Squashes of plant tissue as substrate for PCR

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Several methods have been developed recently that allow the rapid isolation of DNA from plant tissue for use in Southern hybridisations and in the PCR (1, 2). Recently, the preference in the development of DNA markers has shifted to the use of the PCR since it offers rapid sample processing and is likely to be amenable to automation (3, 4). Nevertheless, it is only being accepted slowly by plant breeders, who require the cheap analysis of very large numbers of plants. Currently the major limiting step is the preparation of DNA. Realistically one person can only make about 50 DNA preparations in one day. We describe here a technique for the preparation of DNA from squashes of plant leaves that would allow one person to process several hundred plants in a day.

The technique is a further development of the 'squash-blot' method used to detect repetitive DNA via hybridisation (5). In the procedure used for PCR, a sheet of Whatmann 3MM paper was placed on a glass plate and soaked with 1M NaOH. Excess NaOH was removed and a piece of nylon membrane (presoaked in 1M NaOH) was lain over the 3MM paper. Only Hybond N from Amersham was found to be satisfactory. A plastic screen, cut from a sheet of thin but firm clear plastic (for example the material used for overhead transparencies), was placed over the membrane. A series of holes of about 5 mm in diameter had been precut into the screen. A small segment of leaf was cut from each plant (less than 1 cm length of leaf was needed but about 5 cm was cut-off the plant for ease of handling) and held over a hole in the plastic and crushed with a stainless steel rod to leave a green circle on the filter. This required only a few quick twists of the metal rod. The metal rod was rinsed in NaOH and wiped dry between crushes. After crushing of all the leaf samples the assembly was dismantled and the membrane rinsed with 1.5M NaCl, 0.5M Tris, 1 mM EDTA, pH 7.0, three times with 1 mM EDTA, 10 mM Tris-HCl, pH 7.5 and finally with water. In this state the membrane can be used immediately for the PCR or it can be dried on the bench and stored.

For the PCR; the circles of extract on the membrane were cut out with scissors or a punch; this does not need to be accurate. The membrane segment was cut in half and pushed into an eppendorf tube and 30 µl water was added. The tube was heated to 94°C for 5 min and then 20 µl of the solution, now containing the DNA, was transferred to a 0.5 ml eppendorf tube for the PCR. Sufficient DNA binds to the membrane and elutes off into the hot water for efficient reactions. The remainder of the PCR follows conventional methods. As shown in Figure 1, the patterns of wheat, subterranean clover and tobacco were identical with purified DNA and the squash-blot DNA.

The main advantage of this technique is speed and simplicity. Many samples can be crushed onto a single membrane piece and a single operator can analyse hundreds of plants in one day. The technique is also likely to have applications for a broad range of other plant tissue and for animal, bacterial or fungal material. The method has a high potential for automation since no centrifugation steps are required and large numbers of samples can be treated simultaneously on a membrane.

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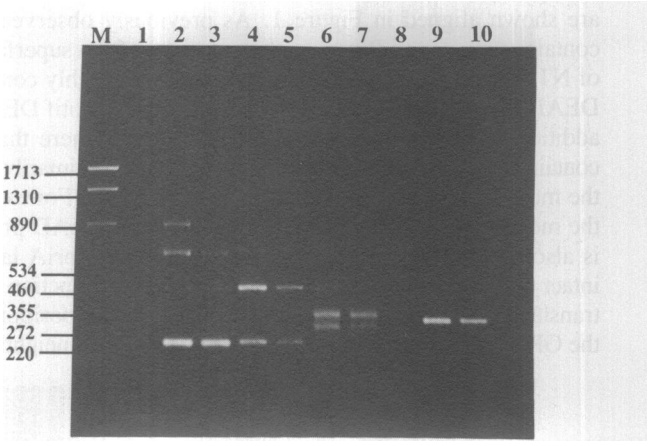

Figure 1. PCR products derived from leaf squashes and purified DNA. PCR was run on DNA prepared from leaves of wheat containing chromosome 6 of rye (Lanes 2, 3, 9 and 10), subterranean clover (Lanes 4 and 5) and tobacco (Lanes 6 and 7). The primers were based on the barley ubiquitin sequence (6) (Lanes 1 to 7) or a rye-specific repeat sequence, pAW173 (Lanes 8, 9 and 10). For each plant, the PCR products are shown for purified DNA (0.4 µg per reaction) (Lanes 2, 4, 6 and 9) or for DNA prepared from the leaf squashes (Lanes 3, 5, 7 and 10). Controls were run with the primers and no added plant DNA (Lane 1, ubiquitin and Lane 8, pAW173). From a total reaction volume of 30 µl, 7 µl were loaded onto a 2% agarose gel. Lane M shows a size marker.

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