Aug 10, 2022

Kasson Lab DNA Extraction

Angie Macias[1], Matthew T Kasson[1], Brian Lovett[1]

[1]West Virginia University

dx.doi.org/10.17504/protocols.io.q26g78413lwz/v1

Brian Lovett
West Virginia University

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.

DOI

dx.doi.org/10.17504/protocols.io.q26g78413lwz/v1

PROTOCOL CITATION

Angie Macias, Matthew T Kasson, Brian Lovett 2022. Kasson Lab DNA Extraction. **protocols.io** https://protocols.io/view/kasson-lab-dna-extraction-bn6tmhen

CREATED

Oct 28, 2020

LAST MODIFIED

Aug 10, 2022

PROTOCOL INTEGER ID

43955

MATERIALS TEXT

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

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 **Sigma Catalog #W292907** .

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

3 Add **600 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 **Sigma Catalog #W292907** to **tubes labeled with "I"**.

Citation: Angie Macias, Matthew T Kasson, Brian Lovett Kasson Lab DNA Extraction
https://dx.doi.org/10.17504/protocols.io.q26g78413lwz/v1

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

Extraction Protocol 1h 10m 3s

6 Sterilize some metal scrapers with flame and **95 % (v/v)** Ethyl Alcohol **Sigma 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 tubes to a floating rack to allow samples to incubate directly in **65 °C** water bath for 30m **00:30:00** .

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

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

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

Citation: Angie Macias, Matthew T Kasson, Brian Lovett Kasson Lab DNA Extraction
https://dx.doi.org/10.17504/protocols.io.q26g78413lwz/v1

12 Protein Precipitation Solution

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

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

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

14 Using a P1000 micropipette, transfer supernatant to each tube containing isopropyl alcohol **Sigma Catalog #W292907** and gently mix by inversion.

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

15 Centrifuge for **00:01:00** at **14.000 rpm** . 1m

16 Carefully pour off the supernatant into waste container.

Be careful to not lose your white DNA pellet!

17 Ethyl

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

18 Centrifuge for **00:01:00** at **14.000 rpm** . 1m

Citation: Angie Macias, Matthew T Kasson, Brian Lovett Kasson Lab DNA Extraction
https://dx.doi.org/10.17504/protocols.io.q26g78413lwz/v1

19 Repeat Step 16.

20 Open and invert tubes onto a clean paper towel.

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

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

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

Citation: Angie Macias, Matthew T Kasson, Brian Lovett Kasson Lab DNA Extraction
https://dx.doi.org/10.17504/protocols.io.q26g78413lwz/v1