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Williams, C E
Ronald, P C

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PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization

Christie E.Williams* and Pamela C.Ronald
Department of Plant Pathology, University of California, Davis, CA 95616, USA

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A protocol is presented that utilizes potassium ethyl xanthogenate to liberate DNA from leaves without the need for tissue homogenization. This quick, single-tube method requires only 1/3 cm^2 of tissue and yields enough DNA to serve as template for 20 polymerase chain reactions. The resulting amplification patterns are indistinguishable from those generated from rigorous large-scale DNA extractions. Because 100–200 samples can be processed per day by a single person, this method allows for the rapid screening of large mapping populations. This protocol was developed for use with rice, but also works well with other species including barley, oat, corn and bean.

Although many quick protocols have been published for small-scale isolation of DNA, certain characteristics of rice and other monocots result in inconsistent yields and poor amplification with the polymerase chain reaction (PCR). Because rice leaves are very fibrous, protocols specifying homogenization of leaf tissue (1) are laborious and undependable when scaled down. Quick protocols that amplify DNA directly from leaf tissue (2, 3, 4) have failed to rupture the tough cell wall of rice leaves sufficiently to liberate template DNA. Fast methods for the extraction of DNA from single seeds of cotton (5) rye and barley (6) and rice (7) have been published. In our hands, these procedures did yield sufficient quantities of rice DNA for PCR (about 100 ng per half-seed). However, PCR amplification was inconsistent, presumably due to the presence of inhibitors like starch. As a result, we designed a quick, small-scale prep that utilizes potassium ethyl xanthogenate (PEX; 8) to dissolve cell walls, degrade proteins and inhibit DNase activity (8). No tissue homogenization is required in order to yield enough DNA for 20 PCR amplifications from a small amount of leaf tissue.

We tested our protocol on young leaves from bean (*Phaseolus vulgaris*), barley (*Hordeum vulgare*), corn (*Zea mays*), oat (*Avena sativa*), rice (*Oryza sativa*), triticale (a wheat/rye hybrid), wheat (*Triticum aestivum*) and durum (*T.durum*). Upon emergence from the soil, the first true leaf was collected on ice in the greenhouse and subsequently stored at −80°C. Older rice tissue, up to 8 weeks after emergence, also was collected. Frozen segments of leaf totalling 1/3 cm^2 were selected and placed in the bottom of a small microcentrifuge tube containing 100 μl of extraction buffer (8). This buffer consisted of 6.25 mM PEX (potassium ethyl xanthogenate or carbonodithioic acid, o-ethyl, potassium salt from Fluka), 100 mM Tris–HCl (pH 7.5), 700 mM NaCl, 10 mM EDTA (pH 8). Dipping the hydrophobic leaf tissue in 70% ethanol prior to placing it in buffer helped to keep it fully submerged. The samples were incubated in a water bath at 65°C for 5 min. Hot samples were transferred directly to a speed-vac and vacuum infiltrated for 2 min (rice, bean, corn and barley) or 6 min (oat, triticale, wheat and durum). After treatment, the leaf tissue was somewhat translucent and the buffer noticeably

