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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; Landgridge 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]
  1.0 g of glass beads (1-mm dia.)[2]
  10-50 mg of plant tissue[3]
- 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[4]
  14.75 μL water
  5.00 μL of each primer (5 μM)
  0.25 μL of 5 units/μL Taq DNA polymerase[5]
  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[6], 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.

4. The 2X KCl-free PCR buffer contains 20 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 0.4 mM dNTP and 0.004% gelatin.
5. The Taq DNA polymerase was purchased from BRL.
6. All the PCR primers used in the experiments are 18-mers and were designed to have an estimated annealing temperature close to 55°C.

This system has been used successfully to analyze tissue samples from all the plant tissues we have tried, including tobacco leaf tissue, soybean leaf tissue, maize leaf tissue, and soybean callus tissue. The reproducibility of the system has also been tested extensively with soybean leaf tissue and two different types of soybean callus tissues, protoplast-derived callus tissue and slow-growing, long-term callus culture. It was found that this system was successful and reproducible for both the soybean leaf tissue and the protoplast-derived soybean callus tissue.

However, when slow-growing, long-term culture lines were used, failure of the system occurred. In practice, such a failure would affect the reliability of the testing results, because the lack of amplification could be due either to the absence of the target sequence in the sample or to the failure of the system itself. To overcome this problem, we introduced an "internal control" into the system. It was known that more than one pair of primers that share similar annealing temperatures can be included in a PCR reaction to co-amplify multiple target sequences (Li et al., 1990). Therefore, besides the test primers, we added another pair of primers, the "internal control" primers, which are specific for a fragment of the genome of the plant species under study. The fragment, which is specific for the internal control primers (the internal control fragment), should be a different size than that of the test primers. This pair of internal control primers can then be included in every miniprep-PCR analysis to monitor the success of each analysis, since whenever the miniprep-PCR system is successful, the internal control fragment will always be amplified.

Using the miniprep-PCR system with an internal control, we have successfully analyzed a collection of slow-growing, long-term transgenic culture lines. For example, in one experiment, 29 slow-growing transgenic soybean callus lines and an untransformed control were tested by PCR amplification for the presence of the neomycin phosphotransferase gene using a pair of primers specific for the gene. A pair of primers specific for the soybean lectin gene, *Le1*, were used as the "internal control primers." The "internal control primers" are expected to produce a 642-bp fragment with any soybean DNA, while the pair of primers specific for the neomycin phosphotransferase gene should amplify a 409-bp fragment. A total of 23 of the 30 reactions resulted in amplification. Among the 23

reactions with amplification, 18 amplified the 409-bp fragment specific for the neomycin phosphotransferase gene and were scored positive while 5 of them, including the negative control, produced only the 642-bp fragment specific for the soybean lectin gene *Le1*, so were scored negative. Southern hybridization results from the gel showed that the 409-bp fragment was indeed not amplified in the five negative samples (including the negative control with wild-type DNA).

In summary, a simple, rapid and reliable procedure has been developed for the preparation of DNA from several plant tissues for PCR analysis. The method takes 2 minutes to homogenize each sample and 15 minutes for boiling and centrifugation of all the samples prepared together. More than 50 samples can be processed in a single working day using this procedure. The use of a Beadbeater, which could process more samples at a time, would speed up the DNA preparation procedure. The method requires only very small amounts of tissue for each analysis. This makes it ideal for the early evaluation of putative transformants when little material is available and for the early evaluation of segregating progeny at the germinating seedling stage. Enough tissue can be obtained for the analysis from germinating seeds on paper towels without the need of growing the plants in the greenhouse or in the field. Most importantly, the entire process is contained within a microfuge tube and only one solution is involved, thus greatly minimizing the possibility of cross-contamination, which can be a big problem for PCR-based analysis. Finally, the introduction of an "internal control" into the system ensures the reliability of the results. The unique feature of this procedure is that plant tissue is homogenized by the fast-moving glass beads inside a microfuge tube and that the nucleases and proteases are inactivated by boiling; it could, therefore, be applicable to any type of plant tissue.

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