PCP
PLANT & CELL PHYSIOLOGY

Letter to the Editor: DNA Purification-Free PCR from Plant Tissues

Zhengnan Jia[1,2,3], Miaomiao Ding[1,2,3], Masahito Nakano[1,2,3], Kunqi Hong[1,2,3], Ruidong Huang[1,2,3], Dieter Becker[4], Jane Glazebrook[5], Fumiaki Katagiri[5], Xiaowei Han[1,2,3] and Kenichi Tsuda[1,2,3,*]

[1]State Key Laboratory of Agricultural Microbiology, Hubei Key Lab of Plant Pathology, Hubei Hongshan Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[2]Shenzhen Institute of Nutrition and Health, Huazhong Agricultural University, Wuhan 430070, China
[3]Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, Guangdong 518120, China
[4]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Carl-von-Linne Weg 10, Cologne 50829, Germany
[5]Department of Plant and Microbial Biology, Microbial and Plant Genomics Institute, University of Minnesota, Twin-Cities, Saint Paul, MN 55455, USA

*Corresponding author: E-mail, tsuda@mail.hzau.edu.cn

(Received 2 June 2021; Accepted 28 July 2021)

Amplification of genomic DNA fragments by polymerase chain reaction (PCR) is essential for plant molecular biology approaches such as genotyping, sequencing and mutant screening (Hamajima 2001, Stepanova and Alonso 2006, Emonet et al. 2007). PCR using plant tissues without DNA purification is often unsuccessful because contaminants from plant tissues inhibit PCR. Therefore, laborious or costly genomic DNA purification is required (Tan and Yiap 2009). Among commercially available DNA purification kits, PlantSaver Whatman Flinders Technology Associates (FTA) card has several advantages. Plant tissue can be pounded to the cellulose-based FTA card and a small punch of FTA card containing plant tissue can be used directly as the PCR template after purifying DNA by several washing steps, eliminating the DNA elution step by directly amplifying DNA from the membrane. Plant samples on the FTA card can be stored at room temperature for years without any special equipment (GE Healthcare 2011). However, the DNA purification step is laborious and costly, undermining throughput.

In order to decrease labor and increase throughput in PCR-based molecular diagnostic, we explored the possibility of eliminating the DNA purification step for PlantSaver FTA card but failed to detect PCR products when we directly used *Arabidopsis thaliana* leaves pounded to PlantSaver FTA card (**Fig. 1B**). We tested known suppressors of PCR inhibitors such as Tween-20, a nonionic detergent (Sekikawa et al. 2008, 2011). Strikingly, we detected PCR products when we added 2–14% Tween at the final concentration in the PCR mixture, allowing us to skip the DNA purification step (**Fig. 1B** and **Supplementary Fig. S1A**). We used 4% Tween-20 condition in the following experiments because it appears to be the optimum condition for PCR and less concentration would be favorable for downstream applications (see Supplementary methods for detail).

Next, we explored the possibility to make a homemade cellulose-based card as a replacement of the expensive PlantSaver FTA card. We selected two different cellulose-based Whatman filter papers and submerged them into buffer containing SDS, which would contribute to tissue lysis and DNA protection. After drying the paper, we pounded plant tissues to the paper and took a small punch of the paper containing plant tissue directly followed by PCR (**Fig. 1A**). These homemade cards worked as well as the PlantSaver FTA card (**Fig. 1B** and **Supplementary Fig. S1A**). This procedure is simple without need for special chemicals or equipment, and therefore, any laboratories would be able to produce their own cards.

To test the robustness of the method, we used two additional primer sets with replications (**Supplementary Fig. S1B**). We found robust amplification of PCR products in all tested primer sets. Furthermore, PCR efficiency did not decrease when we used cards on which plant tissues were pounded 3 or 6 months ago (**Fig. 1B** and **Supplementary Fig. S1B**). These plant-tissue-pounded cards were stored in a desk drawer at room temperature. Thus, DNA on these papers was PCR-compatible for a long period, likely for several years without any special conditions (GE Healthcare 2011).

To demonstrate the utility of our method, we genotyped three *A. thaliana* T-DNA insertion mutants. We were able to distinguish wild-type and T-DNA insertion mutant plants in all tested cases (**Fig. 1C** and **Supplementary Fig. S1C**). In addition to these T-DNA insertion lines, we routinely genotype various lines, indicating the high utility of this method (**Supplementary Table S1**).

