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A Rapid and Efficient DNA Minipreparation Suitable for Screening Transgenic Plants

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Abstract. We present a modified method for DNA minipreparation suitable for large-scale screening of transgenic plants. The method is rapid and efficient—one person can prepare DNA from approximately 50 samples per day. The average yield was about 40 μg DNA per 100 mg of fresh tissue, and the A_{260}/A_{280} was 1.89-2.03. The total DNA extracted by this method could be used for PCR, restriction enzyme digestion, and Southern blotting.

Full text†: This manuscript, in detail, is available only in the electronic version of the Reporter.

Key words: DNA extraction, SDS, transgenic plants

Introduction

In plant genetic engineering, screening of transgenic plants is laborious work requiring isolation of genomic DNA from large numbers of plants. Fast efficient extraction methods to produce high quality DNA are required. To date, numerous methods have been developed for DNA extraction from various plant species (Dellaporta et al., 1983; Guillemaut et al., 1992; Aljanabi et al., 1997; Chang et al., 1997; Chen et al., 1999). However, most of the protocols are either time consuming or produce low yields of DNA. Some require large quantities of plant material. Very rapid DNA isolation methods have been developed; however, they are applicable only to PCR (Dilworth et al., 2000; Mannerlöf et al., 1997).

Here we describe an efficient mini-scale DNA extraction method modified from Pich et al. (1993). The extracted DNA is sufficient for hundreds of PCR reactions and several enzyme digestions. One person can finish the entire 50-sample procedure in one day.

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Materials and Methods

Plant material

Transgenic tobacco plants (*Nicotiana tabacum* cv. Wisconsin 38) were *in vitro* cultured for 3 wk on MS medium (Murashige and Skoog, 1962). Leaves for DNA extraction were cut from seedlings on a clean bench.

Reagents

- DNA extraction buffer: 100 mM Tris-HCl (pH 8); 50 mM EDTA (pH 8); 500 mM NaCl; 2% SDS (w/v); 2% β-mercaptoethanol (v/v). Autoclave and add 10% PVP to a final concentration of 1% (w/v) before use.
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- Isopropanol
- 70% ethanol
- TE buffer: 10 mM Tris-HCl (pH 8); 1 mM EDTA (pH 8)
- 10 mg/mL RNase A (Sigma)
- *Taq* DNA polymerase (Promega)
- *Hin*d III (Fermentas)
- DNA size markers (Takara)

DNA extraction protocol

1. Place 25-100 mg of leaves into a 1.5-mL Eppendorf tube. Cap the tube and throw it into liquid nitrogen for 10 s. Use a 200-μL pipette tip to homogenize the leaves into a fine powder immediately.
2. Add 600 μL of DNA extraction buffer and mix thoroughly. Incubate the tube at 65ºC in a water bath for 15 min.
3. Centrifuge at 12,000 *g* for 10 min at 4ºC. Transfer the supernatant to a new tube.
4. Add 2 μL of RNase A and incubate at 37ºC for 10 min.
5. Add an equal volume of phenol:chloroform:isoamyl alcohol mixture and invert several times gently. Centrifuge at 12,000 *g* for 3 min. Carefully pipet the upper phrase to a new tube.
6. Repeat the phenol:chloroform:isoamyl alcohol extraction.
7. Precipitate the DNA by adding 0.6 volume of ice-cold isopropanol and place the tube at -20ºC for 10 min.
8. Centrifuge at 12,000 *g* for 10 min at 4ºC. Discard the supernatant.
9. Wash the DNA pellet twice with 1 mL of ice-cold 70% ethanol.
10. Air dry and dissolve the DNA in 50 μL of TE buffer.
11. Store at -20ºC until use.

DNA purity and concentration detection

The DNA was diluted with TE buffer (1:500). The absorbance at 260 nm and 280 nm were measured in a UV-3000 spectrophotometer (Shimadzu). DNA purity was judged by the absorbance ratio of A_{260}/A_{280}. The DNA concentration was calculated from the 260 nm absorbance.

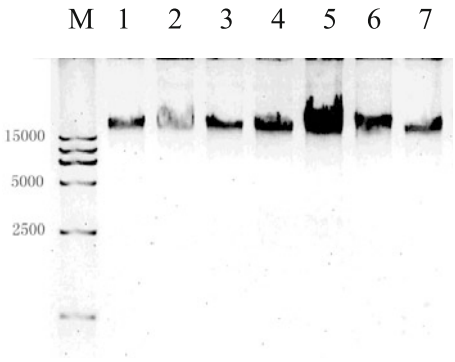

Figure 1. Total DNA prepared from different transgenic tobacco lines. M, DL-15000 DNA size marker; lanes 1-7, DNA of transformant lines. DNA was run on an 0.8% agarose gel at 5 V/cm for 1 h.

