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Note

A Quick and Safe Method for Fungal DNA Extraction

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DNA-based studies, including cloning and genotyping, have become routine in fungal research laboratories. However, preparation of high-quality DNA from fungal tissue requires much time and labor and is often a limiting step for high-throughput experiments. We have developed a quick and safe (QS) DNA extraction method for fungi. Time efficiency and safety in the QS method were achieved by using plate-grown mycelia as the starting material, by eliminating phenol-chloroform extraction procedures, and by deploying a simple electric grinder. This QS method is applicable not only to a broad range of microbial eukaryotes, including true fungi and oomycetes, but also to lichens and plants.

Keywords : fungi, genomic DNA extraction, high-throughput

A number of methods have been developed for genomic DNA extraction from fungal tissues. In general, DNA extraction methods consist of several steps, such as preparation of starting materials, generation of cell lysates, elimination of contaminants, and collection of DNA. Conventional fungal DNA extraction methods that are derived from plant genomic DNA extraction methods (Rogers and Bendich, 1985) require fresh mycelial powder as a starting material; deploy Proteinase K and detergent to generate cell lysates; and use phenol, chloroform, and isoamyl alcohol to eliminate protein residues. Preparation of liquid-cultured fresh mycelia requires relatively long incubation times and much lab space, and harvested mycelia are usually lyophilized and ground. The problems with time and space may become more serious when one is dealing with a large number of samples. Furthermore, Proteinase K treatment and phenol-chloroform extraction procedures require multiple sample transfers, and the remaining Proteinase K or organic solvent in the DNA solution often results in failure of following enzyme reactions. In addition, deploying hazardous materials requires additional expenses for protective equipment and waste disposal.

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We developed a quick and safe (QS) method for fungal DNA extraction. The step-by-step procedure is given in Table 1. The QS method applies plate-grown mycelia as a starting material to increase time and space efficiency. The method could save 3-4 days of extra-incubation and lyophilization for preparing the fungal mass. In addition, colonies on agar plates can be stored at 4°C for up to 2 months without degradation of the DNA. The QS method requires a small amount of fungal mass, so fungal colonies can be grown at the user's convenience. In rice blast fungus (*Magnaporthe oryzae*), ~50 fungal colonies (30-40 mm^2 colony size) can be grown on a single plate (80 mm in

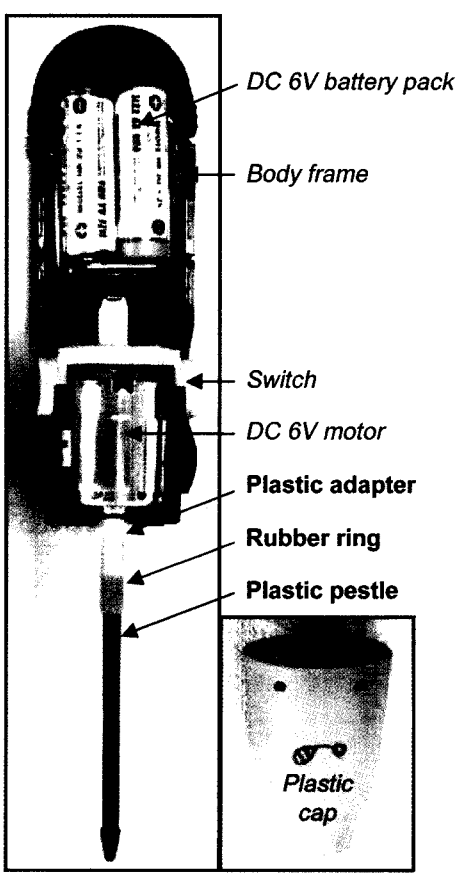

Fig. 1. Inner structure of an electric grinder. Parts from commercial electric driver are indicated in italic, and parts modified in this study are indicated in bold.

