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Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques

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ABSTRACT

A very simple, fast, universally applicable and reproducible method to extract high quality megabase genomic DNA from different organisms is decribed. We applied the same method to extract high quality complex genomic DNA from different tissues (wheat, barley, potato, beans, pear and almond leaves as well as fungi, insects and shrimps' fresh tissue) without any modification. The method does not require expensive and environmentally hazardous reagents and equipment. It can be performed even in low technology laboratories. The amount of tissue required by this method is ~50–100 mg. The quantity and the quality of the DNA extracted by this method is high enough to perform hundreds of PCR-based reactions and also to be used in other DNA manipulation techniques such as restriction digestion, Southern blot and cloning.

To study the molecular systematics of any organism, high quality DNA is required. So far there is no one common and simple procedure for genomic DNA extraction that can be used on a large scale for different eukaryotic organisms. Usually different (tricky) tissues required different protocols and different tissue preparation steps. The need for a universal procedure is urgent especially when hundreds of samples need to be analyzed. We have applied PCR-based techniques for phylogenetic and mapping studies such as AP-PCR (1,2) and cycle sequencing (3) on collections of wheat (*Triticum aestivum*), barley (*Hordium vulgaris*), potato (*Solanum tuberosum*), beans (*Vicia faba*), pear (*Pyrus syrica*), wild almond (*Prunus amygdalus*), fungi (*Nomuraea relieye, Alternaria spp.*), grasshoppers (*Schistocerca pallens*), and shrimps (*Pandalus borealis*), lettuce (*Lactuca sativa*) and eucalyptus (*Eucaliptus grandis*). Most if not all genomic DNA extraction protocols such as the plant DNA extraction protocol of Murray and Thompson (4) and its many derivatives so far, human genomic DNA extraction methods (5,6) and animal genomic DNA extraction (7), require the use of liquid nitrogen and/or freeze-drying (lypholization) of the tissue for the initial grinding which are difficult to obtain in regions of the world where most germplasm collections of many organisms have evolved or can be sampled. We present here a protocol for DNA extraction from fresh tissue that is universally applicable on a variety of organisms regardless of the complexity of their genomes.

About 50–100 mg (1 cm^2) of young field or greenhouse-grown plant leaves, filtered and dried mycelium, the muscle of one back leg of a grasshopper and shrimp muscle were used for DNA extraction. The fresh tissue was homogenized in 400 µl of sterile salt homogenizing buffer (0.4 M NaCl 10 mM Tris–HCl pH 8.0 and 2 mM EDTA pH 8.0), using a Polytron Tissue Homogenizer, for 10–15 s. Then 40 µl of 20% SDS (2% final concentration) and 8 µl of 20 mg/ml protenase K (400 µg/ml final concentration) were added and mixed well. The samples were incubated at 55–65°C for at least 1 h or overnight, after which 300 µl of 6 M NaCl (NaCl saturated H_2O) was added to each sample. Samples were vortexed for 30 s at maximum speed, and tubes spun down for 30 min at 10 000 *g*. The supernatant was transferred to fresh tubes. An equal volume of isopropanol was added to each sample,

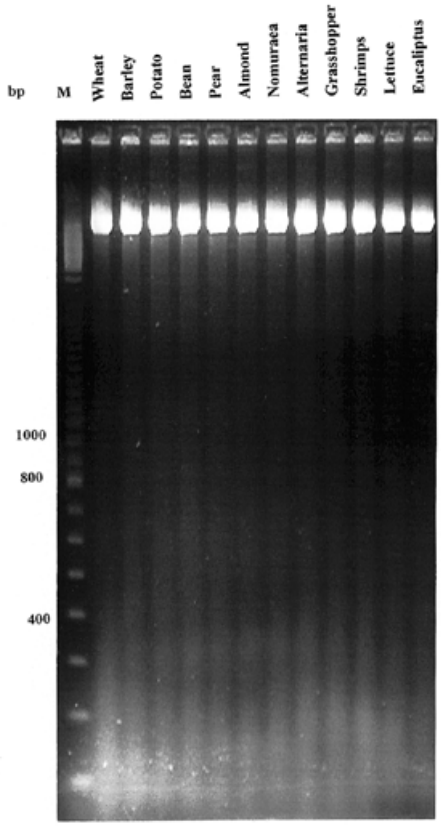

Figure 1. Example of an agarose gel electrophoresis of undigested genomic DNA of all organisms where 3 µg of genomic DNA was loaded from each sample.

