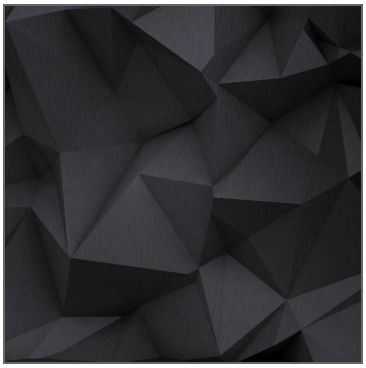

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Modified Promega Wizard Extraction for Barcoding Macrofungi V.3

Stephen Douglas Russell[1]

[1]Mycota Lab / The Hoosier Mushroom Society

Stephen Douglas Russell

ABSTRACT

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

The quality of a DNA extraction method is a primary limiting factor in the total number of samples that will return a result with nanopore barcoding of fungi. The "quick" extraction protocol will often yield a positive result for 80-85% of general fungal collections (less if biased with polypores and recalcitrant species). Utilizing this extraction protocol pushes that number to nearly 100%. It is more time consuming and utilizes more expensive chemicals, but may be worth considering for important specimens that fail with the quick extraction protocol.

Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 96058

MATERIALS

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**
Protein Precipitation Solution 350ml **Promega Catalog #A7953**
Isopropanol **IBI Scientific**

PROTOCOL MATERIALS

Nuclei Lysis Solution, 1000ml **Promega Catalog #A7943** Step 2
Protein Precipitation Solution 350ml **Promega Catalog #A7953** Step 6
Isopropanol **IBI Scientific** Step 7

1 Place tissue from your specimens into each tube using tweezers. Utilize a piece about the size of a grain of rice or smaller. It should easily drop to the bottom of the tube. 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.

2 Add 600uL of Nuclei Lysis Solution, 1000ml **Promega Catalog #A7943** to 1.5mL eppi tubes containing your tissue.

3 Grind the tissue well in each tube using a sterile pestle.

4 Heat the tubes at 65 °C for at least 00:15:00 . It is fine to leave it in longer. I often use one hour. 15m

5 Centrifuge the tubes for 00:03:00 at max rpm. 3m

6 Transfer the supernatant (liquid on top) to a new 1.5mL eppi tube. Label your tubes. 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 at max rpm.

7 Transfer the supernatant (liquid on top) to a new 1.5mL eppi tube. Label your tubes 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.