We tested whether we could amplify longer fragments by our method. Although we could detect PCR products longer than 2 kb using purified genomic DNA, the card-based systems were less efficient to amplify longer fragments, especially for homemade cards (**Supplementary Fig. S1D**). While this is a limitation of our method, primers can be designed to generate shorter fragments for genotyping. Besides, we do not recommend our method for cloning genes, as we do not

Plant Cell Physiol. 62(10): 1503–1505 (2021) doi:https://doi.org/10.1093/pcp/pcab120, Advance Access publication on 19 August 2021, available online at https://academic.oup.com/pcp

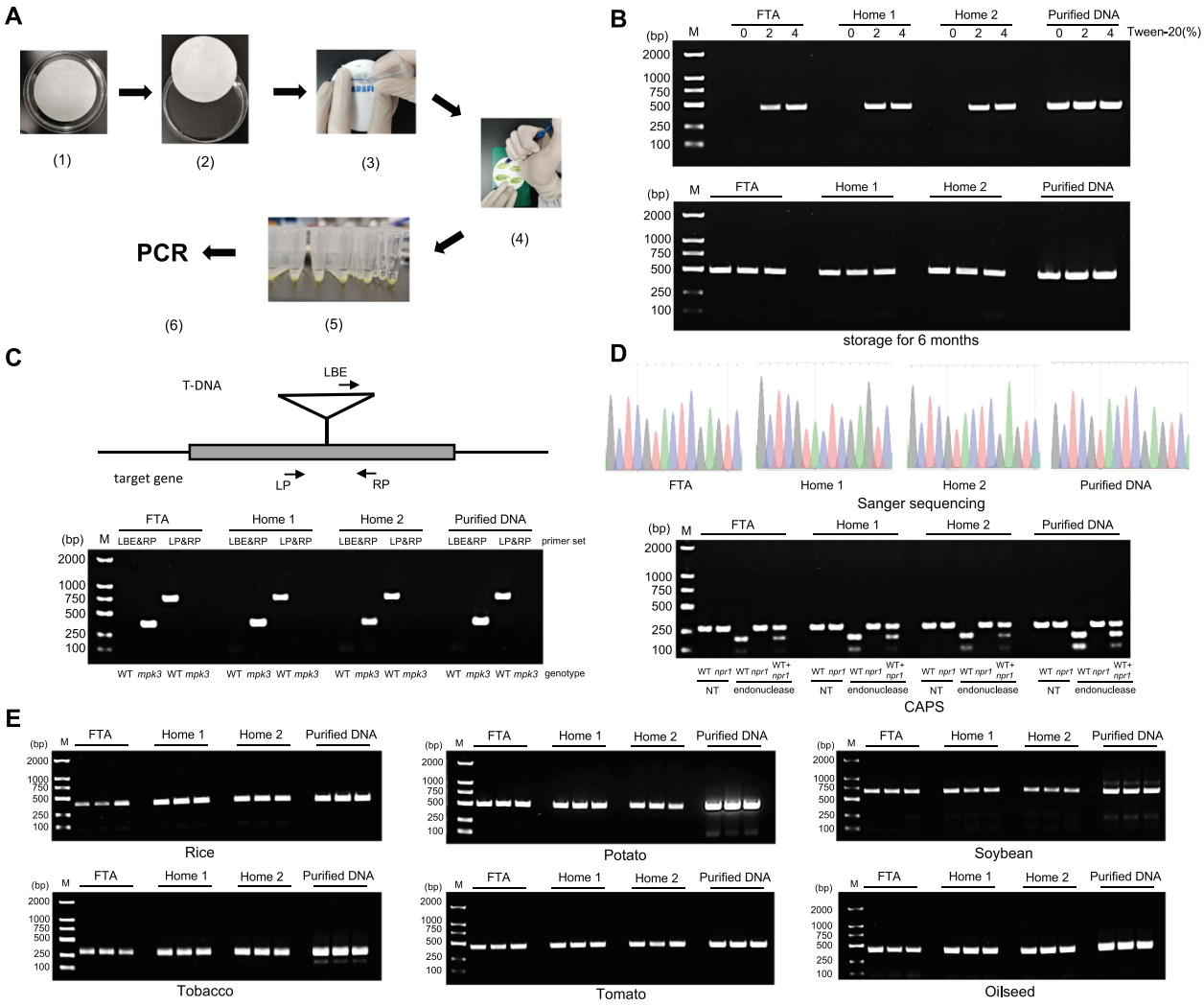

Fig. 1 (A) Schematic representation of sample preparation (for detail, see Supplementary methods). (1) Submergence of a filter paper in the soaking buffer. (2) Air-drying the filter paper at room temperature (RT). (3) Applying leaf samples to FTA or the homemade paper card using parafilm followed by air-drying at RT. (4) Punching a disk with *A. thaliana* leaf samples. (5) (6) Adding the disk into PCR mixture directly followed by PCR. (B) Tween-20 facilitates PCR without DNA purification. A disk from the same PlantSaver FTA Card (FTA) or homemade card (Home 1 or Home 2) or purified *A. thaliana* genomic DNA was added to PCR mixture with 0–4% of Tween-20 as template to amplify *A. thaliana PBS3* gene fragment (upper). The card with *A. thaliana* tissue stored for 6 months was used to amplify (4% Tween-20) the same *PBS3* gene fragment. Three replicates (three different disks from the same plant leaf sample on the same card) are shown (bottom). (C) Schematic representation of T-DNA insertion (upper) and genotyping of *A. thaliana* T-DNA insertion mutants (bottom). (D) Sanger sequencing (upper) and CAPS marker analysis of *A. thaliana npr1-1* mutant (bottom). PCR products (*PBS3* gene fragment) without purification were analyzed by Sanger sequencing and 88–101 bases are visualized as examples (upper). PCR products for *NPR1* gene fragment were directly digested with restriction endonuclease NlaIII, NT, PCR products without endonuclease digestion (bottom). (E) The cellulose-based card system is widely applicable to multiple plant species. A disk of three different plant leaf samples on the same PlantSaver FTA Card (FTA) or homemade card (Home1 or Home 2) or purified plant genome DNA was used for PCR in PCR mixture containing 4% Tween-20.

know the error rate introduced during PCR in high Tween-20 concentrations.

