Electronic Supplementary Information for

Tracing Phosphate Ions Generated During DNA Amplification and Its Simple Use for Visual Detection of Isothermal Amplified Products

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Experimental Section

Preparation of DNA templates. DNA templates were prepared as described in our previous work from seeds of GM rice (*O. sativa*) Huahui 1. The concentration was determined with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) and diluted into 10^3 copies / μL afterwards before use.[1, 2]

Primers used in this work. Primers targeting T-*Nos* gene were designed with Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA) and synthesized from Sangon, Shanghai, China. Sequences are as follows:

Table S1. Primers used in this work

Category	Order	Primer Name	Sequence
Primers used in	1	NOSF3	tgcgggactctaatcata
CPA assay	2	NOSB3	TCGTTCAAACATTTGGCA
	3	NOSCPF	TGAATTACGTTAAGCATGTAATaacccatctcataaataacg
	4	NOSCPR	acagaaattatatgataatcatTAAAGTTTCTTAAGATTGAA
	5	NOSDF5F	cgcaagaccggcaacagg
	6	NOSDR5B	AATTAACATGTAATGCATG
Primers used in	1	NOSF3	tgcgggactctaatcata
PCR assay	2	NOSB3	TCGTTCAAACATTTGGCA

CPA assay for Pi-induced visual detection. CPA assay was carried out at 63 ℃ for 1h in a 50 μL reaction mixture containing 1 × buffer for Gsp Fast DNA polymerase (Ustar Biotech Co., Ltd., Hangzhou, China), 0.4 mM dNTP each (Sangon, Shanghai, China), primer-mix (with 0.1 μM NOSF3, 0.1 μM NOSB3, 1 μM NOSCPF, 0.8 μM NOSCPR, 0.3 μM NOSDF5F and 0.5 μM NOSDR5B), 3 mM $MgCl_2$ (Sigma, St Louis, MO, USA), 6 U Gsp Fast DNA polymerase (Ustar Biotech Co., Ltd., Hangzhou, China) and 10^3 copies of DNA templates. 0.1 U thermostable inorganic pyrophosphatase (New England Biolabs, Ipswich, MA) was added when necessary. The same reaction mixture without template DNA was used as negative sample. For the endpoint assay of Pi generation, reactions were conducted in a MyiQ2 Real Time PCR Detection System (BioRad, Hercules, CA, USA) and added with syto9 (with a final concentration of 1μM, Invitrogen, Carlsbad, CA, USA) for the purpose of monitoring CPA amplification making sure reactions have been performed normally.

PCR assay for Pi-induced visual detection. PCR assay was carried out with the following thermal-cycled process: 98 ℃ for 2 min, 40 cycles at 98 ℃ for 10 s, 55 ℃ for 30 s and 72 ℃ for 1 min, then 72 ℃ for 5 min in a MyiQ2 Real Time PCR Detection System (BioRad, Hercules, CA, USA). It was carried out in a 50 μL reaction mixture containing 1 × buffer for Taq DNA polymerase(Takara Biotechnology Co., Ltd., Dalian, China), 0.2 mM dNTP each (Sangon, Shanghai, China), primer-mix (with 0.5 μM NOSF3, 0.5 μM NOSB3), 1 U Taq DNA polymerase (BioRad, Hercules, CA, USA) and 10^3 copies of DNA templates. 0.1 U thermostable inorganic pyrophosphatase (New England Biolabs, Ipswich, MA) was added when necessary. The same reaction mixture without template DNA was used as negative sample. For the endpoint assay of Pi generation, reactions were added with syto9 (with a final concentration of 1μM, Invitrogen, Carlsbad, CA, USA) for the purpose of monitoring PCR reaction making sure the reactions have been conducted normally.

Electrophoresis assay. Amplified products were electrophoresed at 90 V on a 3 % (w/v) agarose gel (pre-supplemented with Goldview, Sbs Genetech Co., Ltd., Shanghai, China) in TAE buffer. A 50 bp or 20 bp DNA ladder (Takara Biotechnology Co., Ltd., Dalian, China) was employed as marker. The gel was photographed with a ChemiDoc XRS+ System (BioRad, CA, USA)

Standard curve for Mo-Sb-Vc detection of Pi. Standard curve for phosphate ion detection was established by adding 50 μL Mo-Sb-Vc coloration reagentsto 50 μL 0, 10, 20, 60, 100 μM phosphate solution respectively, making a 100 μL solution with a final phosphate ions concentration of 0, 5, 10, 30, 50 μM in a 96-well plate. The Mo-Sb-Vc coloration reagents described above consist of two separate parts: Vc solution (ascorbic acid with a final concentration of 0.4 %) and Mo-Sb solution (containing 1.68 mM ammonium molybdate, 0.08 mM potassium antimonyl tartrate, 0.4 M sulphuric acid). The Vc solution and Mo-Sb solution were added orderly into samples for coloration analyse. After about 10 min, the absorbance of sample at 700 nm was measured with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). All the samples in 96-well plate were sealed with 50 μL mineral oil.

