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A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues

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Molecular approaches have become an important tool for mycologists studying fungal systematics, molecular evolution, population genetics or plant–fungus interactions. For this purpose the analysis of restriction fragment length polymorphisms (RFLPs), PCR-based techniques like amplification of polymorphic sequences by one random primer (RAPDs) or amplification of defined genomic regions are applied. Prerequisite for such studies is the rapid isolation of pure genomic DNA of high molecular weight not only allowing simultaneous processing of large numbers of samples but also providing reproducibility with regard to restriction, ligation, and PCR. Most common problems encountered in fulfilling these conditions are the rather high production of polysaccharides by some fungal cultures, and extraction of insufficient amounts of DNA from fruit bodies or from lignified and/or infected plant tissues. Few published protocols include steps for efficient removal of polysaccharides (Do and Adams 1991). Moreover, most procedures contain phenol–chloroform treatments (e.g. Garber and Yoder 1983, Blaiseau *et al.* 1992) and/or more than one precipitation (e.g. Rodriguez and Yoder 1991: four precipitations) — both latter steps are possible causes of shearing. The short protocol given below combines inactivation of proteins by SDS/Proteinase K with precipitation of acidic polysaccharides by hot CTAB in the presence of SDS and high salts (Kim *et al.* 1990) and only a single selective precipitation of DNA with isopropanol (Marmur 1961, Cryer *et al.* 1975). The method is appropriate for simultanous processing of many samples because all steps can be done in Eppendorf tubes, but it can also be scaled up easily for bulk preparations. A treatment with methanol containing 0.1% mercaptoethanol preceding DNA extraction was performed when basidiocarps and plant tissues contained large amounts of reactive compounds (resins, phenolics) interfering with solubilization of DNA. Methanol extraction was found to be superior over other solvents such as acetone (Schneiderbauer *et al.* 1991). In this way we isolated DNAs from a wide variety of fungi including *Fusarium, Pseudocercosporella, Phytophthora, Setosphaeria, Armillaria, Heterobasidion, Boletus, Russula, Lactarius, Suillus, Cortinarius*, and from conifer roots and needles (*Picea abies, Pinus sylvestris*). DNAs were subjected to Pulsed Field Gel Electrophoresis to verify their high molecular weight: the majority of DNA molecules was in the size range of 50–150 kbp and only insignificant amounts of DNA <50 kbp were present (Figure 1, lane 1). DNAs are suited as substrate for restriction, ligation and PCR (Figure 1, lanes 4–6 and 8–10). Yields depend on the species/isolate. For example, of *Fusarium* spp. yield averaged 100 μg from 30 mg dried mycelium with an average purity measured as $A_{260/280}$ of 1.78 ± 0.05. Needles and basidiocarps yielded less (10–30 μg).

DNA extraction from fungi

For culturing fungi media which do not stimulate production of polysaccharides should be used whenever possible. 30–60 mg dry material or a corresponding amount of fresh weight are sufficient for microextraction in 500 μl buffer:

1. Grind lyophilized mycelium with fine sand in a mortar (or without sand in a mixer mill or grind fresh material in liquid nitrogen).

