A simple and rapid method for the preparation of plant genomic DNA for PCR analysis

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The polymerase chain reaction (PCR) has revolutionised the rapid analysis of mammalian genomic DNA (1). However, PCR is less useful in the analysis of plant DNA due to the difficulties in extracting nucleic acids from limited amounts of plant tissue. We have developed a method for the rapid extraction of small amounts of plant genomic DNA suitable for PCR analysis. The method is applicable to a variety of plant species and has the added advantage of not requiring any phenol or chloroform extraction. Thus it is possible to complete an extraction within 15 minutes without handling any hazardous organic solvents.

Samples for PCR analysis (usually leaf tissue) are collected using the lid of a sterile Eppendorf tube to pinch out a disc of material into the tube. This ensures uniform sample size and also reduces the possibilities of contamination arising from handling the tissue. DNA is extracted as follows: The tissue is macerated (using disposable grinders from Bel-art Products: Scienceware, Pequannock, NJ, 07440 USA. catalog no 992) in the original Eppendorf tube at room temperature, without buffer, for 15 seconds. 400 µl of extraction buffer (200 mM Tris HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) is added and the sample vortexed for 5 seconds. This mixture can then be left at room temperature until all the samples have been extracted (>1 hour). The extracts are centrifuged at 13,000 rpm for 1 minute and 300 µl of the supernatant transferred to a fresh Eppendorf tube. This supernatant is mixed with 300 µl isopropanol and left at room temperature for 2 minutes. Following centrifugation at 13,000 rpm for 5 minutes, the pellet is vacuum dried and dissolved in 100 µl 1×TE. This DNA is stable at 4°C for greater than one year. 2.5 µl of this sample is sufficient for a standard 50 µl PCR (Figure 1). When older tissue is used this may be increased to 25 µl without any deleterious effect on the PCR. Using this protocol we have found it possible to process hundreds of individual samples in a single working day.

In order to demonstrate one application of the method, genomic DNA was prepared from five *Brassica napus* plants putatively transformed with the β-glucuronidase (GUS) reporter gene construct pJIT119 (2, 3). PCR was then performed with 35S promoter and GUS gene specific primers in a Techne PHC1, using the following conditions: 95°C for 45 seconds, 66°C for 30 seconds, 73°C for 2.5 minutes, for 35 cycles. One half of the PCR reaction was then analysed on a 1.5% agarose gel and visualised by ethidium bromide staining (see Figure 1). In order to confirm that the observed PCR products correspond to the presence of the GUS gene construct, the individual plants were also subjected to a standard GUS enzyme assay (2). The plants in lanes 4, 5, 7 and 8 were shown to have high levels of GUS enzyme activity whereas no GUS enzyme activity could be detected in plants 3 and 6 (Data not shown), confirming the results obtained with the PCR analysis.

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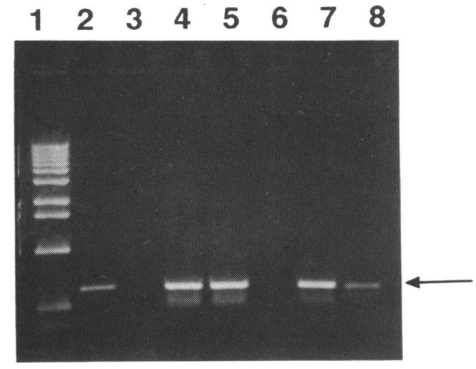

Figure 1. Separation of PCR products following amplification. Lane 1 contains BRL 1 kb molecular weight markers. Lane 2 contains PCR product from 1 pg pJIT119 plasmid DNA (positive control). Lane 3 contains PCR product from an untransformed plant. Lanes 4–8 contain PCR products from individual putatively transformed plants. The expected PCR product (580 bp) is indicated with an arrow.