

DNA extraction from fungal mycelium using Extract-n-Amp

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Abstract

This is a modified protocol for extracting DNA from fungal mycelium using the Sigma-Aldrich Extract-N-Amp Plant Kit published by J.M. U'Ren et al. (2016) Contributions of North American endophytes to the phylogeny, ecology, and taxonomy of Xylariaceae (Sordariomycetes, Ascomycota). *Molecular Phylogenetics and Evolution* 98: 210-232.

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Protocol

Step 1.

Turn heat block on and preheat to 95C.

Step 2.

1. Working at the lab bench, add ½ scoop of the 0.2 mm zirconium oxide beads to a sterile 1.5 ml tube. Close the lid and label the top and side of each tube with the sample name (e.g., AK0013).

Step 3.

1. In either the laminar flow hood or biosafety cabinet, remove a small piece (no bigger than 0.5 cm²) of mycelium from the culture tube/plate and transfer to the corresponding labeled 1.5 ml tube. Try to minimize the amount of agar.
3. ***Note: The second step must be done in a sterile environment to ensure the cultures are not contaminated, but the remaining steps can be done on the lab bench.

■ ANNOTATIONS

Ming-Min Lee 15 Sep 2016

In this step (3) the second sub-step is blank. The 3rd sub-step is ambiguous and may refer to Step 2 (adding the beads) or to this step (3), which I think is the intent...?

Step 4.

1. Add 100 ul of Extraction Buffer (making sure the mycelium is completely submerged in the liquid; you may need to centrifuge) and firmly close tube lids.

Step 5.

1. Place tubes in the Bullet Blender Storm (making sure the lid is for the 1.5 ml tubes not the screwcap tubes), close the lid, and bead beat for 1 minute on speed 10. (Alternatively you can grind the tissue with a sterile blue pestle.) *Use ear protection when using the bead beater.*

Step 6.

1. Briefly (<30 sec) centrifuge to remove liquid from the lid of the tube.

****Do not leave samples in the Extraction Buffer >25 minutes before proceeding to the heating step.*

Step 7.

1. Place tubes in heat block at 95°C for 10 minutes; then briefly centrifuge after incubation to remove condensation.

Step 8.

1. Add 100 ul of Dilution Buffer and vortex to mix.

Step 9.

1. Store the tube at 2-8°C until PCR, and then store at -20° to -80°