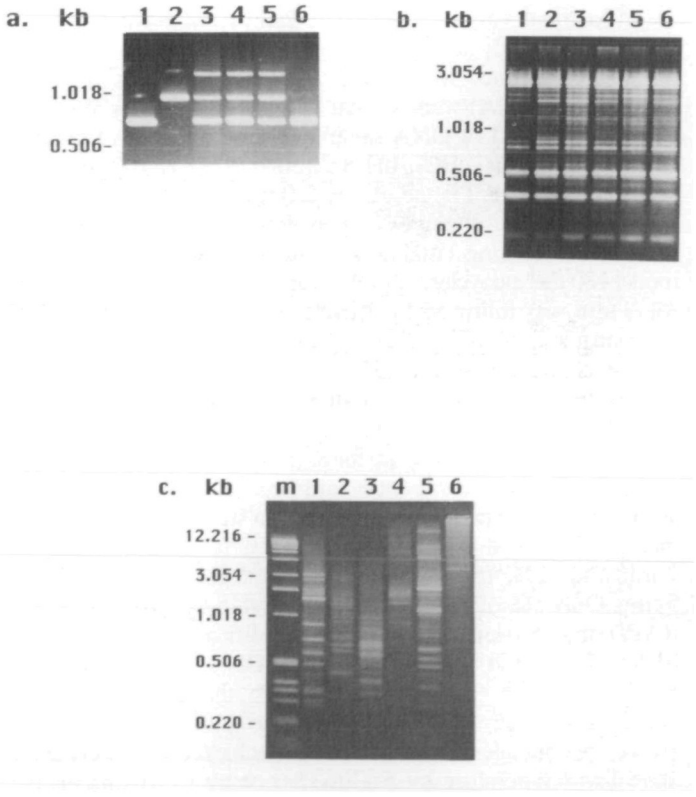

Figure 1. Separation of PCR products on gels containing 1% Synergel (Diversified Biotech) and 0.6% agarose (BRL) in 0.5× TBE buffer. **A)** Lane 1 and 2 contain DNA amplified from large-scale isolations of parental plant DNA. Subsequent lanes contain DNA amplified from PEX isolations of DNA from F_2 plants. Amplification with 24-base STS primers B7 and B8 (7) resulted in single PCR products from parental classes of homozygotes (lanes 1, 2 and 6) and the two parental plus one heteroduplex product from heterozygotes (lanes 3, 4 and 5). The heteroduplex is a PCR artifact that does not migrate in accordance with its actual molecular weight (12). **B)** Lanes are in the same order as those in A. Amplification with 10-base RAPD primer A02 (Operon, Inc.) produced no polymorphisms among the samples. **C)** RAPD primer AB09 generated discrete amplification products from bean (lane 1), barley (lane 2), oat (lane 3), corn (lane 4), rice (lane 5) and triticale (lane 6). Lane m contains 1 kb ladder.

* To whom correspondence should be addressed

green, indicating that cell lysis had occurred. The samples were returned to the 65°C water bath for 15 min or longer, during which time a second set of samples was processed. After incubation, the samples were vortexed for 10 sec, the leaf segments were removed and 10 μl of 3 M sodium acetate (pH 5.2) plus 200 μl of cold ethanol were added for precipitation. Tubes were incubated until the alcohol became viscous, about 10 min, in a dry ice/ethanol bath before centrifugation for 20 min on the high setting of a microcentrifuge. All liquid was then removed from the samples and barely visible amounts of clear residue (containing DNA) remained scattered up the side of the tube. The precipitate was then resuspended by vortexing in 20 μl of modified TE buffer (10 mM Tris−HCl, pH 7.5 and 0.1 mM EDTA, pH 8). The samples were centrifuged (as before) for 5 min to pellet any debris before the liquid was transferred to a new tube for storage at −80°C. DNA was used directly in PCR without quantification. However, initial quantification of DNA yield may be useful if this protocol is to be applied to other plants. For the purpose of this report, rice DNA concentrations were determined for fifteen representative samples. Fluorometry detected 6 ng of DNA or less as the total yield for the 20 μl final volume. The DNA yield from 8-week-old rice tissue was slightly less than from younger plants.

PCR conditions for RAPD markers (random amplified polymorphic DNA) were similar to those reported by Williams *et al.* (9). One μl of DNA sample was added to 24 μl of PCR mix (10 mM Tris−HCl, pH 8.2; 50 mM KCl; 100 μM each of dATP, TTP, dCTP and dGTP; 2.0 mM $MgCl_2$; 400 nM ten-base primer; 40 u/ml Taq DNA polymerase). DNA was amplified under rapid cycling conditions (3 hr total) in a Perkin Elmer model 480 thermocycler. A preliminary denaturation step of 94°C for 1 min was followed by 3 cycles of 94°C for 1 min, 35°C for 1 min and 72°C for 2 min. This was followed by the rapid cycling phase consisting of 32 cycles of 94°C for 10 sec, 35°C for 30 sec, and 72°C for 1 min plus a final extension step at 72°C for 5 min (11). The PCR mix for STSs (sequence tagged sites; 10) was the same as that for RAPDs except that two 24-base primers were used at 500 nM concentration. The STS amplifications began with a denaturation step of 94°C for 1 min that was followed by 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, plus an extension step at 72°C for 5 min. Only 5% of all rice samples failed to amplify with either RAPD or STS primers. In general, amplification products from PEX DNA and large-scale rice DNA isolations were indistinguishable (Fig.1a and b), with the exception of an occasional primer showing decreased product with PEX DNA. However, equivalent amplification was achieved by lowering the annealing temperature by 5°C (STSs) or by increasing primer concentration to 500 nM (RAPDs). DNA isolated from young and old rice tissue worked equally well in PCR (data not shown). In determining the usefulness of this protocol with plants other than rice, 4 RAPD primers were tested. Discrete and reproducible amplification products were detected in reactions containing DNA from bean, barley, oat, corn and triticale (Fig. 1c). Although ample DNA to serve as PCR template was isolated from wheat and durum, only a high molecular weight smear was detected after amplification and electrophoresis. In our experience, amplification of PEX DNA can often be improved by altering DNA concentration and PCR conditions or by using primers known to permit good amplification in the target species (the 4 primers that we tried may be suboptimal for *Triticum*).

Map-based cloning efforts require high resolution genetic analysis of a few tightly linked DNA markers in large segregating populations. This work can be greatly facilitated by the PEX DNA protocol which yields small quantities of DNA which amplify well in PCR. The amplification patterns generated from the PEX DNA were both reproducible and comparable to patterns generated from DNA isolated in large preps that included tissue homogenization, phenol and chloroform extractions, and multiple precipitation steps. This method is applicable to other projects in which small quantities of DNA must be isolated from many individuals.

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