Compared to purified genomic DNA, PCR products using our card-based system may contain compounds that inhibit downstream applications. Therefore, we tested whether PCR products can be directly used for Sanger sequencing without purification of the PCR product. We observed good quality signals with the card-based systems as purified DNA (**Fig. 1D** and **Supplementary Fig. S1E**). Next, we tested if we could perform Cleaved Amplified Polymorphic Sequences (CAPS) marker analysis directly using PCR products without purification. We could distinguish wild-type, homozygous mutant and pseudo-heterozygous (mixed wild type with homozygote) mutant plants (**Fig. 1D** and **Supplementary Fig. S1E**). Until now, we tested 15 restriction enzymes for CAPS marker-based genotyping (**Fig. 1D**, **Supplementary Fig. S1E**

and **Supplementary Table S2**), all of which were successful. Taken together, these results indicate that PCR products generated by this method could be directly used for downstream applications such as Sanger sequencing and CAPS marker analysis.

We investigated whether our method can be used for other plant species. We used 11 additional plant species including major crops, such as rice, potato, soybean and tobacco plants and found robust PCR amplification for all tested species except for tea plants (**Fig. 1E** and **Supplementary Fig. S1F**). Thus, our method can be used to amplify genomic DNA fragments from many plant species if not all.

Our cellulose-based card system has a wide applicability to PCR-based molecular approaches including genotyping T-DNA insertion mutants, Sanger sequencing and CAPS marker analysis. Our method has advantages over existing methods. First, our method is simple and rapid. A punch of plant tissue pounded to the paper card is directly put into the PCR mixture, eliminating a DNA purification step. This dramatically increases throughput, which would allow a researcher to analyze over 1,000 samples per day. Second, our method is low cost. We have shown that ordinary cellulose membrane can be transformed to a DNA storage card with a simple procedure and plant tissues on the card can be stored at room temperature without any special equipment. This also allows researchers to perform multiple PCR amplification from the same sample, which is required, for instance, for generation of high-order and combinatorial plant mutants (Tsuda et al. 2009). Simple, rapid and inexpensive sampling procedure followed by PCR in our method also allows for molecular diagnostic assays involving DNA amplification by PCR from plant genomes in limited resource conditions such as classrooms, field conditions and developing countries.

Supplementary Data

Supplementary data are available at PCP online.

Data Availability

The data underlying this article are available in the article and in its online supplementary material.

Funding

This work was supported by the Fundamental Research Funds for the Central Universities (Program No. 2662020ZKPY009) (to K.T.), the Huazhong Agricultural University Scientific and Technological Self-innovation Foundation (to K.T.), and Joint Funding of Huazhong Agricultural University and Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences (SZYJY2021007) (to K.T. and X.H.).

Acknowledgements

We thank Tsuda lab members for critical comments on the manuscript and Jin Hu, Zhiheng Wu, Xiubao Hu, Lan Wei, Dabing Yang, Xinying Wu and Yuanfang Jiao for providing plant materials.

Disclosures

The authors have no conflicts of interest to declare.

References

Emonet, S.F., Grard, G., Brisbarre, N.M., Moureau, G.N., Temmam, S., Charrel, R.N., et al. (2007) Long PCR Product Sequencing (LoPPS): a shotgun-based approach to sequence long PCR products. *Nat. Protoc.* 2: 340–346.

GE Healthcare. (2011) FTA Cards Manual. https://www.lobov.com.ar/downloads/FTA%20Cards2.pdf (August 5, 2021, date last accessed).

Hamajima, N. (2001) PCR-CTPP: a new genotyping technique in the era of genetic epidemiology. *Expert Rev. Mol. Diagn.* 1: 119–123.

Sekikawa, T., Kawasaki, Y., Katayama, Y. and Iwahori, K. (2008) Suppression of *Bst* DNA Polymerase Inhibition by nonionic surfactants and its application for *Cryptosporidium parvum* DNA detection. *Japn. J. Wat. Treat. Biol.* 44: 203–208.

Sekikawa, T., Kawasaki, Y., Katayama, Y. and Iwahori, K. (2011) A simple method for extracting DNA from Cryptosporidium oocysts using the anionic surfactant LSS. *N. Biotechnol.* 29: 139–143.

Stepanova, A.N. and Alonso, J.M. (2006) PCR-based screening for insertional mutants. *In* Arabidopsis Protocols. pp. 163–172.Humana Press, New Jersey.

Tan, S.C. and Yiap, B.C. (2009) DNA, RNA, and protein extraction: the past and the present. *J. Biomed. Biotechnol.* 2009: 1–10.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J. and Katagiri, F. (2009) Network properties of robust immunity in plants G. P. Copenhaver, ed. *PLoS Genet.* 5: e1000772.