Commentary

Preparation of Plant DNA for PCR Analysis: A Fast, General and Reliable Procedure

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The advent of PCR technology has provided scientists with a very simple and reliable diagnostic test for the presence or absence of a particular nucleic acid (DNA or RNA) sequence in a sample. This technology has been used not only for the confirmation of transformation, but also for studies of variant or polymorphic sites for genotyping, and for the construction of genetic maps in the course of genome analysis (Weber and May, 1989; Williams et al., 1990; Caetano-Anolles et al., 1991; Huang et al., 1992).

PCR itself is simple and rapid, and hundreds of DNA samples can be easily analyzed if purified DNA is available. Thus, the preparation of DNA from plant tissue for the analysis becomes the limiting factor for the potential of this technology. Recently, several DNA isolation methods intended to overcome this problem have been reported. The simplest of them is the method reported by Berthomieu and Meyer (1991) where fresh tobacco leaf or root tissues are placed directly into the PCR reaction buffer to initiate the amplification reactions. This method, however, was not successful in our hands. The other methods (Tai and Tanksley, 1990; Edwards et al., 1991; Landridge et al., 1991, Oard and Dronavalli, 1992), although reliable, are still too complicated to match the ease of the PCR technology for the analysis of a large number of samples in a relatively short period of time. Furthermore, most of the procedures are multi-step processes and require several solutions, increasing the possibility of cross-contamination, which is one of the biggest problems of PCR-based analysis.

We, therefore, developed a simple, rapid, and reliable DNA miniprep procedure for the preparation of DNA for PCR analysis, which has been successfully used with leaves and tissue cultures. The entire system, which uses miniprep DNA for PCR analysis, has been designated as the "miniprep-PCR system" and is described as follows:
Miniprep of plant genomic DNA
- To a 2-mL microfuge tube, add
  0.2 mL of 0.5 M KCl
  1.0 g of glass beads (1-mm dia.)
  10-50 mg of plant tissue
- Tighten the cap of the microfuge tube.
- Place the microfuge tube in a Mini-Beadbeater (Biospec Products, Bartlesville, Oklahoma, USA) and homogenize the tissue for 120 seconds using the high-speed setting.
- Drop the tube into liquid nitrogen.
- Boil the samples together for 10 minutes in a water bath.
- Centrifuge for 5 minutes at top speed in a microfuge.
- Use the supernatant directly for PCR or store the tube at -20°C until use.

PCR amplification with miniprep DNA
- For each 50-μL reaction, add the following ingredients to a 0.5-mL microfuge tube:
  25.00 μL of 2X KCl-free PCR buffer
  14.75 μL water
  5.00 μL of each primer (5 μM)
  0.25 μL of 5 units/μL Taq DNA polymerase
  5.00 μL of the miniprep DNA
- Add about 20 μL mineral oil to cover the reaction mix.
- PCR program:
  5 cycles: 94°C, 1 minute; 55°C, 2 minutes; 72°C, 2 minutes.
  42 cycles: 94°C, 1/2 minute; 55°C, 1 minute; 72°C, 1 minute.
  1 cycle: 72°C, 5 minutes

Analysis of PCR-amplified products
- Mix 20 μL of the reaction mix with 4 μL of 6X loading buffer (10 M urea, 0.1% bromophenol blue, 0.1% xylene cyanol) and load into a well of a 2% agarose gel.
- Run the gel at 12 volts/cm for one hour in 1X TBE buffer (0.09 M Tris-borate (pH 8.0), 2 mM EDTA).
- Stain the gel with ethidium bromide solution.

Notes
1. When old plant tissues or slow-growing, long-term tissue cultures are used, 0.3 M 2-mercaptoethanol should be included in this homogenization solution.
2. The glass beads purchased from the manufacturer should be cleaned by soaking in nitric acid for 20 minutes followed by washing with distilled water thoroughly before use.
3. The optimal amount of tissue depends on the type of tissue and can be determined by a trial experiment. In general, the younger the tissue, the better the result.