Inhibitor-free DNA extraction from soil and sediment samples V.2

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

ABSTRACT

This protocol describes how to extract inhibitor-free DNA from soil and sediment samples. 5 g of soil or up to 10 g of sediment can be processed in one extraction, but there is also a miniaturized version for 250 mg of input material, if less DNA is required. The protocol is based on the DNeasy PowerMax Soil Kit but costs much less. A lot of the buffers can be found in the following patent https://patents.google.com/patent/US7459548B2/en

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 the supply situation.

Chemicals:
Sodium phosphate dibasic

Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0876-100G**

Guanidinium thiocyanate

Guanidinium thiocyanate **Fisher Scientific Catalog #10503345**

Sodium phosphate monobasic Sodium phosphate monobasic

Sodium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0751-100G**

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

Sodium chloride Sodium chloride **Fisher Scientific Catalog #10616082**

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

Hydrochloric acid fuming 37%

Hydrochloric acid fuming 37% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1003171011**

Ammonium acetate Ammonium acetate **Carl Roth Catalog #7869.2**

Aluminium ammonium sulfate dodecahydrate

Aluminium ammonium sulfate dodecahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2140-500G**

Guanidine hydrochloride

Guanidine hydrochloride **Fisher Scientific Catalog #10543325**

Isopropanol 2-Propanol **Carl Roth Catalog #1HPK.1**

Tween 20 Tween 20 **Carl Roth Catalog #9127.1**

Acetic acid Acetic acid **Carl Roth Catalog #7332.1**

Ethanol absolute Ethanol absolute 99.8% p.a. **Carl Roth Catalog #9065.1**

Labware:

50 mL centrifuge tubes, Ultra-High Performance

Centrifuge tubes Ultra-High Performance **VWR International Catalog #525-1098**

Garnet Sharp Particles

Garnet Sharp Particles **BioSpec Products Catalog #11079103gar**

Vortex Adapter for 2 (50 ml) tubes **Qiagen Catalog #13000-V1-50**

Econospin Maxi Spin column

EconoSpin® DNA Only Maxi Spin Column **Epoch Life Science Catalog #2040-050**

2 mL screwcap tubes 2 mL screwcap tube **Sarstedt Catalog #72.693**

The EconoSpin® All-In-One DNA Only Mini Spin Column

The EconoSpin® All-In-One DNA Only Mini Spin Column **Epoch Life Science Catalog #1920-250**

Stock solutions:

1 L SDS stock solution | [M] 10 Mass / % volume

- 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 sodium chloride stock solution | [M] 5 Molarity (M)

- Add 292.2 g sodium chloride to a beaker
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution | [M] 1 Molarity (M) | pH 8

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O
- Adjust pH to pH 8 with HCl
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

500 mL sodium acetate stock solution | [M] 3 Molarity (m) | pH 5

- Add 123 g sodium acetate to a beaker
- Adjust volume to 400 mL with ddH2O
- Adjust ph to pH 5 with acetic acid
- Adjust volume to 500 mL with ddH2O
- Sterilize by filtering and store at Room temperature

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 ddH2O
- Adjust pH to pH 7.5 with HCl
- Adjust volume to 1 L with with ddH2O
- Sterilize by filtering and store at Room temperature

[M] 1 Molarity (m) pH 8.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O
- Adjust pH to pH 8.5 with HCl
- Adjust volume to 1 L with with ddH2O
- Sterilize by filtering and store at Room temperature

1 L wash buffer stock solution ([M] 50 millimolar (mM) Tris) pH 7.5

- Add 50 mL Tris stock solution pH 7.5 to a beaker
- Adjust volume to 1 L with with ddH2O
- Sterilize by filtering and store at Room temperature

Working solutions:

500 mL bead-beating solution ([M] 180 millimolar (mM) sodium phosphate , [M] 120 millimolar (mM) guianidinium thiocyanate) pH 8

- Add 12.8 g sodium phosphate dibasic to a beaker
- Add 7.1 g guanidinium thiocyanate
- Adjust volume to 490 mL with ddH_2O
- Adjust pH to pH 8 by adding sodium phosphate monobasic
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL lysis solution ([M] 150 millimolar (mM) sodium chloride , [M] 4 Mass / % volume SDS , [M] 500 millimolar (mM) Tris) pH 8

- Add 200 mL of [M] 10 Mass / % volume SDS stock solution to a beaker
- Add 15 mL of [M] 5 Molarity (m) sodium chloride stock solution
- Add 250 mL of [M] 1 Molarity (m) Tris stock solution pH 8
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL ammonium acetate buffer ([M] 130 millimolar (mM) ammonium acetate)

- Add 5 g ammonium acetate to a beaker
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL inhibitor removal solution (
[M] 120 millimolar (mM) aluminum ammonium sulfate dodecahydrate)

- Add 27.2 g aluminium ammonium sulfate dodecahydrate to a beaker
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL DNA binding buffer ([M] 5 Molarity (M) Guanidine hydrochloride , [M] 40 % (v/v) isopropanol , [M] 0.05 % (v/v) Tween 20 , [M] 115 millimolar (mM) sodium acetate) pH 5

- Add 238.8 g guanidine hydrochloride to a beaker
- Add 200 mL isopropanol
- Add 250 µL Tween 20
- Add 20 mL [M] 3 Molarity (m) sodium acetate stock solution pH 5
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L wash buffer ([M] 10 millimolar (mM) Tris , [M] 80 % (v/v) Ethanol) pH 7.5

- Add 200 mL was buffer stock solution
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L elution buffer ([M] 10 millimolar (mM) Tris) pH 8.5

- Add 10 mL Tris stock solution pH 8.5 to a beaker
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

SAFETY WARNINGS

Buffers containing guanidine produce highly reactive compounds when mixed with bleach. Don't mix the extraction waste with bleach or solutions that contain bleach.
Reagents are potentially damaging to the environment. Dispose waste as mandated.