Table 1. Step-by-step procedure for quick and safe DNA extraction from fungi

1. Culturing mycelium * Time: ~1-2 days
Inoculate fungal strains on agar medium and incubate plates until the colony diameter reaches 5-10 mm under appropriate propagation conditions. For *Magnaporthe oryzae*, mycelia propagated for 2-3 days on the complete medium at 25°C in the dark.
2. Preparing reaction tubes * Time: ~10 s per sample
Prepare and label two sets of 1.5-ml Eppendorf tubes. Fill one set of tubes with 0.5 ml extraction buffer (1M KCl, 100 mM Tris-HCl, 10mM EDTA), and the other with 0.3 ml 2-propanol. Use a multi-channel pipette for large numbers of samples.
3. Harvesting fungal tissues * Time: ~10 s per sample
Various types of mycelia and conidia can be used as starting materials. Collect 10-20 mg fungal mass (20-40 mm^2 mycellial tissue) with toothpicks from the growing colony and put it into the extraction buffer. **Note:** Agar fragments in the mycelial tissues do not affect this method. Harvested mycelia should not be left for long periods in the extraction buffer. Rapid process is recommended for a better yield and quality.
4. Mechanically pulverizing fungal tissues * Time: ~5 s per sample
Pulverize the fungal mass with the electric grinder shown in Figure 1. Gently push down the mycelial tissues with the plastic pestle and operate the grinder for 1-2 s. Complete pulverization is not required. To prevent cross-contamination, wash the pestle tip between samples (dip the pestle tip in 70% EtOH and operate the machine for 1-2 s, then dip the pestle tip into sterile distilled water and operate it again. Drying the pestle tip is not required). **! Caution** Do not push the pestle too hard against the tube or operate the grinder for more than 5 s because heat from friction may damage the tube or pestle tip. Do not operate the grinder without a protective cap. Do not take the pestle off the tube while the machine is operating.
5. Eliminating cell debris and contaminants * Time: 10 min + 10 s per sample
Centrifuge cell lysates at 5,000 rpm for 10 min. Decant supernatant directly into the tubes of 2-propanol that were prepared in Step 2. Discard remaining lysates (~0.1 ml) and cell debris after decanting. **Note:** Some debris may contaminate the supernatant, but this has no effect on the method.
6. DNA precipitation * Time: 10 min + 10 s per sample
Mix the lysate and 2-propanol by inverting the tube several times. Centrifuge the tube at 12,000 rpm for 10 min. Discard as much of the supernatant as possible. (Optional: Wash the tube with 0.8 ml 70% EtOH)
7. Dissolving DNA pellet * Time: 15 min + 5 s per sample
Evaporate any remaining EtOH by incubation at 37°C for 15 min. Add 50 µl distilled water or 1X TE and dissolve the DNA pellet by tapping or vortexing at low speed. Complete desiccation is not required because the remaining solution will be free of EtOH after 15 min incubation. Using a desiccator or freeze-drier may reduce drying time.

diameter) to save incubation space. Use of multi-well plates is recommended to prevent cross contamination for high sporulating species. Disruption of the fungal cell wall and removal of cell contaminants are indispensable for high yield and purity of DNA. In the QS method, mechanical pulverization is used to generate the cell lysates, because it is faster than procedures using cell wall degrading enzymes or high temperatures (Lugert et al., 2006). Mechanical pulverization in the QS method can be further accelerated using a simple electric grinder. The inner structure of the grinder is shown in Fig. 1. This grinder was made by replacing the driver head of an electric screwdriver (OEA-0411-HD1, Orange, China) with a conical plastic pestle (F199230001, Bel-Art, Pequannock, NJ). Manual pulverization with a plastic pestle also works, but use of the electric grinder is recommended for large-scale experiments, as this device enables a researcher to process ~1,000 samples per day. An extraction buffer containing 1 M KCl (Thomson and Henry, 1995) is used for salting out contaminants from the cell lysates. Because KCl is compatible with the PCR reaction, the cell lysates can be directly used for PCR reaction. Organic extraction procedures are omitted in the QS method. This reduces potential risks and additional expenses from use of hazardous materials so that DNA extraction can be done on laboratory benches, thus providing improved space utilization and convenience.

We tested yield and quality of DNA extracted with the QS method. Various weights (1-30 mg) of *M. oryzae* mycelia from 3-day-old colonies on the complete agar medium were used, and the extracted DNA was quantified (Fig. 2). There was a linear correlation between amounts of fungal mass and DNA yield, and the regression equation was $y = 0.3893x$ ($R^2 = 0.8194$). This suggests that the minimum threshold of fungal mass to extract >2 µg DNA is 10 mg,

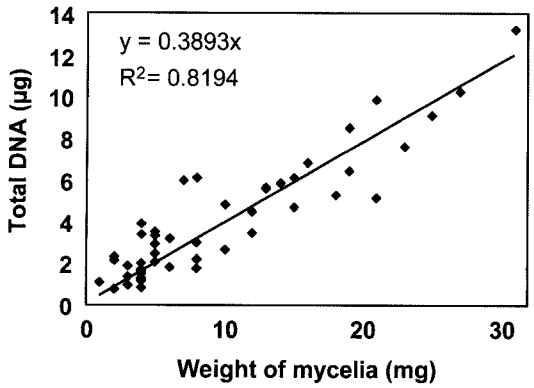

Fig. 2. Correlation between amount of fungal mass and total DNA extracted with the QS method. The regression equation was generated from 51 samples in total.

