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**Abstract**
Isolation of DNA from filamentous fungi for PCR analysis is usually time consuming and involves use of toxic chemicals such as phenol/chloroform. In *Saccharomyces cerevisiae*, PCR assay can be performed with yeast colonies (Huxley et al. TIG 1990 **6**:236). Here we describe a PCR protocol which uses *M. grisea* conidia directly for PCR analysis without extraction of DNA.

# Assessment of *Magnaporthe grisea* mating type by spore PCR

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Isolation of DNA from filamentous fungi for PCR analysis is usually time consuming and involves use of toxic chemicals such as phenol/chloroform. In *Saccharomyces cerevisiae*, PCR assay can be performed with yeast colonies (Huxley et al. TIG 1990 **6**:236). Here we describe a PCR protocol which uses *M. grisea* conidia directly for PCR analysis without extraction of DNA.

The PCR assay is performed with conidia produced on an oatmeal culture (Crawford et al. 1986 Genetics, **114**:1111-1129) that has been grown at room temperature for one week. To collect conidia, 5 ml sterile water is added to each 0.5 ml microtube. A flat-ended toothpick is wetted in sterile water and used to gently scrape conidia from the surface of the culture with both sides of the toothpick. Care should be exercised to avoid transferring medium since agar may inhibit the PCR reaction. Conidia can be released by rotating the toothpick briefly in the water in the microtube. The samples are frozen in liquid nitrogen or in a -80 C freezer for 10 min.

For *M. grisea*, 10(4)-10(6) spores per reaction give the best result. Too many spores (over 10(6)) will inhibit the PCR reaction. PCR is carried out by adding to each tube of conidia 45 ml of 10 mM Tris-HCl (pH 9.0 at 25 C) containing 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl2, 0.2 mM each dNTP, 1.0 mM each primer, 1.25 units of Taq DNA polymerase (Promega). Samples are overlaid with 50 ul mineral oil and subjected to the following PCR condition: 95 C for 5 min; 30 cycles of 95 C for 1 min / 52 C for 2 min / 72 C for 2 min; followed by 72 C for 5 minutes.

PCR primers used for *M. grisea* are A1 (AGCCTCATCAACGGCAA) and A5 (GTAGCGTACAAGCACGG) for Mat1-1, B15 (CTCAATCTCCGTAGTAG) and B16 (CATCCGATATGACGACA) for Mat1-2. The expected PCR products are 372 bp for Mat1-1 and 376 bp for Mat1-2 (Courtesy of Dr. Seochang Kang). For other fungi, the 18S rDNA primers NS1 and NS2 (White et al., 1989. PCR protocols, Academic Press) were used in spore PCR tests.

This spore PCR protocol allows the rapid determination of the mating type (*Mat1*-1 and *Mat1*-2) of *M. grisea*. It is particularly useful for scoring mating type when low fertility or sterility is a problem in crossing or analyzing field collections. The mating type alleles determined by spore PCR are consistent with mating tests on 58 progeny of a cross between strains 4375-R-26 X 4136-4-3. Fungal mycelium can be used instead of spores for PCR, but it may be more difficult to collect mycelium than spores from petri dish culture without touching the agar.

The same protocol has been used successfully with *Neurospora crassa, Fusarium moniliforme* and *Aspergillus nidulans* with 18S rDNA primers NS1 and NS2. This spore PCR protocol can be applicable to other fungi and may be useful for rapid PCR assay without DNA preparation in analyzing transformants and field collections.