



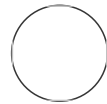
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WORKS FOR ME

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Modified Promega Wizard Extraction for Barcoding Macrofungi

DOI

dx.doi.org/10.17504/protocols.io.rm7vzb3p4vx1/v1[Stephen Douglas Russell](#)¹¹The Hoosier Mushroom Society

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COMMENTS 0

ABSTRACT

This protocol is best used when preparing macrofungal specimens for Sanger sequencing or as a secondary extraction protocol for ONT nanopore barcoding.

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PROTOCOL CITATION

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71350

MATERIALS TEXT

Equipment:

Tube Racks for 1.5uL eppi tubes
Tweezers
Pestles
Heat Block
Vortexer
Centrifuge

Consumables:

1.5uL eppi tubes
Molecular water
70% ethanol
Kimwipes

Reagents:












☒ Nuclei Lysis Solution, 1000ml **Promega Catalog #A7943** Step 1

☒ Protein Precipitation Solution 350ml **Promega Catalog #A7953** Step 6

☒ Isopropanol **IBI Scientific** Step 7

- 1 Add 600uL of ☒ Nuclei Lysis Solution, 1000ml **Promega Catalog #A7943** to 1.5mL eppi tubes. One tube for each specimen you are planning an extraction for.
- 2 Place tissue from your specimens into each tube using tweezers. Utilize a piece about the size of a grain of rice. The tissue can be either fresh or dried. Label the tube with the appropriate number. Wipe the tweezers off with a Kimwipe or paper towel in between each specimen. These tubes can be stored at room temperature until they are ready to be used.
- 3 Grind the tissue in each tube using a sterile pestle.
- 4 Heat the tubes at ☒ 65 °C for ☒ 00:15:00 .

15m

- 5 Centrifuge the tubes for  00:03:00 . 3m
- 6 Transfer the supernatant (liquid on top) to a new 1.5mL eppi tube. 6m 20s
- Add  200 μL of  Protein Precipitation Solution 350ml **Promega Catalog #A7953** to the tube.
- Vortex the tube for  00:00:20 .
- Centrifuge the tube for  00:06:00 .
- 7 Transfer the supernatant (liquid on top) to a new 1.5mL eppi tube. 1m
- Add  600 μL of 100%  Isopropanol **IBI Scientific** to the tube. This precipitates the DNA.
- Centrifuge the tube for  00:01:00 . The DNA will now be in a pellet stuck to the bottom of the tube.
- Discard the supernatant. It can just be poured out of the tube into a waste container.
- 8 Add  600 μL of 70% ethanol to the tube. 16m
- Centrifuge the tube for  00:01:00 .
- Discard the supernatant. It can just be poured directly out of the tube into a waste container.
- Place the tube upside down on a Kimwipe for at least  00:15:00 , or until all of the ethanol has evaporated from the tube. I usually leave the tube to dry overnight.
- 9 Add 30uL of molecular water to the tube.
- Your DNA template is now ready for amplification.