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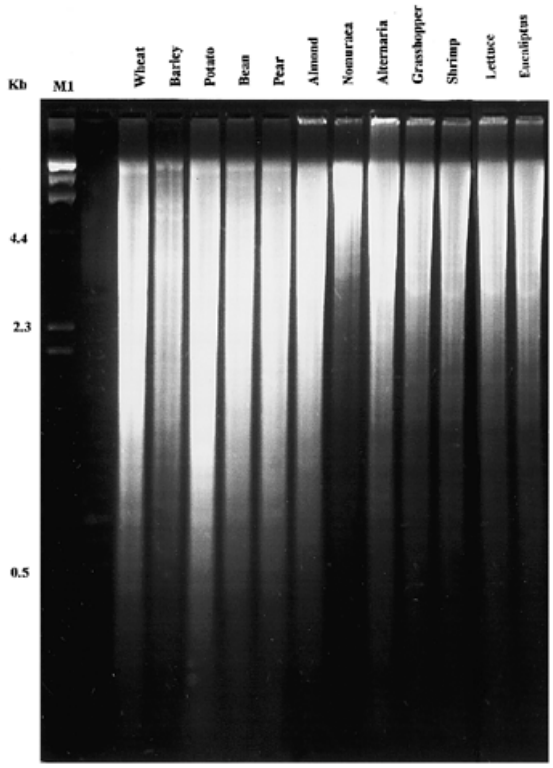

Figure 2. Example of agarose gel electrophoresis of *Bam*HI restriction digest of genomic DNA extracted by our protocol from all organisms under study. M1, λ/*Hin*dIII molecular weight marker.

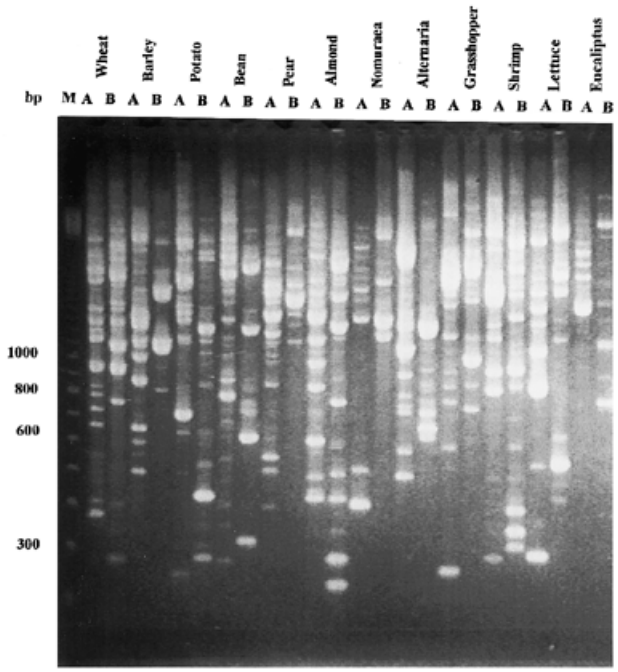

Figure 3. RAPD fingerprints of all DNAs with primers OPB-18 (5′-CCA CAG CAG T-3′) and OPJ-10 (5′-AAG CCC GAG G-3′) and 2 U Stoffel fragment *Taq* DNA polymerase. The amplification program is as previously described (1). The amplification products were resolved on 2% agarose gel. The organism is indicated at the top of the figure. A, primer OPB-18; B, primer OPJ-10. M1, 100 bp molecular weight DNA ladder (BRL/Gibco).

mixed well, and samples were incubated at –20°C for 1 h. Samples were then centrifuged for 20 min, 4°C, at 10 000 *g*. The pellet was washed with 70% ethanol, dried and finally resuspended in 300–500 µl sterile dH_2O. Genomic DNA (5–15 ng) in 10 µl of ddH_2O was used for RAPD amplification of genomic DNA (1). Ten micrograms of each genomic DNA sample was incubated overnight with 5 U *Bam*HI, *Eco*RI, *Hin*dIII and *Cla*I, and analyzed on 1.5% agarose gels. The samples were incubated for 16 h to ensure complete digestion and also to allow the detection of nuclease activity.

The purity of the DNA, determined from the A260/A280 ratio averaged >1.77 for all organisms. There was no RNA contamination in all samples nor any sign of degraded DNA during preparation (Fig. 1). The yield of DNA ranged from 500 to 800 ng/mg fresh weight for all individuals sampled. The amount of tissue required for this method is minimal, but we scaled up the amount of tissue 10-fold without any reduction in DNA quality and quantity. The average number of PCR reactions that can be performed using DNA extracted from 50 mg tissue was >3000.

Figure 2 shows one example of an agarose gel of a *Bam*HI restriction digestion reaction of genomic DNA extracted by our method (we used also *Eco*RI, *Hin*dIII and *Cla*I, data not shown). DNA was completely digested with the restriction enzymes and there was no evidence of the presence of nucleases in any sample. All DNA samples were subjected to PCR amplification using 10mer random primers (Operon Technologies, Alameda, CA) (Fig. 3). All genomic DNAs produced a clear, sharp and reproducible PCR product pattern. We repeated the PCR experiment over a period of 3 months and obtained the same banding pattern which indicates the reproducibility of the results and the integrity of the DNA (8).

The efficiency, speed, universality and requirement of no expensive facilities or toxic chemicals makes the present method an attractive alternative to the existing methods of genomic DNA extraction; none of which is ideal or universal (4–7,9). These results show that the DNA produced by our universal, simple, low cost, fast and safe protocol is of high quality and can be used reliably in DNA manipulation and PCR-based techniques on a wide range of organisms even in low technology laboratories.

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