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Protocols

A Rapid DNA Minipreparation Method Suitable for AFLP and Other PCR Applications

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Abstract. A rapid DNA minipreparation method was developed for rice and other plant species. This method uses an Eppendorf tube and 1-ml pipette tip to grind plant tissues, and requires only one transfer for DNA isolation. In a single day, one person can complete DNA isolation from more than 120 leaf samples. The yields of the DNA samples ranged from 2.3 to 5.2 μg from 25–50 mg fresh leaf tissue. DNA samples extracted using this method from rice were completely digested with five restriction enzymes (*Eco*R I, *Eco*R V, *Hin*d III, *Mse* I and *Pst* I) and were successfully used for AFLP and other PCR applications.

Key words: CTAB, DNA extraction

Introduction

PCR-based methods are widely used in plants for marker-assisted breeding and high-resolution mapping. Because these studies require analysis of large populations, a DNA extraction method, which is fast, inexpensive and yields high quality DNA, is desired.

Rapid and efficient rice DNA extraction methods suitable for PCR have previously been developed (Williams and Ronald, 1994; Zheng et al., 1995). These methods, however, do not work well for AFLP analysis as yield is low, quality is poor and there are risks of cross contamination (Chen and Ronald, unpublished).

A rapid CTAB DNA isolation technique for extracting DNA from five plant species and one fungus has been proven effective for PCR analysis

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(Stewart and Via, 1993). Barley DNA prepared using this method was successfully used for AFLP analysis and high-resolution mapping of the barley *MLO* locus (Büschges et al., 1997). However, since rice leaves are composed of high quantities of cellulose, lignin, silica cells, wax, etc., leaf samples cannot be easily homogenized using this method (Chen and Ronald, unpublished). We have now modified the CTAB method for rapid isolation of DNA from rice and other species. The DNA is suitable for AFLP analysis and other PCR-based applications. Using this method, high quality DNA samples from a segregating population consisting of 2200 rice plants were extracted by a single person in less than twenty days.

Materials and Methods

Leaves of rice, grape, maize, squash, tomato, peppermint, and walnut were collected and set in ice. An Eppendorf 1 ml plastic pipette tip (Out Patient Service, Inc) was bent by pressing the tip against the bench. The bent pipette tip was then mounted on a disposable pellet pestle (VWR Scientific Cat. KT749520-0000) attached to a drill (Black & Decker (US) Inc) to serve as the homogenizing pestle. A fragment of leaf sample (25–50 mg) was rounded into a ball on the end of a forceps and put into a 1.5-ml Eppendorf tube on ice. The leaf segment must be folded or rounded rather than cut into pieces before being placed in an Eppendorf tube to avoid losing the leaf tissue when adding the liquid nitrogen. Using forceps, the uncapped tube with leaf or root tissue was dipped into liquid nitrogen to allow the liquid nitrogen enter the tube. It was then homogenized for 20 s at full speed. Immediately after homogenization, the tissue powder was added to 700 μl pre-warmed (65 °C) extraction buffer [2% w/v CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 2% w/v polyvinylpyrrolidone (PVP-40), 5.0 mM ascorbic acid, 4.0 mM diethyldithiocarbamic acid (Doyle and Doyle 1990)]. 7 μl Rnase A (20 mg/ml) was added and the mixture stirred and incubated at 65 °C for approximately 5 min. 570 μl of a chloroform: isoamyl alcohol mixture (24:1) was then added, and the mixture was shaken by hand and centrifuged at a full speed (13,000 rpm) for 10 min at room temperature. The upper DNA containing phase was transferred to a new Eppendorf tube. DNA was precipitated by adding 0.7 vol of isopropanol, mixing and immediately spinning at full speed for 5 min. The DNA pellet was washed with 70% ethanol, air-dried and suspended in 15 μl of TE. The extracted DNA was subjected to five restriction enzyme digestions (*Eco*R I, *Eco*R V, *Hin*d III, *Mse* I and *Pst* I) and to AFLP (Vos et al., 1995) and PCR analysis.

