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Abstract
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PCR from fungal spores after microwave treatment

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The polymerase chain reaction (PCR) has been widely used for diverse genetic analyses in fungi, including population genetics and phylogenetic studies. For some of these analyses a large number of DNA samples are needed. Although several simplifications of DNA extraction protocols have been reported, most of them involve the mechanical disruption of mycelial tissue, use of organic solvents and various centrifugation steps. Direct PCR from fungal spores is not readily suitable to all fungi mainly because of difficulties in rupturing the cell wall. Although spores from some fungi can be directly amplified after a prolonged initial step at 94oC in the PCR (Aufauvre-Brown et al.1993, Curr. Genet. **24**:177-178), spores from different fungi can be less susceptible to heat denaturation in aqueous solution (Saupe S. and Ferreira A.V.B., unpublished results). Direct spore PCR was performed after freezing the samples at very low temperatures (Jin-Rong and Hamer, 1995 Fungal Genet. Newsl., **42**:80) but it was shown that an excessive number of spores can inhibit the PCR.

Microwave irradiation has proven to be useful in DNA extraction protocols from different eukaryotes (Goodwin and Lee 1993, Biotechniques **15:**438-444). It is presumed that microwave irradiation acts by exposing the DNA normally protected by the cell structures. Here we describe a simple 10-minute template preparation method for PCR amplification from minute quantities of fungal spores using microwave irradiation.

Vegetative spores from five filamentous fungal species were taken from an agar slant or plate with a loop and transferred to an empty 500 ul Eppendorf tube. *Neurospora crassa* sexual spores were also tested. Each tube was closed and the dry spores were irradiated in full power (700W) for 5 min in a domestic microwave oven (Danby D811). A beaker containing water was present during the irradiation to avoid damage to the oven. Thirty microliters of TE (10 mM Tris-HCl pH 7.5, 1 mM EDTA) was added to the tube, the tube was vortexed and then spun in a microfuge for 1-5 min at 14,000 RPM. The supernatant liquid was collected and transferred to a clean Eppendorf tube. Five microliters of the supernatant liquid was used in a 50 ul PCR. An example of the results of a PCR using the internal transcribed spacer (ITS) ribosomal region primers (White et al. 1990, PCR Protocols, Academic Press.) is shown in Figure 1. DNA prepared from five different fungal species using the microwave method was amplified under the following conditions: 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 5% glycerol, 0.2 mM of dNTPs, 5 units *Taq* DNA polymerase and 0.02 mM of each of the ITS1 and ITS4 primers. After 40 cycles of 95 C, 55 C and 72 C for 1 min. each, half of the reaction mixture was subjected to electrophoresis in 1.5% agarose gels. A control PCR is shown (lane 8) performed with 100 ng of phenol:chloroform-purified *N. crassa* DNA.

We have used this method to screen *N. crassa* transformants for homologous recombination at specific sites using a variety of different primers. Amplification from samples prepared in this manner was efficient even for a single copy gene. At least 6 PCR reactions can be performed if starting with approximately 107 spores (based on *N. crassa*). The time of microwave irradiation could be changed for different species as we have had satisfactory amplifications of *N. crassa* DNA after only one minute (but not 30 sec) of irradiation (not shown). This method drastically reduces manipulation and template preparation time and therefore minimizes the possibility of contamination of the samples with extraneous DNA.

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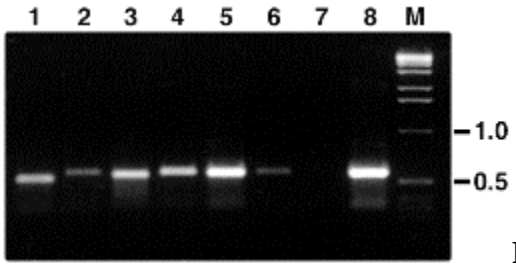

Figure 1. PCR of ITS from filamentous fungi after microwave treatment. Lanes (1) *Fusarium oxysporum*; (2) *Penicillium expansum*; (3) *Alternaria alternata;* (4) *Cochliobolus bicolor*; (5) *N. crassa* vegetative and (6) sexual spores; (7) no DNA control and (8) *N. crassa* phenol:chloroform- extracted DNA. (M) marker, BRL 1 Kb ladder.

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