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A miniprep procedure for isolating genomic DNA from Magnoporthe grisea

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Abstract

We have developed a simple miniprep procedure for the isolation of genomic DNA from the ascomycete *Magnaporthe grisea*. This pathogen of many grasses, including rice, has a moderate growth rate and produces intermediate to low numbers of conidia when grown in culture. Thus, in our previous DNA preparation procedure we inoculated swirling liquid cultures with mycelium that had been fragmented in a blender rather than with conidia. The mycelium obtained from these cultures was ground in liquid nitrogen for DNA extraction. Though the quantity and quality of DNA obtained by this method is satisfactory, the technique is too laborious for analysis of many strains. We developed the procedure described below to eliminate the need to fragment mycelium in a blender to inoculate cultures and to eliminate the need to grind mycelium in liquid nitrogen for DNA extraction. The new procedure, which relies on the enzymatic removal of cell walls and the lysis of protoplasts, should be readily adaptable to other filamentous fungi with growth characteristics similar to those of *M. grisea*.

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A miniprep procedure for isolating genomic DNA from *Magnaporthe grisea*

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We have developed a simple miniprep procedure for the isolation of genomic DNA from the ascomycete *Magnaporthe grisea*. This pathogen of many grasses, including rice, has a moderate growth rate and produces intermediate to low numbers of conidia when grown in culture. Thus, in our previous DNA preparation procedure we inoculated swirling liquid cultures with mycelium that had been fragmented in a blender rather than with conidia. The mycelium obtained from these cultures was ground in liquid nitrogen for DNA extraction. Though the quantity and quality of DNA obtained by this method is satisfactory, the technique is too laborious for analysis of many strains. We developed the procedure described below to eliminate the need to fragment mycelium in a blender to inoculate cultures and to eliminate the need to grind mycelium in liquid nitrogen for DNA extraction. The new procedure, which relies on the enzymatic removal of cell walls and the lysis of protoplasts, should be readily adaptable to other filamentous fungi with growth characteristics similar to those of *M. grisea*.

1. Inoculate a mycelial plug into complete medium (3 g yeast extract, 3 g casamino acids, 10 g glucose/liter) in wells of tissue culture plates (24-well dishes, six columns with four rows, Corning #25820, Cell WellsTM, 16 mm diameter wells). Using 1.5 ml of complete medium per well, grow each strain in three wells in the dark at 24-28°C without shaking until a mycelial mat covers the well (4-6 days for *M. grisea*).

2. Remove the mycelial mats from the wells, blot them on a towel to remove excess medium, and place all three mats in a well containing 1.2 ml of osmotically-stabilized enzyme solution [0.7 M NaCl, 3.3 mg NovoZymTM 234/ml (Novo Industrias, Bagsvaerd, Denmark)] for two hours at room temperature. Perform this step in a well in the same column as the one that was used for mycelial growth, thereby eliminating sample numbering for the enzyme step.

3. Pipette the digested mycelium to a microfuge tube. (The mycelial mat disintegrates with pipetting.) Pellet the protoplasts for two minutes in a microfuge at full speed. Decant the supernatant.

4. Resuspend the pellet in 600 µl of lysis buffer (50 mM Tris.HCl, pH 7.5, 100 mM EDTA, 0.5% SDS, 0.3 M sodium acetate). Heat the lysed protoplasts at 65°C for 30 minutes.

5. Extract the lysed protoplasts with 600 µl of phenol equilibrated with TE (10 mM Tris.HCl, pH 8.1, 1 mM EDTA).

6. Remove the aqueous phase to clean microfuge tubes containing 500 µl of isopropanol. Mix several times by inversion and spin immediately for 5 minutes in a microfuge.

7. Wash the pellet in 70% EtOH and air dry.

8. Dissolve the nucleic acid pellet in 100 µl TE containing 1 µg RNAse A/ml (Sigma, St. Louis).

We have successfully used this procedure to analyze several hundred transformants. One person can easily complete fifty minipreps in one day. Ten microliters of the DNA obtained (1/10 the preparation) cuts readily in 25 µl reaction mixtures with all enzymes tested (including *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Sst*I and *Xho*I). This quantity provides a strong signal on Southern blots when 4 mm gel lanes are used. The small variation in the quantity of DNA obtained from different samples allows preliminary screening without DNA quantification. - - - Central Research and Development Department, Du Pont Experimental Station, P.O. Box 80402, Wilmington, DE 19880-0402.