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A Rapid Micro-method for the Isolation of Total DNA from Barley (*Hordeum vulgare* L.) Tissues

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Abstract

The high yielding and reproducible micro-method of barley total DNA isolation was developed. A strong buffer was used for the maximum yield of DNA. RNA was removed by treating DNA with DNase-free pancreatic RNase A and polysaccharide contamination was removed by ethanol precipitation. DNA isolated from callus or leaf tissue was proved enough for restriction enzyme digestion and Southern blotting.

Highlights

- Protocol is most suitable for the isolation of DNA from small quantity of tissues
- Strong buffer yielded high quantity of DNA.

Keywords: Barley, callus, DNA, isolation, leaf tissues, tissue culture

An important factor in the isolation of plant DNA is the enough disruption of the plant cell wall. Unfortunately, many procedures for breaking open cells also shear DNA and reduce the DNA quantity. As plant extracts often contain large amounts of polysaccharides, tannins and pigments that are difficult to separate from DNA. These can slacken the activity of most DNA modifying enzymes, including restriction enzymes, which causes difficulties during analysis of DNA by Southern blotting. In the present protocol, plant cells are usually disrupted by highly concentrated buffer that reduces these problems and obtained DNA can successfully be digested by the restriction enzymes.

Materials and Methods

Callus initiation and plant regeneration

Callus cultures were initiated from immature embryos of barley cv Dissa. The *in vitro* plant regeneration was obtained from the somatic embryos of calli of immature embryos (Tiwari, 1992).

Procedure for the isolation of total DNA from barley tissues

This method of total DNA isolation was based on the method of Dellaporta *et al.* (1983), which was high yielding and reproducible.

1. Barley leaf or callus tissue (0.5 g) was frozen in liquid nitrogen and ground in a pestle and mortar to a fine powder.
2. Liquid nitrogen was added to the mortar and the sample was poured into a 30 ml screw-capped Nalgene tube (Technate Ltd; Old Wolverton, Milton Keynes, MK 12 5QL) containing 10 ml of extraction buffer (0.1M sucrose, 150 mM Tris HCl; pH 8, 800 mM NaCl, 75 mM EDTA, 0.1% 2-mercapto-ethanol). Tubes were left on ice until completely thawed, then mixed by vigorously shaking.
3. Tubes were incubated at room temperature for 10-15 min, followed by the addition of

0.15 volume of 20% (w/v) sodium dodecyl sulphate (Sigma).

4. Tube contents were mixed by vigorous shaking and incubated at 65°C for 20 min after which the suspension changed to pale yellow. Four ml of 5M potassium acetate added and tubes were incubated on ice for 20 min.
5. Tubes were centrifuged at 12,000xg (20 min, 4°C) and the supernatant was filtered through a double layer of miracloth (Calbiochem, La Jolla, California, USA) into a clean Nalgene tube containing 8 ml of ice-cold isopropanol. Tubes were incubated at -20°C for 20 min.
6. DNA was pelleted by centrifugation at 12000xg (15 min, 4°C).
7. The DNA pellet was rinsed with 70% ethanol and tubes were placed upside down on tissue paper for 30 min, or vacuum desiccated for 10 min, to remove traces of ethanol.
8. The pellet was dissolved in 200-500 µl of TE buffer (50 mM Tris HCl, 10 mM EDTA, pH 8.0) and transferred to 1.5 ml eppendorf tubes. Incubate at 65°C in a water bath with gentle inversion every 15-20 min or until completely dissolved. Finally, DNase free pancreatic RNase A (Sigma) was added to a final concentration of 50 µg/ml and samples were incubated at 37°C for 45 min in a water bath.
9. DNA was used in restriction endonuclease digestions or stored at -20°C. If the DNA failed to cut, the ethanol precipitation (step 8 and 9) was repeated and 0.1 volume of 50 mM spermidine (Sigma) was added to the restriction digest mixture.

