



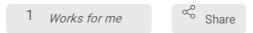


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# Sample preparation and lysis of homogenized malaise trap samples

## Dominik Buchner<sup>1</sup>

<sup>1</sup>University of Duisburg-Essen, Aquatic Ecosystem Research



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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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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

**X** 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%

Solycerol 87 % for molecular biology Panreac

AppliChem Catalog #A3739,1000

#### Labware:

2 mm dia zirconia beads

Products Catalog #11079124zx



**⊠**2 mL screwcap

2 mL screwcap tubes T tube Sarstedt Catalog #72.693

Wide-bore tips

Scientific Catalog #2079G

Disposable PES Bottle Top Filters

Scientific Catalog #15973307

#### Stock solutions:

- □1 L Tris stock solution [M]1 Molarity (M) p+7.5
- Add **121.14 g Tris ultrapure 99.9%** to a beaker
- Adjust volume to **■800 mL** with ddH<sub>2</sub>O
- Adjust pH to p+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<sub>2</sub>0
- Sterilize by filtering and store at § Room temperature
- □1 L EDTA stock solution [M]0.5 Molarity (M) p+8
- Add **186.12 g EDTA disodium salt** to a beaker
- Adjust volume to □1 L with ddH<sub>2</sub>0
- Adjust pH to pH8 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 11 L with ddH<sub>2</sub>0
- 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 ) p+7.8

- Add ⊒50 mL of [M]1 Molarity (M) Tris stock solution p+7.5
- Add **333 mg calcium chloride**
- Add **500 mL** of glycerol 87%
- Adjust volume to **□900 mL** with ddH<sub>2</sub>0
- Adjust pH to pH7.8 with sodium hydroxide
- Adjust volume to □1 L with ddH<sub>2</sub>0
- 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 ,p+7.5 )

- Add **50 mL** of [M] 1 Molarity (M) Tris stock solution p+7.5
- Add ■80 mL of [M]5 Molarity (M) NaCl stock solution
- Add **40 mL** of [M]0.5 Molarity (M) EDTA stock solution p+8
- Add ⊒50 mL of [M]10 Mass / % volume SDS stock solution
- Adjust volume to **300 mL** with ddH<sub>2</sub>O
- 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 22 g of Proteinase K in 200 mL Proteinase K storage buffer
- Store at A -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  $\blacksquare 800~\mu L$  of the small size fraction and  $\blacksquare 200~\mu L$  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 **\$\pi**11.000 x g, 00:03:00

3m

- 4 Remove as much ethanol as possible with a □1000 μL pipette.
- 5 Add  $\blacksquare 900~\mu L$  of TNES buffer and  $\blacksquare 100~\mu L$  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 **41400 rpm**, **56°C**, **00:20:00**
- 8 Store at 8-20 °C until DNA extraction.

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