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Tools and techniques

High molecular weight genomic DNA mini-prep for filamentous fungi

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ABSTRACT

Purification of high quality genomic DNA (gDNA) from filamentous fungi suitable for whole genome sequencing has previously involved many steps. Here, we report a simple and easy-to-follow mini-preparation protocol for high molecular weight (~20 kb) gDNA from filamentous fungi including *Aspergillus* and *Eurotium*. This comprehensive protocol includes graphic step-by-step instructions for inoculation, homogenization, and purification of gDNA. The most critical step is a thorough 3–5 min homogenization of the freeze-dried mycelium using a motorized hand-held homogenizer with a mini spatula inserted. Approximately 20 mg of the fine mycelial powder is then subjected to a modified procedure for the DNeasy Plant Mini Kit (Qiagen). This Qiagen spin column protocol avoids precipitation, dryness, and resuspension of gDNA, which can cause shearing and loss of gDNA. Final gDNA yields from ~20 mg of fine mycelial powder are 8 to 20 μg with a consistent 260/280 nm absorbance ratio of ~1.9. All 30 gDNA samples we purified using our method were of high molecular weight (~20 kb). Whole genome sequencing of these DNA samples resulted in 160–260 X coverage with 2 × 150 reads using NextSeq 500. These gDNAs are also of a suitable quality for Southern blotting and PCR-based amplification of various genes in filamentous fungi.

1. Introduction

Extraction of high-quality genomic DNA (gDNA) from filamentous fungi for use in PCR amplification and whole genome sequencing applications has been a challenge due to the complex multi-layered cell wall and the high concentrations of polysaccharides and pigments that bind to and/or co-precipitate with the gDNA. While our previously reported gDNA isolation protocol using a bead beating method can provide high yields of gDNA suitable for PCR analyses, it unavoidably fragments the gDNA, resulting in gDNA ranging from 2 to 10 kb in size (Yu et al., 2004).

The most commonly cited fungal gDNA isolation method involves manual grinding of the freeze-dried mycelium in a microcentrifuge tube in liquid nitrogen using a hand-held micro-pestle followed by shearing, multiple precipitations, chloroform extraction, and resuspension (Al-Samarrai and Schmid, 2000). While one can extract high yields (8–32 μg) of high molecular weight (HMW) gDNA in 1 h using this method, results may be somewhat inconsistent depending on the skills of the user. Other fungal gDNA extraction protocols involve either organic solvent extractions or generation of protoplasts, requiring additional time and skills for gDNA preparation (Al-Samarrai and Schmid, 2000; Gonzalez-Mendoza et al., 2010; Tapia-Tussell et al., 2006; Vazquez-Angulo et al., 2012).

In this report, we present a simple and reproducible method for HMW gDNA preparation, which can be easily followed by undergraduate and high-school researchers. We have simplified the grinding of the lyophilized mycelium by implementing a motorized hand-held grinder with a micro-spatula to effectively generate fully homogenized hyphae. Starting with about 20 mg of fine mycelial powder that is subjected to a modified DNeasy Plant Mini Kit (Qiagen, 2015) procedure, one can consistently obtain 8 to 20 μg of HMW gDNA (~20 kb in size) with a 260/280 nm absorbance ratio of 1.84–1.99 (± 0.05). The recovered gDNA samples are of a suitable quality for next generation sequencing (NGS), Southern blotting, and PCR-based amplification of various genes of fungal genomes.

2. PROTOCOL (~1 h)

2.1. Reagents and equipment required

- Complete medium (CM)(Pontecorvo et al., 1953).
  20 g of D-glucose, 50 ml of 20× nitrate salt solution, 1 ml of trace

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element solution, 1 g of yeast extract, 2 g of Bacto Peptone, 1.5 g of Casamino acids Add ddH_2O to a final volume of 1 L. Adjust pH to 6.5 with 1 N NaOH.

Potato dextrose broth (PDB) can be used instead of CM, though the fungus may grow more slowly.

- 2 ml screw cap microcentrifuge tubes (Thermo-Fisher, Catalog No. 21-403-201) → Any microcentrifuge can be used here.
- Cordless Pestle Motor (VWR Catalog No. 47747-370) → We found that the VWR motor has a rubber connector which can accommodate the microspatula.
- Handi-Hold™ Microspatula (Fisherbrand™, Catalog No. S67384): This replaces the disposable pellet mixers for the Pestle Motor.
- DNeasy Plant Mini Kit (Qiagen, Catalog No. 69104)
- A microcentrifuge
- A water bath at 65 °C
- A freeze-dryer

2.2. Notes before the experiment

Centrifugation steps are performed at RT (18–25 °C).

Add ethanol (99.5%, Anhydrous) to the AW1 and AW2 buffers as directed in the DNEasy kit instructions.