Southern blotting

The isolated DNA was digested with *Hind* III and *Bgl* 1, *Hind* III and *EcoRI*, electrophoresed and blotted on nylon membrane respectively as described by Maniatis *et al.* (1982). DNA was hybridised with pBG35 flax ribosomal DNA probe according to Maniatis *et al.* (1982). Probe DNA was kindly provided by Dr R Waugh, SCRI, Invergowrie, Dundee, U.K.).

Results and Discussion

Using this protocol, yields of 200-400 µg DNA isolated from 0.5 g of callus tissue. RNA contamination is removed by treating the DNA with DNase free pancreatic RNase A and polysaccharide contamination was removed by ethanol precipitation. It was observed that the addition of 50 mM spermidine to the restriction endonuclease digestion mixture helps in the digestion of DNA. The hybridisation results indicated that 20kb and 20kb and 8kb gene fragments contained in the lane 2 and lane 3 respectively (Fig.1 B). This method reports high yield DNA that could be cut by restriction endonucleases. This protocol proved to be the most effective of 4 methods (Murray and Thompson, 1980; Mettler, 1987; Paszkowski *et al.* 1984; Dellaporta *et al.* 1983) for the extraction of DNA from barley leaf and callus tissues. Hence, it is believed that this protocol provides information to assist in the progress of barley molecular biology and biotechnology.

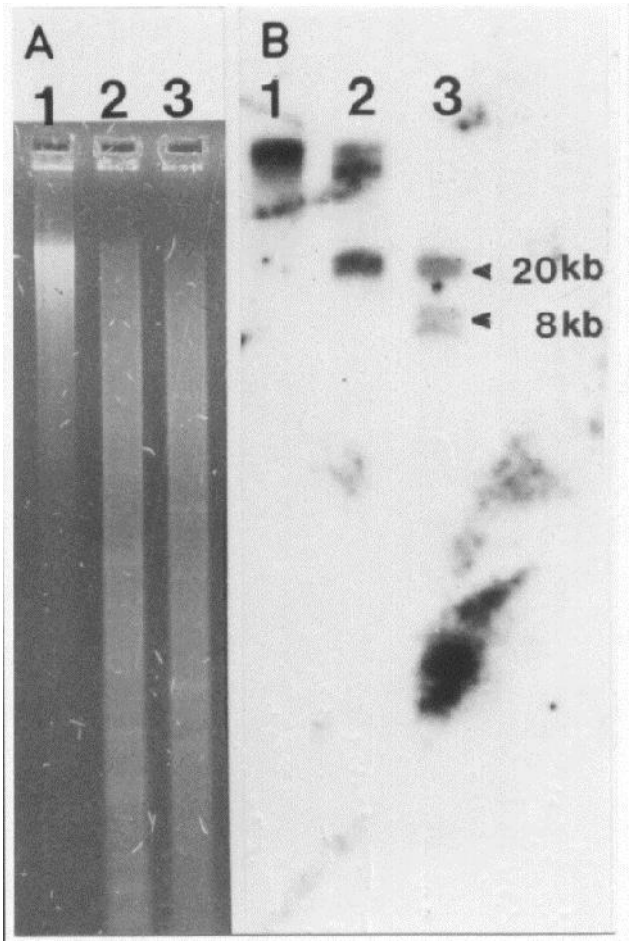

Fig. 1. (A-B) Agarose gel electrophoresis and Southern blot analysis of DNA isolated from barley callus. (A) Ethidium bromide staining agarose gel (0.8% w/v). Lane 1, undigested barley callus DNA. Lane 2, barley callus DNA digested with *Hind* III and *EcoRI*. Lane 3, barley callus DNA digested with *Hind* III and *Bgl* 1 (B) Autoradiograph of Southern blot probed with the flax ribosomal DNA probe, pBG35. Lanes 1-3, same as in A.

Film was exposed for 24 h at -70°C. The size of DNA fragments is indicated in kbp.

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