# An alternative, rapid method of plant DNA extraction for PCR analyses

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In spite of the possibility of analysing large samples and the rapidity of Polymerase Chain Reaction (PCR) technology, the usual protocols for DNA plant extraction remain time-consuming, slow and even hazardous. New protocols, which are both rapid and inexpensive, have recently been proposed to facilitate the integration of 'DNA genetic markers' in plant breeding programs (1, 2). The tremendous advantage of PCR-derived technology versus the Southern technique is its ability to tolerate 'low quantity' and 'bad quality' DNA: 5 to 40 ng are needed for PCR against 3 to 5 grammes for RFLP markers. In the same way, only the sequence to be amplified has to be intact for PCR whereas the DNA size after extraction must be of 50 Kb for good results with Southern method. Thus, another method, inspired from the YAC protocols and using agarose plugs (3), is proposed here for extraction of DNA from germinating seeds which allows several hundreds of individuals to be extracted per day. Even small seedlings such as those of the *Brassica* species can be analysed after 2 days of germination, instead of up to one month of plant growth used for RFLP analyses, reducing at the same time the greenhouse costs and the delay. Different constraints: 'no liquid nitrogen, no centrifugation, no solvents and no notation' were chosen during the conception of this protocol for an easier handling during routine operations and for smooth automatisation.

The entire seedling is put in a 96 microplate flat bottom well in the case of 'small seeds' such as *Brassica napus* (2 day seedlings). For larger seeds such as *Helianthus annuus* (5 day seedlings) two pieces of 3–5 mm of stem are used. 100 µl of lysis buffer( 50 mM Tris HCl — 20 mM EDTA — 1% Sarkosyl — 1 mg/ml Proteinase K — pH = 8) are added to each well. The tissues are crushed, either manually with a few quick twists of a plexiglass rod, or using a mini-drill. The rod is washed with water between each crush. The microplate is then placed in an oven at 55°C for one hour. Approximately 50 µl of the lysate is then easily removed and mixed in a new flexible microplate, usually used for PCR analyses, containing 50 µl of a 2% low melting agarose maintained in liquid on a PCR apparatus at 55°C. The extracts, still inside the plate, are first allowed to solidify at room temperature and next are dialysed against water changed 3 times at 30 minutes intervals, and then overnight. The plugs are preserved in a microplate covered with adhesive film, or with an oil drop on each plug, at 4°C, until use for at least 9 months.

The advantage of using microplates during the different stages of this technique is that the samples can be identified by the symbols marked on the plates. The DNA quantity, estimated on agarose gel, is about 5 ng/µl of plug.

Before each amplification, the agarose plugs are melted on the PCR apparatus at 68°C for 5 to 10 minutes. 1–5 µl are extracted with a micropipette and placed in the microplate where the PCR is to be performed. 5–100 µl of the mix containing all the constituents for PCR is then loaded and covered with a mineral oil drop. The presence of agarose does not appear to disturb amplification as the temperatures used, during the PCR cycles, are higher than 38°C. For analyses, 10 to 20 µl of PCR products can be loaded on agarose gel with no problem.

Up to 40 PCR reactions could be performed with one extract. Before each amplification, the plugs are melted, then solidified and preserved again until another sampling at 4°C.

PCRs with different types of primer were performed successfully as is shown in Figures 1 and 2: short primers (10mers) for RAPD, long primers with GC clamp (65mers) used in DGGE.

## REFERENCES

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2. Edwards,K. *et al.* (1991) *Nucleic Acids Res.* **19**, 1349.
3. Guidet,F. *et al.* (1990) *Nucleic Acids Res.* **18**, 4955.

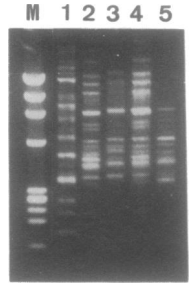


**Figure 1.** 1% agarose gel electrophoresis of products of amplification with a short primer (10mers) for RAPD: lane M contains size standards obtained by digesting (ΦX174 DNA with HaeIII. Lane 2 contains PCR products of a mustard seed. Lanes 3–5 contain PCR products of 3 different rape seeds and lane 6 of a cauliflower seed.

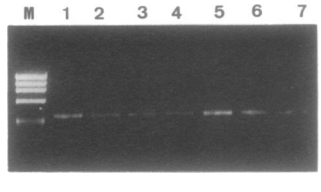


**Figure 2.** 1% agarose gel electrophoresis of products following amplification with long primer with a GC clamp (65mers) used in DGGE. Lane M contains ΦX174 DNA digested by HaeIII. Lanes 2–7 contain PCR products of sunflower individual seed. The expected PCR product is a 320 bp fragment.