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Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride

Heng Zhu, Feng Qu and Li-Huang Zhu*
Institute of Genetics, Academia Sinica, Beijing 100101, China

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With increasing uses of DNA fingerprinting, restriction fragment length polymorphism (RFLP) analysis, and PCR-based techniques like random-amplified polymorphic DNA (RAPD) in genome research, the difficulty of isolating high molecular weight DNA, especially from plants, fungi, and other organisms with cell walls, becomes a main obstacle. Quite a few protocols have been reported. But almost all of them include freezing with nitrogen and homogenizing with mortal and pestle or a mechanical homogenizer (1, 2). Some of them involve phenol–chloroform extraction (3), while others require gradient sedimentation with cesium chloride or precipitation with CTAB (4). All these treatments are time-consuming and tedious. Here, with the use of benzyl chloride, a simple, fast procedure is developed. Benzyl chloride can destroy cell walls of plants, fungi and bacteria through its effect to react with -OH residues in polysaccharides, including cellulose, hemicellulose, etc. Thus, genomic DNA is released with the least mechanical shearing. Furthermore, benzyl chloride has the property, similar to phenol, that it can also extract proteins and other cell debris from the aqueous phase.

Our procedure is as follows: 1 g of fresh plant leaves is cut to small pieces of approximately 1 cm^2 each. For fungi and bacteria, 1 g of pellet harvested by centrifugation is used. To each sample, 5 ml extraction buffer (100 mM Tris–HCl, pH 9.0, 40 mM EDTA), 1 ml 10% SDS and 3 ml benzyl chloride are added. The tube is vortexed and incubated in 50°C 30 minutes

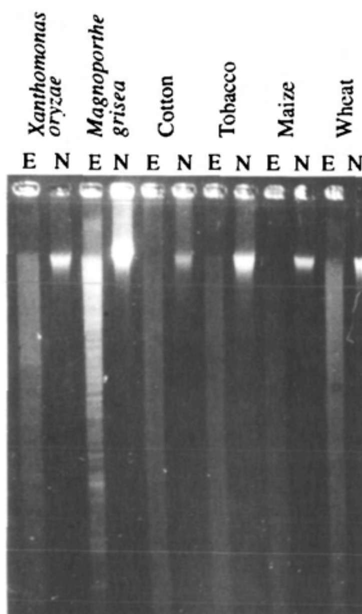

Figure 1. Genomic DNAs isolated from different organisms and restrictive digestion analysis of them using *Eco*RI. The preparation method has been described in the text. The samples were checked with 0.8% agarose gel. The 'N' and 'E' refer to undigested and digested genomic DNAs, respectively. The proportion of DNA from each preparation loaded per lane was: 1/5 for Wheat and maize, 1/10 for tobacco and cotton, 1/20 for *Magnaporthe grisea* and *Xanthomonas oryzae*.

Table 1. The yields of genomic DNAs isolated from different organisms (μg/g)

Organisms	yields	Organisms	yields
Plant		Tobacco	60–80
Wheat	10–12	Fungi	
Rice	10–12	*Magnaporthe grisea*	100–110
Maize	20–30	*Candida albicans*	90–100
Cotton	20–30	Bacteria	
Soybean	60–80	Xanthomonas oryzae	100–120

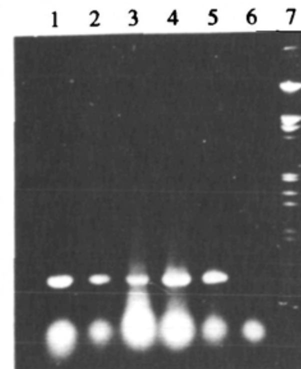

Figure 2. PCR amplification of a part of 5'-noncoding region of phyA gene using the rice genomic DNAs prepared with our method as template. No. 1–4 correspond the PCR products amplified from 4 different DNA preparations. In lane 5, the template DNA was prepared using a conventional method. Lane 6 is a negative control without template DNA in the reaction. Lane 7 is Lamda DNA digested with HindIII as molecular weight marker.

* To whom correspondence should be addressed

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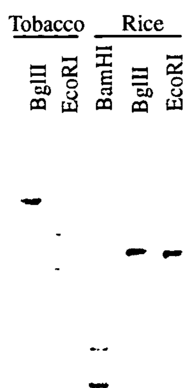

Figure 3. Southern analysis of rice and tobacco DNAs isolated with our method. DNAs were digested with enzymes shown in the figure, separated on a 0.80% agarose gel, and transferred to Hybond N+ membrane. A 18s rDNA clone of soybean was labeled nonradioactively (Dig-Luminescent Detection Kit, Boehringer Mannheim, Germany) and used as probe for hybridization.

with shaking or repeated vortexing at 5 minute intervals to keep the two phases thoroughly mixed. Then 3 ml 3 M NaOAc, pH 5.0 and the tube is kept on ice for 15 minutes. After centrifugation at 6,000 g, 4°C for 15 minutes, the supernatant is collected, and DNA is precipitated with isopropanol.

For monocot plants, when a high yield is particularly desired, it is recommended that young leaves be immersed in 1 N NaOH, 0.5 N Na_2CO_3 solution for 15 minutes to remove the silicon-containing cuticle, then rinsed with tap-water and dried with tissues. After that, the leaves are cut, and the procedure described above is followed.

Using this method, we have isolated genomic DNAs from plants (e.g. wheat, rice, maize, soybean, cotton and tobacco), fungi (*Magnaporthe grisea*, *Candida albicans*) and bacteria (*Xanthomonas oryzae*) with reproducible yields (See Table 1). The quality of isolated DNA is tested with restriction endonuclease digestion (Figure 1), PCR amplification (Figure 2) and RFLP analysis (Figure 3).

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