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General Fungal DNA Extraction V.2

Version 1 is forked from Kasson Lab DNA Extraction

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

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Abstract

This is a routine protocol for extracting DNA from various fungi. This extraction method is suitable for follow-up molecular work such as PCR amplification.

Materials

Sterile micropestles, isopropyl alcohol, ethyl alcohol, cell lysis buffer, protein precipitation buffer, elution buffer, metal scraper.

Protocol materials

- isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W292907
- Cell Lysis Solution, 1000ml (for Wizard Genomic) **Promega Catalog #**A7933
- Nuclei Lysis Solution, 1000ml **Promega Catalog #**A7943
- isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W292907
- Elution buffer pH 8.0 (250 mL) **Alfa Aesar Catalog #**J61558
- Ethyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023
- Cell Lysis Solution, 1000ml (for Wizard Genomic) **Promega Catalog #**A7933
- Protein Precipitation Solution 350ml **Promega Catalog #**A7953
- isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W292907
- Ethyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023
- Elution buffer pH 8.0 (250 mL) **Alfa Aesar Catalog #**J61558
- Elution buffer pH 8.0 (250 mL) **Alfa Aesar Catalog #**J61558
- Cell Lysis Solution, 1000ml (for Wizard Genomic) **Promega Catalog #**A7933

Troubleshooting

Before you begin

1 Turn on hot water bath, set to 65 °C.

2 Pull two Eppendorf 1.5 mL centrifuge tubes per sample.

2.1 Label both sets of tubes with (short) sample names.

2.2 Label one tube set for each sample with an "I" for isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W292907.

Sketch of "I"-labeled tubes (drawing from Angie Macias).

3 Add 200 µL of Cell Lysis Solution, 1000ml (for Wizard Genomic) **Promega Catalog #**A7933 (or Nuclei Lysis Solution, 1000ml **Promega Catalog #**A7943) to **tubes without "I"**.

4 Add 600 µL of isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W292907 to **tubes labeled with "I"**.

5 Place tube with Elution buffer pH 8.0 (250 mL) **Alfa Aesar Catalog #**J61558 into 65 °C water bath.

Extraction Protocol

1h 10m 3s

6 Sterilize some metal scrapers with flame and [M] 95 % (v/v) Ethyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023 .

7 Add 1/2 pea-sized amount of fungal tissue (young hyphae) to each tube containing Cell Lysis Solution, 1000ml (for Wizard Genomic) **Promega Catalog #**A7933 .

7.1 Flame-sterilize and cool scrapers between samples.

7.2 Alternatively, pellet a pea-sized amount of mycelium grown in liquid culture and transfer to each tube.

8 Macerate each sample with a new, sterile micropestle until tissue is homogenous.

9 Add 400 µL of Cell Lysis Solution, 1000ml (for Wizard Genomic) **Promega Catalog #**A7933 (to 600 µL total volume added).

10 Add tubes to a floating rack to allow samples to incubate directly in 65 °C water bath for 00:30:00 . 30m

11 Remove samples and vortex for 00:00:03 before returning to 65 °C water bath for 00:30:00 . 30m 3s

11.1 Place a sufficient aliquot of Elution buffer pH 8.0 (250 mL) **Alfa Aesar Catalog #**J61558 in water bath to warm for Step 21.

12 Remove samples and allow them to cool on the bench for 00:05:00 . 5m

13 Add 200 µL of Protein Precipitation Solution 350ml **Promega Catalog #**A7953 to each tube and vortex for 10 seconds.

14 Centrifuge samples for 00:03:00 at 14.000 rpm . 3m

> Note
>
> Proteins will form a large pellet: unload samples carefully into rack.

15 Using a P1000 micropipette, transfer supernatant to each tube containing isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**W292907 and gently mix by inversion.

> Note
>
> It's better to leave some liquid than to carry bits of the protein pellet into the next step.

16 Centrifuge for 00:01:00 at 14.000 rpm . 1m

17 Carefully pour off the supernatant into waste container.

> Note
>
> Be careful to not lose your white DNA pellet!

18 Add 600 µL of [M] 70 % (v/v) Ethyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023 to each tube and mix gently by inversion.

19 Centrifuge for 00:01:00 at 14.000 rpm . 1m

20 Repeat Step 16.

21 Open and invert tubes onto a clean paper towel.

Note

A tube rack can be placed on the tube lids to secure inverted tubes onto the paper towel.

22 Add 100 µL of warmed Elution buffer pH 8.0 (250 mL) **Alfa Aesar Catalog #**J61558 to each tube.

23 Store fully-labeled tubes in a box (not a tube rack) in the -20 °C freezer.