equal to ~20 mm^2 of fungal colony. To evaluate DNA quality, we applied the QS-extracted DNA to PCR amplification and Southern hybridization. About 50 ng genomic DNA (1 μl) extracted from *M. oryzae* transformants was used for PCR reactions. The mutant allele (4 kb) and/or the wild-type allele (2 kb) bands were clearly amplified from all tested transformants (Fig. 3). For Southern hybridization analysis, QS-extracted DNA was compared with DNA extracted using a conventional method (Rogers and Bendich, 1985). Genomic DNAs from four *M. oryzae* transformants (TF#1-4), which contained several copies of the hygromycin phosphotransferase gene (*HPH*), were prepared by two methods. The genomic DNA was digested with restriction enzymes (*Xho*I, *Sac*I, or *Bam*HI), and 0.6 k *HPH* gene fragment was used as a probe. Band patterns and intensities from both DNA samples were identical (Fig. 3), indicating that QS-DNA is suitable for restriction enzyme reactions and Southern hybridization.

The QS method was applied to a broad range of eukaryotic organisms. Genomic DNA was successfully extracted from eight true fungi (*Fusarium oxysporum*, *F. graminearum*, *F. verticilioides*, *Colletotrichum gloeosporioides*, *C. acutatum*, *Phomopsis* spp., *Botryosperia* spp. and *M. oryzae*), nine oomycetes (*Phytophthora europaea*, *P. cactorum*, *P. quercina*, *P. citricola*, *P. citrophthora*, *P. cambivora*, *P. cactorum*, *P. botryose* and *Pythium* sp.), five uncharacterized lichens, and four plant species (*Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum* and *Brassica oleracea*).

Although several simplified methods for fungal DNA extraction have been developed, they provide genomic DNA applicable only for PCR (Borman et al., 2006; Cenis, 1992; Liu et al., 2000; Thomson and Henry, 1995), use phenol and chloroform (Cassago et al., 2002; Guo et al., 2005), or require expensive instruments and supplies (Loeffler et al., 2002; Muller et al., 1998). The QS method provides not only efficiency in time, space, and cost but also high quality and quantity of DNA with organic solvent-free procedures. This QS method could be more applicable for high-throughput experiments in fungal genomics studies.

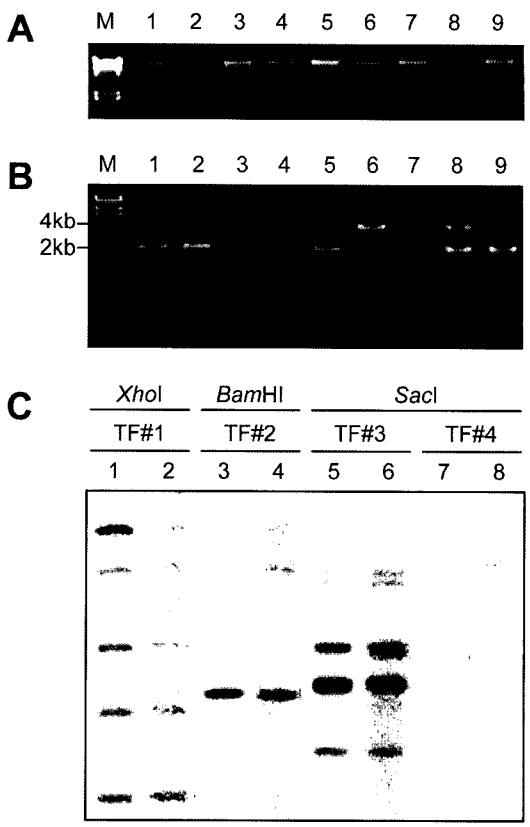

Fig. 3. Genomic DNA extracted with the QS method and their applications in PCR reactions and Southern hybridization. (A) DNA samples extracted from different transformants of *Magnaporthe oryzae*. One microliter of DNA solution (out of 50 μl) for each sample was electrophoresed on a 0.7% agarose gel. (B) PCR amplification using DNA prepared by QS method. Five microliter of each PCR product (out of total 10 μl) was visualized. The 2 kb and 4 kb bands indicate wild type and mutant alleles, respectively. (C) Southern hybridization using DNA samples from conventional method and QS method. One microgram of genomic DNA from 4 different transformants (TF#1-4) isolated by each method was digested with *Xho*I, *Bam*HI and *Sac*I. Odd and even lanes indicate DNA samples from conventional and QS methods, respectively.

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