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A Simple and Rapid DNA Microextraction Method for Plant, Animal, and Insect Suitable for RAPD and Other PCR Analyses

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PCR and specifically random amplified polymorphic DNA (RAPD) analyses permit the analysis of hundreds of DNA samples per day after purified DNA is available. DNA preparation from the selected tissues, whether they are of animal, plant, or insect origins, is the time-limiting factor to fully exploit the potential of the technology. Numerous DNA isolation methods addressing this problem have been published,[1–5] but most of them, although simple, are designed either for animal or plant, or more often for specific species or tissues, and none has been reported for universal applications, including minute insects (<500 μm). Here, we report a simple microextraction method for total DNA suitable for PCR-based DNA analyses in all species tested. The two key improvements are the use of a very high salt concentration in the extraction buffer that presumably "salts out" several PCR inhibitors, and the tissue disruption technique that renders the DNA extraction process simple, fast, and inexpensive. It provides highly repeatable results and uniform DNA yield. We use it routinely for extracting DNA from leaf tissues of *Brassica* plant species and from minute insects (smaller than 500 μm) for both site-specific PCR amplification and RAPD analyses.

The procedure is as follows: A 5-mm-diameter leaf disc for plant tissues, 10–20 mg of animal tissues, or a single intact insect (microhymenoptera; *Trichogramma* species) was immersed in 160 μl of extraction buffer [200 mM Tris-HCl (pH 8.0), 70 mM EDTA, 2 M NaCl, 20 mM sodium metabisulfite] in a microcentrifuge tube (Kontes). Tissue breakage was achieved by physical grinding using an electric motor-driven Kontes pestle until

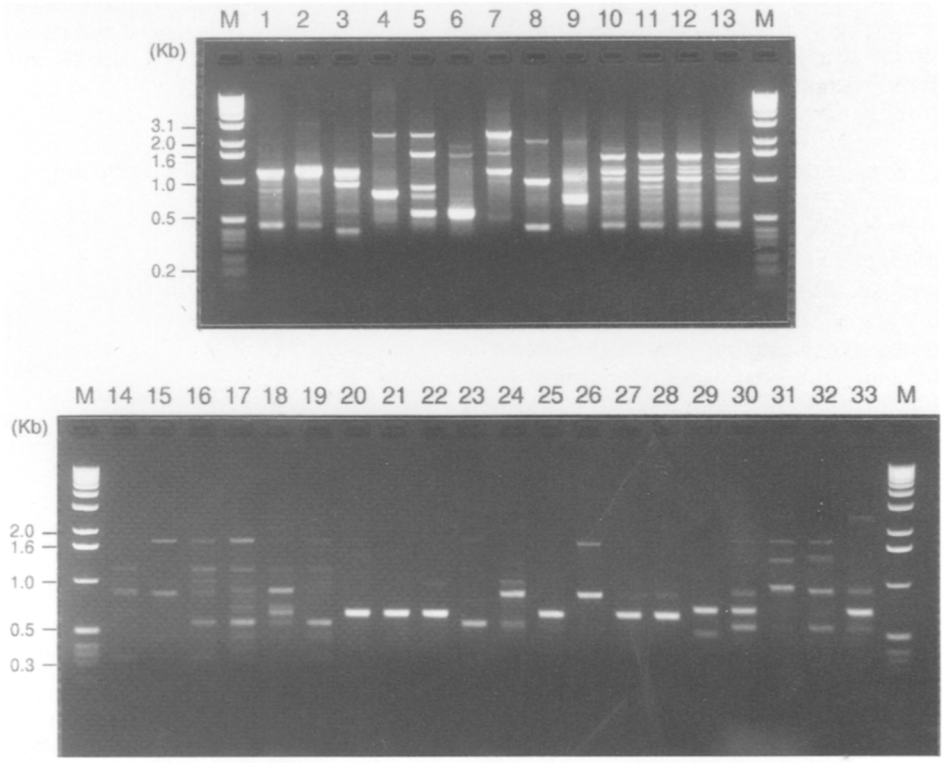

FIGURE 1 RAPD results using primer OPB04 (Operon Technologies, Alameda, CA) on the DNA extracted from nine plant species, four different rat tissues, and primer OPA18 on four species of microhymenoptera in the following lanes: (*1*) *Brassica oleracea* (cabbage); (*2*) *B. oleracea* (rapid cycle line); (*3*) *Zea mays* (maize); (*4*) *Solanum tuberosum* (tetraploid potato); (*5*) *S. chacoense* (diploid potato); (*6*) *Malus pumila* (apple); (*7*) *Lycopersicon esculentum* (tomato); (*8*) *Phaseolus limensis* (lima bean); (*9*) *Fragaria ananassa* (strawberry); (*10*) *Rattus norvegicus* (rat liver); (*11*) rat brain; (*12*) rat kidney; (*13*) rat skin; (*14–18*) five haploid males of *Trichogramma dendrolini* (microhymenoptera); (*19–23*) five haploid males of *T. pretiosum*; (*24–28*) five haploid males of *T. minutum*; (*29–33*) five haploid males of *T. evanescens*. One-twentieth of the total extraction product from each species or tissue was used for RAPD reaction. (M) 1-kb ladder (BRL) used as size markers.

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no visible pieces of tissue remained. In the case of leaf tissues, the release of chlorophyll into the buffer was a good indication of effective breakage of the plant cell wall. Cells were lysed further by addition of 40 µl of a 5% sarcosyl solution and were incubated at 60°C for 1 hr. The lysate was then centrifuged for 15 min at 16,000*g* to remove the cell debris. DNA was precipitated from the clear supernatant by the addition of 90 µl of 10 M ammonium acetate and 200 µl of isopropanol at room temperature for 15 min for plant and rat DNA but at −20°C for 2 hr for insect DNA because of the minute quantity of the latter. Total DNA was pelleted by centrifugation at 16,000*g* in the microcentrifuge for 15 min and then washed with 70% ethanol. The pellet was dried briefly and resuspended in 50 µl of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA] with RNase added at 10 µg/ml. This protocol yields 500–750 ng of plant and rat DNA and ~20 ng of insect DNA of sizes >23 kb, which is enough for at least 20 site-specific PCR or 50 RAPD reactions. Figure 1 shows the RAPD results with the DNA extracted from nine plant species, four different rat tissues, and four species of *Trichogramma*; not shown are similar results from the plant *Brassica napus* (canola) and two other species of microhymenoptera (*Anaphes sordidatus* and *Anaphes* sp. nov.). Both the quantity and quality of DNA obtained by this procedure are comparable with DNA isolated by more time-consuming methods. As for any RAPD reactions, slight variations in intensity and the presence of faint DNA bands can be seen among tissues of the same organism (Fig. 1, lanes 10–13). This is the result of variations in the DNA yield from one tissue to another. However, within the same tissue, DNA yield was uniform and no variation in the DNA amplification profile was detected (data not shown). The advantages of this method are that expensive enzymes, phenol, and chloroform are not required; and at the same time it is rapid, applicable to different types of plant and animal tissues, and can treat a large number of samples simultaneously with possible scope for automation.

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