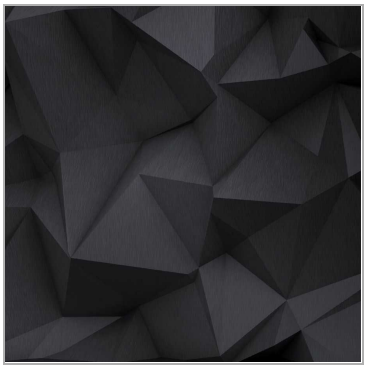

VERSION 2

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

Created: Mar 14, 2023

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78721

Modified Promega Wizard Extraction for Barcoding Macrofungi V.2

In 1 collection

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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. 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.

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**

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.