2.3. Part 1: Fungal inoculation and collecting the mycelium

1. Pour ~10 ml of liquid CM (or PDB) onto a sterile petri dish.
2. Inoculate a loopful of spores (10^3–10^4/loop) from the solid culture of a fungus using an inoculation loop (Fig. 1) and gently swirl the plate to spread the spores. → For slow-growing fungus, proceed with a liquid-shake culture to obtain sufficient amount of vegetative cells.
3. Incubate the plate at the optimal growth temperature (~30 °C for *Aspergillus*) for 12–15 h until a fine semi-transparent mycelial mat without signs of development is formed on the surface of the liquid medium (Fig. 1). → Sporulation can cause degradation of gDNA and unwanted pigmentation.
4. Collect the mycelial hyphal mat from the surface of the liquid medium, wash briefly with sterile ddH_2O, and squeeze-dry thoroughly on a paper towel (Fig. 2).
5. Roll and transfer the squeezed-dried sample (100–200 mg mycelia) into a 2 ml screw cap microcentrifuge tube and place it in a microcentrifuge tube rack (Fig. 2).
6. Freeze the sample by pouring liquid N_2 onto the rack, or placing the sample at −80 °C for at least 1 h.
7. Freeze-dry the samples for 12–14 h. One can simply wrap the top of the open micro-centrifuge tubes with a sheet of Kimwipe and place the rack into a freeze-dryer (Fig. 2).

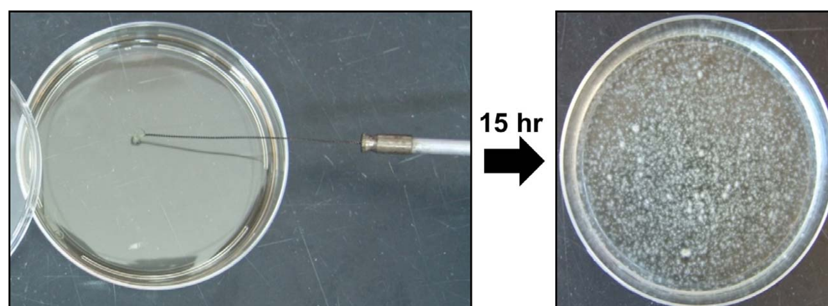

Fig. 1. Inoculation. Inoculate a loopful of spores into 10 ml liquid CM in a petri dish (Left panel). Hyphal mat will form on the surface of the medium in 12–15 h (Right panel).

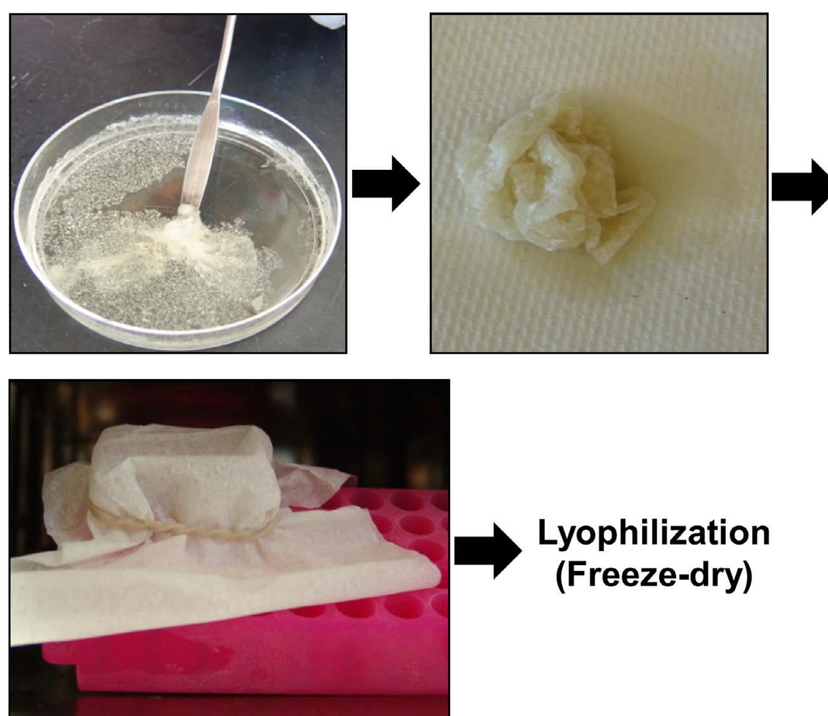

Fig. 2. Collecting mycelia. Collect the hyphal mat from the surface using a spatula (Top left panel), wash, and squeeze-dry using paper towels (Top right panel). Roll a dried sample into a tube and cover the open tubes in a rack with a Kimwipe (Bottom panel). Freeze-dry the frozen samples for 12–14 h.

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