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Sample preparation and lysis of homogenized malaise trap samples

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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.

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PROTOCOL CITATION

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PARENT PROTOCOLS

In steps of

Guanidine based DNA extraction with silica-coated beads or silica spin-columns

GUIDELINES

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

MATERIALS TEXT

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

Tris ultrapure 99.9% 99.9% **Diagonal Catalog #A1086.1000**

Sodium chloride **Fisher**

Sodium chloride **Scientific Catalog #10112640**

EDTA disodium salt **Sigma**

EDTA disodium salt **Aldrich Catalog #E5134-50G**

Sodium dodecyl

SDS ultrapure sulfate **Diagonal Catalog #A1112.0500**

Proteinase

Proteinase K K **7BioScience Catalog #RP100B**

Hydrochloric acid fuming 37%

Hydrochloric acid fuming 37% **Sigma**

Aldrich Catalog #1003171011

Sodium hydroxide - pellets **Fisher**

Sodium hydroxide **Scientific Catalog #S/4920/60**

Calcium chloride 94% **Carl**

Calcium chloride **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

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2 mL screwcap

2 mL screwcap tubes T 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 Molarity (M)** 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 Molarity (M)**

- 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 Molarity (M)** 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** ,

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[M]3 millimolar (mM) CaCl2 [M]50 % (v/v) glycerol) pH7.8

- Add 50 mL of [M]1 Molarity (M) Tris stock solution pH7.5
- Add 333 mg calcium chloride
- Add 500 mL of glycerol 87%
- Adjust volume to 900 mL with ddH_2O
- Adjust pH to pH7.8 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 , pH7.5)

- Add 50 mL of [M]1 Molarity (M) Tris stock solution pH7.5
- Add 80 mL of [M]5 Molarity (M) NaCl stock solution
- Add 40 mL of [M]0.5 Molarity (M) EDTA stock solution pH8
- Add 50 mL of [M]10 Mass / % volume SDS stock solution
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH7.5 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.

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BEFORE STARTING

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.

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.

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