Nucleic Acids Research, 1993, Vol. 21, No. 4 ***1051–1052***

Tissue grinding with ball bearings and vortex mixer for DNA extraction

Joseph C.Colosi and Barbara A.Schaal[1,*]
Biology Department, Allentown College of St Francis de Sales, Center Valley, PA 18034 and
[1]Department of Biology, Campus Box 1137, Washington University, St Louis, MO 63130-4899, USA

Received November 2, 1992; Revised and Accepted January 18, 1993

Many of the current applications of DNA technology require either the isolation of uncontaminated DNA from small tissue samples or the processing of a large number of samples. We are currently analyzing genetic variation in *Panicum miliaceum* L. and *Manihot esculenta* Christz by RAPD (Random Amplified Polymorphic DNA) (1). For RAPD analysis we require a technique that both quickly extracts DNA from a large number of accessions, and also minimizes the chance of contamination by extraneous DNA which could be amplified and give false results. Furthermore, in applications which analyze genetic variation in rare and endangered species, only small samples can be used. For example, in a current study of *Asclepias medii* Torr., an endangered species, existing plants are so small that removing a full leaf for DNA analysis would greatly compromise photosynthetic ability. We report here a simple, quick method of grinding plant tissue for DNA extraction. This procedure takes minutes per sample, yields finely ground leaf tissue, and minimizes the chance contamination by extraneous DNA because materials are used only once.

Plant material is collected with a paper punch which cuts out 6 mm diameter leaf discs; from 1 to 30 are used per sample (0.005–0.15 g fresh wt.). The leaf discs arc put into a microfuge tube. Three washed and autoclaved, 4 mm diameter, stainless steel ball bearings (available from supply companies for about $4 per hundred) are added on top of the leaf discs (only one ball bearing is used to grind a single leaf disk). The tube is filled with liquid nitrogen. The liquid nitrogen is allowed to boil off, thus freezing the plant tissue and cooling the ball bearings. When all of the liquid nitrogen has evaporated, the tube is placed in a Styrofoam holder (3×3×4.5 cm, with a conical bottom to fit the cup of the vortex mixer and a hole in the centre to fit the tube) which insulates the tube and sample, keeping them cold. A small, flat piece of Styrofoam wrapped in a laboratory wipe is held on top of the tube by finger pressure and the tube is vortexed on a standard vortex mixer at maximum velocity for 20 to 60 seconds. The progress of grinding can be monitored by lifting the tube out of the Styrofoam holder and observing the tube from the side. More finely ground tissue falls to the bottom and unground tissue rises to the top of the mass along with the ball bearings. After vortexing, the ball bearings are poured off before the tube is capped and stored in a −80°C freezer until DNA is extracted. The entire sample is then processed in the microfuge tube by standard mini prep CTAB extraction (modified from reference 2). To eliminate the chance of contamination from sap that may accumulate on the paper punch, several holes are punched in a sheet of paper by the paper punch between leaf samples. To completely eliminate any chance of contamination from one leaf sample to another, a paper punch is not used and the leaves are torn into strips. A fixed weight of leaf tissue, such as 0.15 g is then inserted into a microfuge tube with the use of an pipette tip. The stainless steel balls and liquid nitrogen are added as described above. Before vortexing, the pipette tip is pushed though the frozen leaf tissue to break up the tissue so that the ball bearings can pulverize the tissue pieces. Both the pipette tip and the weighing paper are discarded after each tissue sample, thus eliminating any chance of contamination of one sample by another.

This technique yields finely ground leaf tissue that yields DNA of high quality for PCR and digestion with restriction enzymes (data supplied but not shown). This method has been used to grind leaf tissue and then extract DNA and amplify DNA by PCR for the following species: *Bucida spinosa* Jennings, *Callistemon acumenatus* Cheel., *Chamelaucium ciliatum* Desf., *Daphne odora* D. Don., *Dissotis rotundifolia* Triana, *Eucalyptus polyanthemos* Schauer., *Eugenia brasiliensis* Lam., *Laguncularia racemosa* (L.) Gaetner f., *Leptospermum scoparium* Forster and Forster f., *Melaleuca linariifolia* Sm., *Myrciaria cauliflora* (DC) 0.Berg, *Myrtus communis* L., *Pimenta dioica* (L.) Merr., *Psidium guajava* (L.), *Punica granatum* Sokotra, *Quisqualis indica* L., *Rhizophora mangle* G.F.W. Meyer, *Syzgium jambos* (L.) Alston, *Tibouchina urvilleana* (DC) Cogn. (S.O'Kane pers. com.). The method works well for thin blades of dicots and grasses. However, larger veins, stems, and hard seeds do not grind sufficiently by this method.

The ball bearings may be used repeatedly by thorough washing, autoclaving, and drying between each use. It is several orders of magnitude easier to clean and dry 1000 ball bearings (enough for over 300 extractions) than it is to thoroughly clean and dry a mortar and pestle between each grinding.

It is important that no water be on the leaf discs and both the tubes and bearings be dry before liquid nitrogen is added. Otherwise the water freezes and impairs the free movement of the ball bearings. Enough liquid nitrogen should be added to thoroughly freeze all of the tissue. Caution should be taken so that the leaf tissue does not bubble out of the tube as the liquid nitrogen boils. Care also should be taken to assure that all of

* To whom correspondence should be addressed

the liquid nitrogen has boiled off before the tube is capped. Residual liquid nitrogen could result in the cap exploding off and part of the sample being lost.

Larger samples of leaf tissue can be ground in larger tubes with larger bearings. For example, 2 g of leaf blade (mid vein removed) grinds to a fine powder in two minutes with five 6.5 mm diameter ball bearings in a plastic tube, 2.5 cm diameter by 10 cm. Likewise, thicker leaf tissue of *Dieffenbachia* sp., *Philodendron* sp. and several species in the Myrtales which did not grind with 4 mm diameter ball bearings in a microfuge tube, ground to a fine powder in the larger tube with 5 6.5 mm diameter ball bearings.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Rockefeller Foundation to B.A.S. J.C.C. is supported by a mid-career NSF Grant # DEB-9203114.

REFERENCES

1. Williams,J.G.K., Kubelik,A.R., Livak,K.J., Rafalski,J.A. and Tingey,S.V. (1990) Nucleic Acids Res. **18**, 6531–6535.
2. Saghai-Maroof,M.A., Soliman,K.M., Jorgensen,R.A. and Allard,R.W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 8014–8018.