Midiprep method for isolation of DNA from plants with a high content of polyphenolics

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The preparation of high quality DNA from polyphenolic-containing plants such as field bean (Vicia faba), tomato (Lycopersicon esculentum), and potato (Solanum tuberosum) was difficult, because of DNA degradation mediated by secondary plant products such as phenolic terpenoids and tannins which may bind to DNA and/or RNA after cell lysis (John 1992).

Common methods for DNA isolation from such plants either include time consuming and expensive procedures (density gradient centrifugation or chromatography on columns) or yield DNA of poor quality.

The method described here is based on modified protocols of Dellaporta et al. (1983) and John (1992). It combines the complexation of polyphenolic compounds by polyvinyl-pyrrolidone (PVP) following cell lysis and selective precipitation and centrifugation for removal of PVP complexes and DNA recovery.

Plant tissues (100 mg leaf, stem) were quickly frozen in liquid nitrogen, powdered with mortar and pestle and transferred into 3 volumes (w/v) extraction buffer (500 mM NaCl, 50 mM Tris/HCl (pH 8.0), 50 mM EDTA, 1% (v/v) β-mercaptoethanol; added immediately before use). The mixture was thawed and ice cold 20% stock solution PVP (25 kd, Serva; stored at -20°C) was added to a final concentration of 6%. The lysate was kept on ice after thawing. Solid SDS was added to a final concentration of 2% (w/v). The extract was slightly mixed and incubated in a waterbath at 65°C for 10 minutes. Then 1/10 vol of 5 M potassium acetate was added after 30 minutes incubation on ice and centrifugation (13000×g, 10 minutes, 4°C). The supernatant was transferred into a new tube, mixed with 0.6 vol isopropanol by inverting the tubes three times and incubated on ice for 10 minutes. After another centrifugation (13000×g, 10 minutes, 4°C) the supernatant was discarded completely. The pellet was washed in 70% ethanol, dried under vacuum and dissolved in 1×TE (pH 8.0). The whole procedure can be performed in 2.0 ml tubes.

We used this method successfully for different plant species (tomato, potato, field bean) and obtained, depending on the species, about 20—40 μg of high quality DNA per 100 mg fresh weight (Figure 1A).

The method is suitable for the quick isolation of many DNA samples and the DNA is clean enough (OD260/280 = 1.68) being used directly for restriction, Southern or PCR analysis (Figure 1B–E). Additionally, we used field bean DNA isolated in the described manner for cloning, e.g. library construction.

REFERENCES


Figure 1. A) Genomic DNA undigested: Lane 1: potato DNA. Lane 2: tomato DNA. Lane 3: field bean DNA. Lanes M: BstEII digested lambda DNA. B) Polymorphic PCR products obtained from genomic DNA using 5S RNA spacer specific primers for all three species. Lane 1: potato DNA. Lane 2: tomato DNA. Lane 3: field bean DNA. Lanes M: BstEII digested lambda DNA. C) Southern hybridisation of digoxigenin-labeled rDNA probe (3.5 kb) to BamHI digested genomic DNA. Lanes 1 and 3: tomato DNA. Lanes 2 and 4: potato DNA. Lanes M: BstEII digested lambda DNA. D) Southern hybridisation of digoxigenin-labeled 5S RNA spacer (0.4 kb) to BamHI digested genomic DNA. Lanes 1 and 2: field bean DNA. E) Detection of low copy gene polymorphism in field bean DNA of five individual plants, using PCR with legumin B3 gene specific primers. Lanes 1—4: PCR products from individual plants (1.09—1.37 kb). Lanes 5—8: Southern hybridisation with field bean specific legumin B3 probe.