



JUL 12, 2023

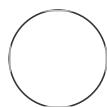
General Fungal DNA Extraction

Forked from [Kasson Lab DNA Extraction](#)

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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.

OPEN ACCESS

Protocol Citation: Angie Macias, Matthew T Kasson, Brian Lovett 2023. General Fungal DNA Extraction. [protocols.io](https://protocols.io/view/general-fungal-dna-extraction-cwn9xdh6) <https://protocols.io/view/general-fungal-dna-extraction-cwn9xdh6>

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


Created: Jul 03, 2023

Last Modified: Jul 12, 2023

PROTOCOL integer ID:
84417


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 (Angie Macias).

3 Add  200 μ L 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 μ L of

 isopropyl alcohol Merck MilliporeSigma (Sigma-Aldrich) Catalog #W292907

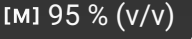


to **tubes**

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  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.

- 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 μ L 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  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.