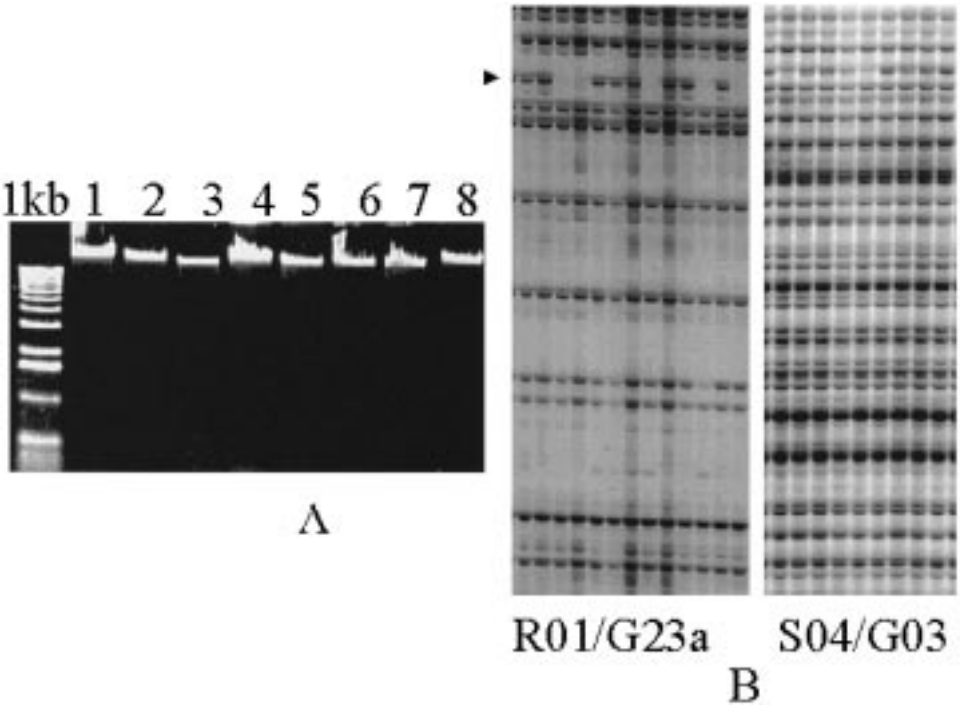

Figure 1. A. Genomic DNA was extracted from leaves of the following plant species using the rapid minipreparation method: 1 = rice from seedling stage, 2 = rice at flowering stage, 3 = adult walnut, 4, 5 = tomato, 6 = grape, 7 = squash, 8 = peppermint. B. Autoradiogram of a polyacrylamide gel showing AFLP markers amplified with primers (R01/G23 and S04/G03) flanking the *Pi5*(*t*) locus. The bands indicated by arrows cosegregate with *Pi5*(*t*).

Results and Discussion

A high yield of good quality DNA was obtained using our CTAB method. One hundred twenty DNA samples were randomly sampled from the 2200 samples and quantified using a DNA fluorometer. The yields of the DNA samples ranged from 2.3 to 5.2 μg from 25–50 mg fresh leaf tissue, enough to conduct 100 to 500 PCRs, or one restriction digestion for RFLP analysis. DNA were completely digested with five different restriction enzymes (*Eco*R I, *Eco*R V, *Hin*d III, *Mse* I and *Pst* I).

Not only was high quality DNA extracted from young rice tissues, this method also worked well for extracting DNA from the flag leaf of mature rice plants, and from leaves of adult plants of maize, tomato, peppermint, squash, grape and walnut (Figure 1).

This method is much more efficient than the fast prep methods previously reported for rice DNA extraction (Williams and Ronald 1995; Zheng et al.,