Visual detection of amplified products. Amplified products were diluted to a volume of 50 μL in a 96-well plate before the visual detection. Diluted samples were then added with 50 μL coloration reagents (including 0.4 % ascorbic acid and 1.68 mM ammonium, 0.08 mM potassium antimonyl-tartrate in 0.4 M sulphuric acid) and sealed with 50 μL mineral oil afterwards. After about 10 min's reaction, they were measured at 700 nm with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) or photographed with camera. For the quantification assay of Pi generation in CPA or PCR, amplified products were diluted from 10 to 80 times and estimated with SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) respectively. These diluted samples within the linear range of standard curve were considered to be effective for Pi quantification and used for the calculation of Pi concentration in the original solution. For qualitative assay, amplified samples were usually diluted 10 times before the addition of coloration reagents.

LAMP assay detected with Pi-induced visual strategy. Commercial LAMP kit (PA101 S, Deaou Biotechnology Co., Ltd., Guang Zhou, China) targeting T-*Nos* was employed to prove the feasibility of Pi-induced visual strategy for the detection of other kinds of isothermal amplification besides CPA. LAMP assay was carried out at 63 ℃ for 1h in a 50 μL reaction mixture containing 44 μL reaction mix, 2 μL Bst DNA polymerase, 0.1 U thermostable inorganic pyrophosphatase ((New England Biolabs, Ipswich, MA), and 10^3 copies of DNA templates. Both of quantitative and qualitative assay for Pi-generation in LAMP were analysed.

Supporting Figures

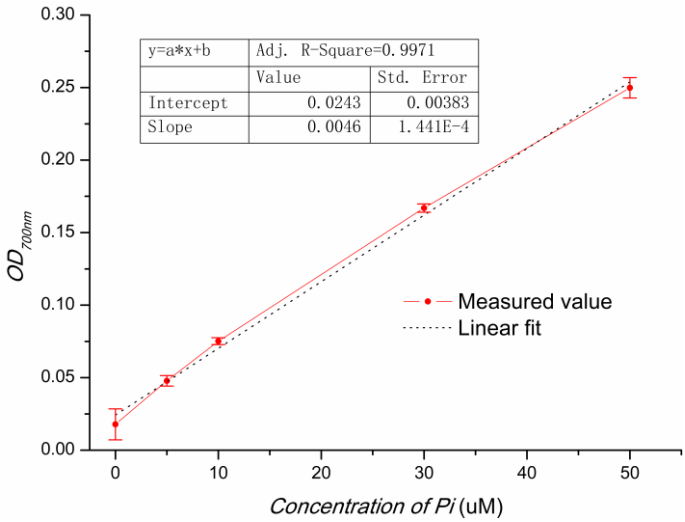

Figure S1. Standard curve for Mo-Sb-Vc detection of Pi. The corresponding plot of Pi concentration against OD_{700nm} was found to be OD_{700nm} = 0.0046 Pi - 0.0243, R^2=0.9971. Value of OD_{700nm} from 0.025 to 0.23 was considered to be effective range for Mo-Sb-Vc quantitation of Pi.

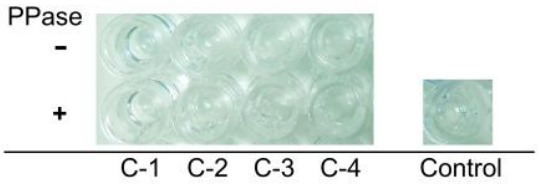

Figure S2. Pictures for the study of dNTPs decomposition under CPA condition. (-) means sample without PPase and (+) means samples with PPase. C-1: samples with dNTPs; C-2 : samples with dNTPs and 10 × buffer for GspF; C-3: samples with dNTPs, 10 × buffer for GspF, and Mg^{2+}; C-4: samples with dNTPs, 10 × buffer for GspF, Mg^{2+} and GspF DNA polymerase; Control: H_2O. All the samples and the control were treated with Mo-Sb-Vc coloration reagent for about 10 min and sealed with 50 μL mineral oil. There was no difference in colour observed with naked eyes between samples and the control, which indicated that almost no template-independent decomposition of dNTP occurred under CPA isothermal condition.

