out at 63 °C for 50-60 min in a simple heat block (MSC-100 Thermo shaker, Hangzhou Allsheng Instruments CO. Ltd., Hangzhou, China). For electrophoresis assay, the 10 μL of CPA products were electrophoresed at constant voltage (90 V) on a 3 % (w/v) agarose gel, which had been supplemented with Goldview (Sbs Genetech Co., Ltd., Shanghai, China) in TAE buffer solution. A 50 bp DNA Ladder (Takara Biotechnology Co., Ltd., Dalian, China) was used. The gel was photographed with a ChemiDoc XRS+ System (BioRad,CA, USA).

Preparation of pH-sensitive screen printed electrode

Home-made screen-printed electrode was prepared on an automatic screen printer (Z-C3050A, Zheng Ting Screen Printing Machine Co. Ltd., Shanghai, China). The PVC substrate (0.7 mm thick, purchased from a local company) was firstly cleaned with ethanol and dried at room temperature. The multistage printing process involved the sequential deposition of silver layer, graphite working layer and an insulating layer. The working electrode was printed with the well mixed conductive graphite ink (Electrodag 423SS), 9,10-phenanthraquinone (PAQ) and 1,1-dimethylferrocene (Fc) at a ratio of 4:1:1. Then, the prepared electrode was heated in an oven for 30 min at 120 °C to evaporate the solvents. Finally, an insulating layer made from insulating ink (Electrodag 452SS) was printed on the surface of carbon film and then solidified by irradiating with 254 nm ultraviolet. The disposable electrode sizes 23.5 mm × 5 mm × 0.7 mm (shown as Figure 4, working area is 2.13 mm^2), and can be directly inserted into the 200 μL micro tubes.

Electrochemical Measurements

Electrochemical measurements were performed on a CHI 440 electrochemical workstation (CH instruments, USA). Square wave voltammetry (SWV) was selected for the end-point detection of pH-CPA with screen printed electrodes. The SWV parameters were as followed: -0.8 V initial voltage, 0.6 V final voltage, 0.004 V increment, 0.025 V amplitude, 25 Hz frequency, 2 s quiet time and 1 μA sensitivity. The samples for endpoint detection were prepared in parallel. Samples to be detected before and after amplification were both detected at room temperature. The ones after amplification were cooled at room temperature about 20 min before pH electrochemical detection.

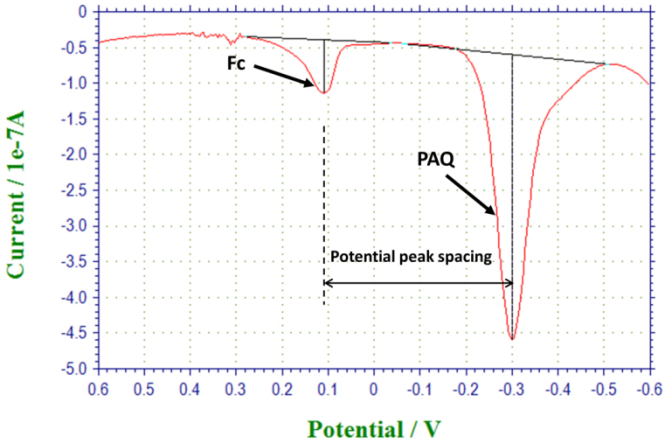

Figure S1 Typical square-wave voltammetry (SWV) response of the pH-sensitive screen-printed electrode in Tis-HCl buffer (50 mM, 9.0).

Standard curve of pH-sensitive printed electrode

Solutions of known Tis-HCl buffer (50 mM) in the range of 7.0-9.0 were prepared for establishing standard curve. The response of this disposable screen-printed electrode was explored over the pH range of 7.0 to 9.0. As shown in Figure S2, the corresponding plot of potential peak spacing of PAQ and Fc against pH was found to produce a value of 75 mV per pH unit at room temperature (25℃) (ΔE_p = 0.075 pH - 0.2674, R^2=0.9918). This 75 mV/pH response is greater than the theoretically predicted Nernstian response 59 mV (PAQ process is known to be 2 H^+ and 2 e^-). Such a difference is also observed in other works and the reasons for it require further investigation.[2, 3]

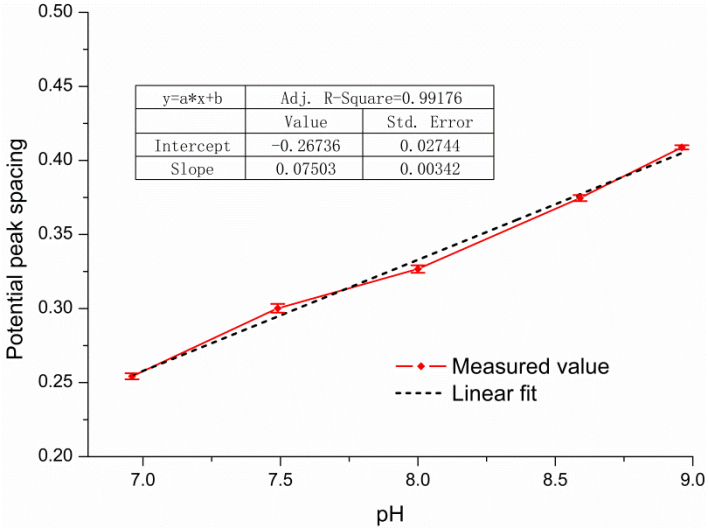

Figure S2 Corresponding plot of the peak potential difference between the PAQ and Fc against pH at 25℃.

A summarized condition for pH-CPA targeting T-Nos gene

The optimized buffer-free condition for pH-CPA targeting T-Nos gene was summarized as below:

Table S2 Optimized buffer-free condition for pH-CPA targeting T-Nos gene.

Component	Final Concentration
NOSF3	0.1 μM
NOSB3	0.1 μM
NOSCPF	1 μM
NOSCPR	0.8 μM
NOSDF5F	0.3 μM
NOSDR5B	0.5 μM
Betaine	1 M
Triton X-100	0.1 %
$MgCl_2$	3 mM
dNTP	0.4 mM
$(NH_4)_2SO_4$	7.5 mM
KOH	3 mM
KCl	47 mM
Gsp Fast DNA polymerase	6 U
Template	100 – 10000 copies

H_2O	Up to 50 ul

References

1. F. Zhang, L. Wang, K. Fan, J. Wu and Y. Ying, *Anal Bioanal Chem.*, 2014, **406**, 3069-3078.
2. A. E. Musa, F. J. del Campo, N. Abramova, M. A. Alonso-Lomillo, O. Domínguez-Renedo, M. J. Arcos-Martínez, M. Brivio, D. Snakenborg, O. Geschke and J. P. Kutter, *Electroanal*, 2011, **23**, 115-121.
3. V. G. H. Lafitte, W. Wang, A. S. Yashina and N. S. Lawrence, *Electrochem Commun*, 2008, **10**, 1831-1834.