# DNA extraction from fungal mycelium using Extract-n-Amp

**Version 2**

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## Abstract

This is a modified protocol for extracting DNA from fungal myelium 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.

**Citation:** Jana U'Ren DNA extraction from fungal mycelium using Extract-n-Amp. **protocols.io** dx.doi.org/10.17504/protocols.io.fugbntw
**Published:** 16 Sep 2016

## Protocol

**Step 1.**
Turn heat block on and preheat to 95C.

**Step 2.**

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).

ANNOTATIONS
**Naupaka Zimmerman** 02 Oct 2016

I believe we have always used the 1 mm beads instead of 0.2 mm?

**Step 3.**

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.

***This step must be donin a sterile environment to ensure the cultures are not contaminated, but the remaining steps can be done on the lab bench.

NOTES
**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.**

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.**

Place tubes in the bead beater.  If using the Bullet Blender Storm make 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.**

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.**

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

**Step 8.**

Add 100 ul of Dilution Buffer and vortex to mix.

**Step 9.**

Store the DNA at 2-8°C for short-term use and -20° to -80° for long-term storage.