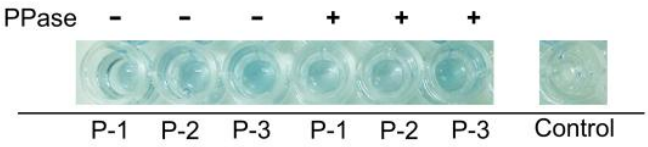

Figure S3. Pictures for the study of dNTPs decomposition under PCR condition. (-) means sample without PPase treatment and (+) means samples with PPase treatment. P-1: samples with dNTPs; P-2: samples with dNTPs and 10 × buffer for Taq (Mg^{2+} plused); P-3: samples with dNTPs, 10 × buffer for Taq, and Taq DNA polymerase; Control: H_2O. All the samples and the control were treated with Mo-Sb-Vc coloration reagent for about 10 min and sealed with 50 μL mineral oil. Both of the P-3 samples with or without PPase were

shown in light blue, which would result in a false positive readout. That is, traditional PCR thermal-cycled condition would cause an obvious decomposition of dNTP and not suitable for the application of Pi-based visual detection of DNA amplification.

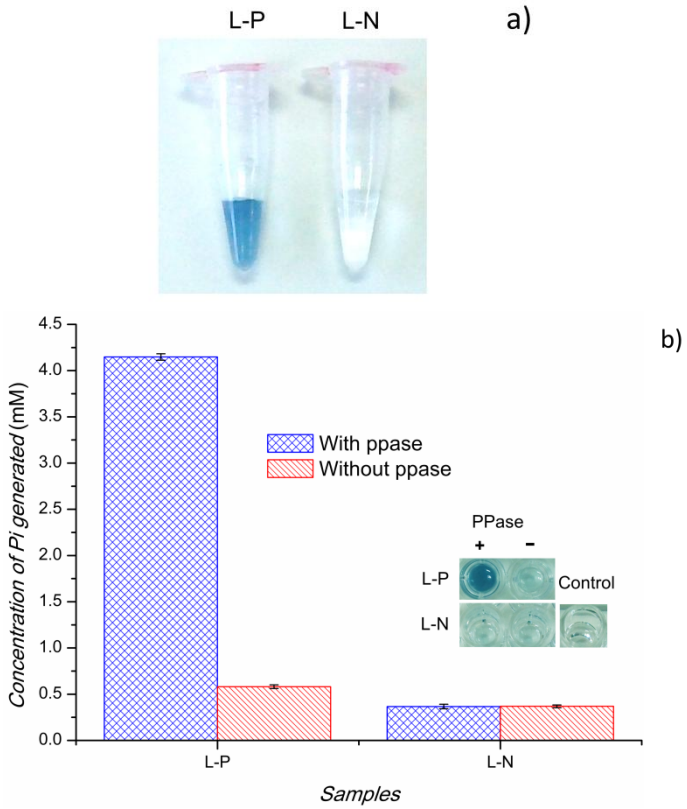

Figure S4. Pi-induced visual detection of LAMP. (a) Visual detection of LAMP amplified products in micro tubes, which were diluted 50 times before detection. (b) Bar graph for the quantitation of Pi generated during LAMP and pictures for the qualitative detection of 10 times diluted samples in 96-well plate. (-) means sample without PPase (red in bar graph) and (+) means samples with PPase (blue in bar graph). During LAMP amplification, with the addition of PPase about 4 mM phosphate ions have been generated in positive sample and less than 0.5 mM in negative one. Similar with the situation in CPA isothermal amplification, there was a significant difference between the freshly amplified samples with or without PPase treatment. The results indicated that with the addition of PPase this Pi-induced visual detection strategy can be successfully used for LAMP amplification detection.

References

1. F. Zhang, J. Wu, R. Wang, L. Wang and Y. Ying, *Chem Commun*, 2014, **50**, 8416-8419.

2. F. Zhang, L. Wang, K. Fan, J. Wu and Y. Ying, *Anal Bioanal Chem.*, 2014, **406**, 3069-3078.