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Sample preparation and lysis of homogenized malaise trap samples

In 4 collections

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We use this protocol and it's working

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Abstract

This protocol describes the steps of sample preparation and lysis before DNA extraction for the Malaise trap metabarcoding protocol of the LeeseLab.

Guidelines

Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

Materials

Materials required:
Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on thesupply situation.

Chemicals:
Tris ultrapure 99.9% Tris ultrapure 99.9% **Diagonal Catalog #**A1086.1000
Sodium chloride Sodium chloride **Fisher Scientific Catalog #**10112640
EDTA disodium salt EDTA disodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E5134-50G
SDS ultrapure Sodium dodecyl sulfate **Diagonal Catalog #**A1112.0500
Proteinase K Proteinase K **7BioScience Catalog #**RP100B
Hydrochloric acid fuming 37%
Hydrochloric acid fuming 37% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**1003171011
Sodium hydroxide Sodium hydroxide - pellets **Fisher Scientific Catalog #**S/4920/60
Calcium chloride Calcium chloride 94% **Carl Roth Catalog #**A119.1
Glycerol 87% Glycerol 87 % for molecular biology **Panreac AppliChem Catalog #**A3739,1000

Labware:
2 mm dia zirconia beads Zirconia Beads 2 mm dia **BioSpec Products Catalog #**11079124zx
2 mL screwcap tubes T 2 mL screwcap tube **Sarstedt Catalog #**72.693
Wide-bore tips ART Wide Bore Tip 1000 uL **Thermo Fisher Scientific Catalog #**2079G
Disposable PES Bottle Top Filters
Fisherbrand Disposable PES Bottle Top Filters **Fisher Scientific Catalog #**15973307

Stock solutions:

1 L Tris stock solution [M] 1 Mass Percent pH 7.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH 7.5 with HCl
- Adjust volume to 1 L
- Sterilize by filtering and store at Room temperature

1 L NaCl stock solution [M] 5 Mass Percent

- Add 292.2 g Sodium chloride to a beaker
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L EDTA stock solution [M] 0.5 Mass Percent pH 8

- Add 186.12 g EDTA disodium salt to a beaker
- Adjust volume to 1 L with ddH_2O
- Adjust pH to pH 8 with sodium hydroxide
- Sterilize by filtering and store at Room temperature

1 L SDS stock solution [M] 10 Mass Percent

- Add 100 g SDS ultrapure to a beaker
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L Proteinase K storage buffer ([M] 50 millimolar (mM) Tris , [M] 3 millimolar (mM) CaCl2 [M] 50 % (v/v) glycerol) pH 7.8

- Add 50 mL of [M] 1 Mass Percent Tris stock solution pH 7.5
- Add 333 mg calcium chloride
- Add 500 mL of glycerol 87%
- Adjust volume to 900 mL with ddH_2O
- Adjust pH to pH 7.8 with sodium hydroxide
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

Working solutions:

1 L **TNES buffer (** [M] 50 millimolar (mM) Tris , [M] 400 millimolar (mM) NaCl , [M] 20 millimolar (mM) EDTA , [M] 0.5 Mass / % volume SDS , pH 7.5)

- Add 50 mL of [M] 1 Mass Percent Tris stock solution pH 7.5
- Add 80 mL of [M] 5 Mass Percent NaCl stock solution

- Add 40 mL of [M] 0.5 Mass Percent EDTA stock solution pH 8
- Add 50 mL of [M] 10 Mass / % volume SDS stock solution
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH 7.5 with HCl
- Sterilize by filtering and store at Room temperature

200 mL Proteinase K working solution ([M] 10 mg/mL Proteinase K)

- Dissolve 2 g of Proteinase K in 200 mL Proteinase K storage buffer
- Store at -20 °C

Safety warnings

Reagents are potentially damaging to the environment. Dispose waste responsibly.

Before start

Make sure all buffers are prepared before starting.

1 For each sample prepare a screwcap tube pre-filled with a few 2 mm zirconia beads.

2 Shake the sample well.
Transfer 800 µL of the small size fraction and 200 µL of the large size fraction to a 2 mL screwcap tube. It might be beneficial to use wide-bore tips or cut off the tip when using regular pipette tips.

3 11.000 x g, 00:03:00 3m

4 Remove as much ethanol as possible with a 1000 µL pipette.

5 Add 900 µL of **TNES buffer** and 100 µL of **Proteinase K working solution**.
Vortex shortly.

Note

Depending on the amount of samples this can be prepared as a mastermix. We usually prepare TNES + Proteinase K in batches for 24 samples. Proteinase K tends to self-digest if the time for samples preparation takes too long.

6 Bead-beat for 00:02:00 at 2400 rpm 2m

7 Incubate 1400 rpm, 56°C, 00:20:00

8 Store at -20 °C until DNA extraction.