

EXTRACTION OF DNA FROM *PHYTOPHTHORA INFESTANS*

Adapted by H. Judelson from a published procedure for extracting DNA from fungi by Raeder and Broda, with modifications based on a method used by B. Tyler for Neurospora. This works best with fresh mycelia, giving DNA that is readily digested with restriction enzymes. It usually works well using lyophilized mycelia as well, but in a few instances poor digestion was observed.

Growing mycelia

1. Fill each of three 100 mm petri plates with 20 ml clarified V8 or rye media. Inoculate each plate with about 3 small tufts of mycelia. Incubate at 18°C until the plate is about 80% full of mycelia (i.e. harvest when the culture is still growing).
2. Collect mycelia by harvesting on filter paper in a Buchner funnel, or by blotting on paper towels until most of the liquid is removed and the mycelia starts to look dry.
3. Either extract immediately (first choice), or for later extractions, freeze at -80°C (second choice), or freeze and lyophilize (third choice)

Extraction of DNA

1. Grind in mortar and pestle to powder. For lyophilized mycelia, grind dry. For fresh or frozen material, precool mortar with liquid nitrogen, fill again with nitrogen, drop in mycelia, grind, and then let nitrogen boil off.
2. Move powder to oak ridge tube. For starting with about 1 g of fresh mycelia or 0.2 g of lyophilized mycelia, add 10 ml extraction buffer (0.2 M Tris 8.5, 0.25 M NaCl, 25 mM EDTA, 0.5% SDS). Invert tube several times until thawed (for fresh material), or invert and let stand 5 minutes (lyophilized material, to allow for rehydration).
3. Add 7 ml of phenol (buffered to about pH 8.0 with 0.1 M Tris-Cl) plus 3 ml of chloroform (24:1 chloroform:isoamyl alcohol). Shake a few times to mix phases. Place tubes on side on orbital shaker and shake for 20 minutes (30 minutes for lyophilized material) at about 90 rpm.
4. Spin 30 min, 10k x g in centrifuge at room temperature; a swinging bucket rotor is preferred at this step.
5. Save supernatant (avoid interface), add an equal volume of chloroform: isoamyl alcohol, and shake a few times to mix phases. Spin again for 5 minutes.
6. Move supernatant to a new tube and add 50 µl of 10 mg/ml RNase A. Incubate 30 min at 37°C.
7. Add 0.6 volumes isopropanol. Invert several times to mix. Let sit 5 minutes (or longer).
8. Centrifuge at 10k x g for 20 minutes. Pour off supernatant and spin again for 30 seconds. Pipet off any remaining fluid. Add 5 ml 70% ethanol, spin 1 minute, pour off supernatant. Dry (air dry or briefly under vacuum).
9. Add TE (10 mM Tris 7.5- 1 mM EDTA); usually about 300 µl. Place at 65°C for one hour to aid resuspension. Usually 50-200 µg of DNA is obtained. Check OD and run a small amount on a gel to check for quality and the extent of RNA contamination.