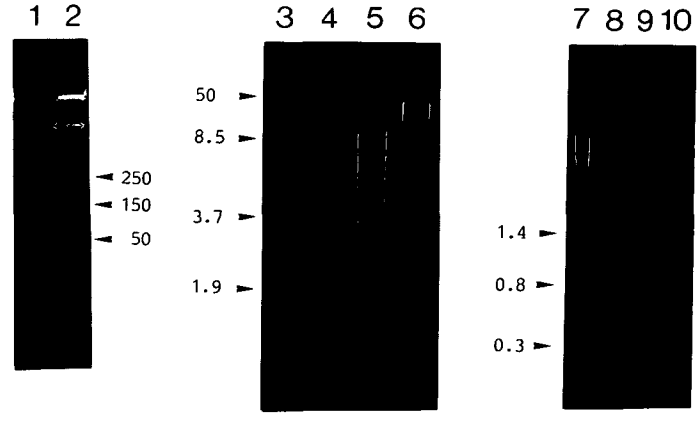

Figure 1. Examples of fungal DNAs prepared by the protocol described here (fragment sizes in kbp). Lane 1: Pulsed Field Gel Electrophoresis (CHEF-DR II, Bio-Rad) of undigested genomic DNA of *Xerocomus chrysenteron* fruitbody; running conditions: 6 V/cm at 14°C for 16 h with pulse times ramped from 3 sec to 45 sec, 1% agarose, 0.5×TBE. Lanes 4, 5, 6: Electrophoresis (1 V/cm, 7 h, 0.8% agarose) of RNase-treated genomic DNA of a plasmid-containing *Fusarium graminearum*; undigested (4), BglII-digested (5), and a religated BglII-digest (6). Lanes 8, 9, 10: Electrophoresis (1.5 V/cm, 6 h, 1.5% agarose) of random amplified polymorphic DNA of *Fusarium avenaceum* (8), *F.culmorum* (9), and *F.graminearum* (10); primer: 5'-GGGTGTGTAG-3'. Lanes 2, 3, and 7 are molecular weight standards (λ-ladder, mixture of undigested and BstEII-digested λ, and 100-bp ladder (Pharmacia), respectively).

2. Put powdered mycelium into a microtube with 500 µl TES (100 mM Tris, pH 8.0, 10 mM EDTA, 2% SDS); add 50–100 µg Proteinase K from an appropriate stock solution; incubate for 30 min (minimum) up to 1 h at 55°–60°C with occasional gentle mixing. Note: if sand is present mixing is easier and more efficient in a 2 ml tube than in the conical 1.5 ml tubes.
3. Adjust salt concentration to 1.4 M with 5 M NaCl (= 140 µl), add 1/10 vol (= 65 µl) 10% CTAB, incubate for 10 min at 65°C.
4. Add 1 vol SEVAG (= 700 µl), mix gently, incubate for 30 min at 0°C; centrifuge for 10 min at 4°C, rpm_{max}.
5. Transfer supernatant to a 1.5 ml tube, add 225 µl 5 M NH_4Ac, mix gently; place on ice for approx. 30 min (the longer the better); centrifuge, 4°C, rpm_{max}.
6. Transfer supernatant to a fresh tube; add 0.55 vol isopropanol (≈510 µl) to precipitate DNA; centrifuge immediately for 5 min, rpm_{max}. If no DNA lumps become visible immediately place samples on ice before centrifugation for approx. 15–30 min. If a compact DNA lump is present transfer of DNA with a hooked Pasteur pipette is also possible.
7. Aspirate off supernatant and wash pellet twice with cold 70% ethanol, dry pellet and dissolve it in about 50 µl TE.

Optional: After step 5 an RNase treatment can be inserted.

DNA extraction from fruit bodies and plant tissues

Extract powdered tissues (step 1) three times with 1 ml methanol containing 0.1% mercaptoethanol and centrifuge between each extraction. Dry pellet at least 1 h at room temperature and continue with step 2 of the above protocol.

ABBREVIATIONS

SEVAG: chloroform: isoamylalcohol, 24:1, v/v; CTAB: cetyltrimethylammoniumbromide.

REFERENCES

1. Blaiseau,P.-L., Kunz,C., Grison,R., Bertheau,Y. and Brygoo,Y. (1992) *Curr. Genet.* **21**, 61–66.
2. Cryer,D.R., Eccleshall,R. and Marmur,J. (1975) *Methods Cell. Biol.* **12**, 39–44.
3. Do,N. and Adams,R.P. (1991) *Biotechniques* **10**, 162–166.
4. Garber,R.C. and Yoder,O.C. (1983) *Anal. Biochem.* **135**, 416–422.
5. Kim,W.K., Mauthe,W., Hausner,G. and Klassen,G.R. (1990) *Can. J. Bot.* **68**, 1898–1902.
6. Marmur,J. (1961) *J. Mol. Biol.* **3**, 208–218.
7. Rodriguez,R.J. and Yoder,O.C. (1991) *Experimental Mycology* **15**, 232–242.
8. Schneiderbauer,A., Sanderman,H. and Ernst,D. (1991) *Anal. Biochem.* **197**, 91–95.