BEFORE START INSTRUCTIONS

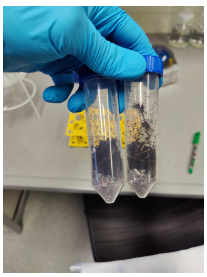
Make sure all buffers are prepared before starting.

Protocol for up to 10 g of input material

20m 30s

1 Prepare one 50 mL centrifuge tube per sample with 15 g of garnet beads.

2 Add up to 10 g of soil to the tube.



Note

The amount of starting material differs from soil type to soil type. For most soil types 2 g of input material is sufficient. If the output is too low with 2 g it can be increased step by step.

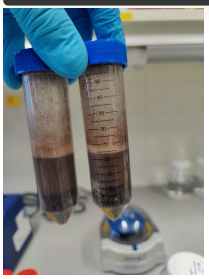
3 Add 15 mL bead-beating solution and 1.2 mL lysis solution. Vortex shortly.

4 Place the samples on a Vortex adapter (e.g. Qiagen) and vortex at maximum speed for 00:10:00 10m

Note

If you want to process more samples, instead of the vortex adapter a Thermoblock can be used. As an alternative, you can incubate the sample for 00:30:00 at 65 °C and at maximum RPM.

5 2500 x g, 20°C, 00:03:00 . Transfer the supernatant to a new tube. 3m

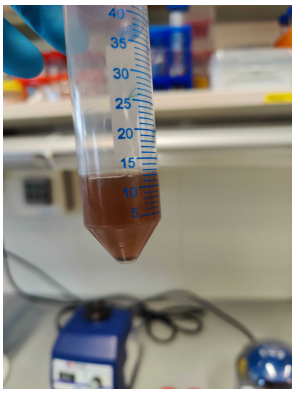


Note

For the large volume protocol, the samples can be carefully poured instead of being pipetted.

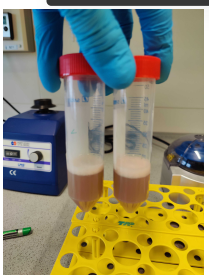
6 Add 5 mL ammonium acetate buffer , vortex shortly, and incubate at 4 °C for 00:10:00 10m

7 2500 x g, 20°C, 00:04:00 . Transfer the supernatant to a new tube avoiding the pellet. The solutio 4m may still be colored, depending on the input material.

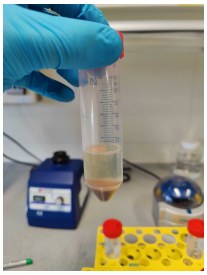


8 Add 4 mL of inhibitor removal buffer . A precipitate may form. Vortex shortly, incubate at 4 °(10m

for 00:10:00 .



9 2500 x g, 20°C, 00:04:00 . The solution will clear up. Avoiding the pellet, transfer up to 15 mL 4m a new tube.



10 Add 30 mL DNA binding buffer . Vortex or invert to mix.

11 Add the mixture to a maxi spin column (e.g. Epoch Life Science) in a 50 mL centrifuge tube.

12 2500 x g, 20°C, 00:00:30 . Discard the flow-through. Repeat once to bind the complete sample volume. 30s

13 Add 10 mL wash buffer . 2500 x g, 20°C, 00:05:00 to wash and dry the column. 5m

14 Transfer the column to a new tube. Add 1 mL elution buffer . Incubate for 00:03:00 at Room temperature . 3m

15 2500 x g, 20°C, 00:01:00 to elute the DNA. DNA eluate should be completely colorless and ready go for downstream analysis. 1m

Protocol for up to 250 mg of input material 50m

16 Prepare one 2 mL centrifuge tube per sample with 750 mg of garnet beads.

17 Add 250 mg of soil or sediment sample.

18 Add 750 µL bead-beating solution and 60 µL lysis solution. Vortex shortly.

19 Place the samples on a Vortex adapter (e.g. Qiagen) and vortex at maximum speed for 00:10:00 10m

Note

If you want to process more samples, instead of the vortex adapter a Thermoblock can be used. As an alternative, you can incubate the sample for 00:30:00 at 65 °C and at maximum RPM.

20 10000 x g, 20°C, 00:03:00 . Transfer the supernatant to a new tube. 3m

21 Add 250 µL ammonium acetate buffer , vortex shortly, and incubate at 4 °C for 00:05:00 5m

22 10000 x g, 20°C, 00:01:00 . Transfer the supernatant to a new tube. 1m

23 Add 200 µL of inhibitor removal buffer . A precipitate may form. Vortex shortly, incubate at 4 °C 5m for 00:05:00 .

24 10000 x g, 20°C, 00:01:00 . Transfer 600 µL of the supernatant to a new tube. 1m

25 Add 1200 µL DNA binding buffer . Vortex to mix.

26 Load 650 µL of the mixture to a mini spin column (e.g. Epoch Life Science).

27 10000 x g, 20°C, 00:00:30 . Discard the flow-through. Repeat two times to bind the complete sample volume. 30s

28 Add 500 µL wash buffer . 10000 x g, 20°C, 00:00:30 to wash the column. Discard the flow-through. 30s

29 10000 x g, 20°C, 00:01:00 to dry the column. Transfer the spin column to a clean 1.5 mL microcentrifuge tube. 1m

30 Add 50 µL elution buffer . Incubate for 00:03:00 at Room temperature . 3m

31 10000 x g, 20°C, 00:01:00 to elute the DNA. DNA eluate should be completely colorless and ready go for downstream analysis. 1m