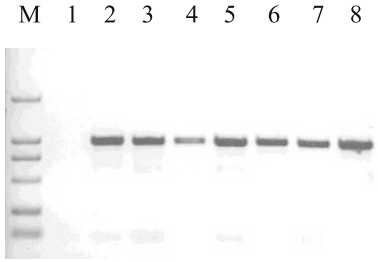

Figure 2. PCR amplification of *hpt II* gene fragment. PCR products were amplified from approximately 50 ng of template DNA by using primer h1 (5'-AAAAAGCCTGAACTCACCGC-3') and h2 (5'-ACTTCTACACAGCCATCGGT-3'). M, DL-2000 DNA size marker; lane 1, control; lanes 2-8, products from different transformants. The PCR products were run on a 1% agarose gel at 5 V/cm for 45 min.

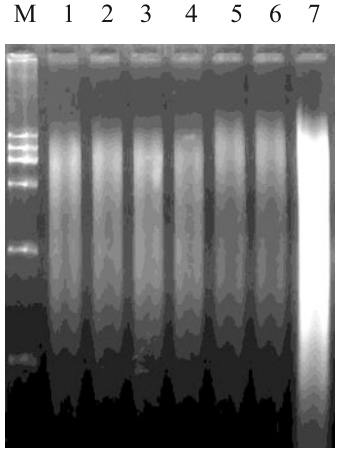

Figure 3. Restriction analysis of total DNA from transgenic tobacco. M, DL-15000 molecular weight marker; lanes 1-6, digestion of 10 μg of total DNA from different lines; lane 7, digestion of 20 μg total DNA. DNA were digested by *Hin*d III and separated on an 0.8% agarose gel at 2.5 V/cm for 3 h.

PCR analysis and restriction enzyme digestion

PCR was carried out in a 20-μL reaction system containing: 50 ng template DNA, 100 pM of each primer, 200 μM of each dNTP, and 1 unit of *Taq* DNA polymerase. Amplification was performed as denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 1.5 min, with a final extension at 72°C for 10 min. The PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide. Extracted DNA was digested by *Hin*d III at 37°C overnight and subjected to electrophoresis (0.8% gel; 2.5 V/cm; 3 h).

Results and Discussion

The above protocol is a modification of the method described by Pich et al. (1993). It consists of the following major steps: (1) grinding of samples, (2) phenol:chloroform:isoamyl alcohol extraction, and (3) DNA precipitation. In the original method, the tissues are ground with a mortar and pestle as generally described (Pich et al., 1993; Pirttilä et al., 2001). This has the disadvantages of labor intensity and easy contamination of DNA. We use a 200-μL (or 1-mL) pipette tip to homogenize tissues in the 1.5-mL tube directly. This avoids the risk of cross contamination during homogenization (Chen et al., 1999). We screen transgenic tobacco in the early seedling stage when the transformants are cultured *in vitro*. In this stage, DNA extraction is easily conducted. Using this method, one person can grind approximately 80 samples within 1 h. The amount of initial material required is minimal; 50 mg of leaf tissue is adequate to isolate total DNA.

Polyvinylpyrrolidone (PVP) was added to prevent oxidation of the secondary metabolites in plant tissues (Kim et al., 1997; Pich et al., 1993). The DNA yield was very high, ranging from 26-50 μg per 100 mg of fresh tissue. The A_{260}/A_{280} was 1.89~2.03, suggesting that the isolated DNA was free of protein. The DNA solution was colorless and could be used directly for restriction digestion. The extracted DNA was not degraded and contained no RNA contamination (Figure 1). Approximately 50 ng of DNA were used as the template to amplify the hygromycin-resistant gene (*hpt II*) fragment. After 35 PCR cycles, the expected band of 970 bp appeared in each transformant line (Figure 2). The genomic DNA was completely digested by *Hin*d III (Figure 3).

We have used this DNA extraction procedure for screening transgenic rice plants (data not shown). We also have used it to extract DNA of high quality and yield from a variety of species, including *Arabidopsis thaliana*, maize, and spinach.

Acknowledgments

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