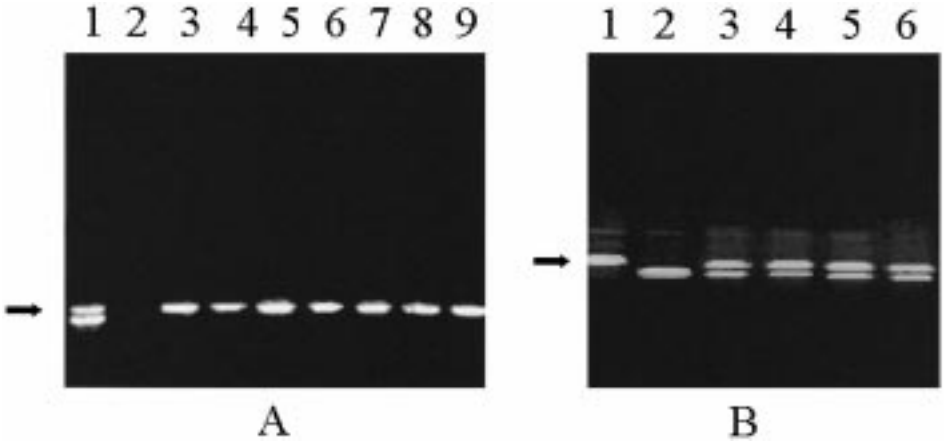

Figure 2. A. PCR detection of *Xa21D* in progeny of transgenic rice with primer pair DR-1and PB822-3. DNA was extracted using rapid minipreparation method. The band indicated by an arrow is a 1.9 kb DNA fragment amplified from *Xa21D* LRR domain. B. PCR detection of *Xa21* in progeny of transgenic rice with primer pair 3′ Xa21 R and H3 Frag F. DNA extracted using rapid minipreparation method. The band indicated by an arrow is a DNA fragment of 1.4 kb amplified from *Xa21* gene. Sample orders in panel A are as follows: 1 = IRBB21; 2 = TP309; 3, 4, 5, 6, 7, 8, 9 = *Xa21* D plants. In panel B, 1 = IRBB21; 2 = TP309; 3, 4, 5, 6 = *Xa21* plants. IRBB21 is the non-transgenic donor of *Xa21* and *Xa21D*. TP309 is the recipient used in transformation experiments.

1995). In a single day, one person can complete DNA isolation from more than 120 leaf samples. DNA from 2200 individuals of a large segregating population for a rice blast resistance gene *Pi5*(*t*) were extracted in less than twenty days using this method.

This method has been routinely used to extract DNA from rice for PCR-based applications in our laboratory. For construction of a high-resolution map of the rice blast resistance gene *Pi5*(*t*), 2200 DNA samples were extracted using this method. AFLP markers flanking *Pi5*(*t*) (R01/G23a and S04/G03) were then used to identify rare recombinants (Chen et al., 1997) (Figure 1). We also used this method to identify transgenic rice lines containing the *Xa21* and *Xa21D* transgenes conferring bacterial blight resistance (Song et al., 1995; Wang et al., 1998). Seedlings carrying *Xa21* or *Xa21*D were quickly identified by PCR analysis at the early seedling stage using specific primers (Figure 2). The presence of *Xa21* or *Xa21*D in these plants was confirmed by inoculating the seedlings with an incompatible strain of *Xanthomonas oryzae* pv *oryze* (Wang et al., 1998). DNA extracted using this method was also used for RAPD analysis for screening closer markers linked to the *Pi5*(*t*) locus (data not shown).

DNA sample contamination associated with re-used homogenizer and vessels (Zheng et al., 1995) and handling during extraction is unacceptable if the DNA extracted is to be used for PCR-based marker analysis, such as RAPD and AFLP. Though the procedure described by Stewart and Via (1993) has only a single Eppendorf tube transfer for DNA extraction, the buffer and sample mixture can be easily spilled over during homogenization. The CTAB method described here uses liquid nitrogen during homogenization greatly reducing the risk of cross contamination.

The procedure described here works well for extracting high quality DNA from all plant tissue samples tested and should be widely applicable for analysis of large populations from virtually all plant species.

References

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F and Schuize-Lefert P (1997) The barley *Mlo* gene: A novel control element of plant pathogen resistance. Cell 88: 695–705.

Chen D, Wang GL and Ronald PC (1997) Location of the rice blast resistance locus *Pi5(t)* in Moroberekan by AFLP bulk segregant analysis. Rice Genetics Newsletter 14: 95–98.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Stewart CN Jr and Via LE (1993) A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14: 748–758.

Song W-Y, Wang G-L, Chen L, Kim H-S, Pi L-Y, Gardner J, Wang B, Holsten T, Zhai W-X, Zhu L-H, Fauquet C and Ronald PC (1995) A receptor kinase-like protein encoded by the rice disease resistance gene Xa21. Science 270: 1804–1806.

Vos P, Hogers R, Bleeker M, Rijans M, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995) A new technique for DNA fingerprinting. Nucl Acid Res 23: 4407–4414.

Wang G-L, Ruan D-L, Song W-Y, Sideris S, Chen L, Pi L-Y, Zhang S, Zhang Z, Fauquet C, Gaut BS, Hhalen MC and Ronald PC (1998) *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10: 765–779.

Williams C and Ronald PC (1994) Rapid, homogenization-free isolation of rice DNA for PCR. Nucl Acids Res 22: 1917–1918.

Zheng K, Subudhi PK, Domingo J, Magpantay G and Huang N (1995). Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genetics Newsletter 12: 255–258.