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Inhibitor-free DNA extraction from soil and sediment samples V.3

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Protocol status: Working

We use this protocol and it's working

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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**

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**

Ethanol absolute  Ethanol absolute **Carl Roth Catalog #9065.4**

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 (Avantor) 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 ddH₂O
- Sterilize by filtering and store at  Room temperature

 1 L sodium chloride stock solution **[M] 5 Mass Percent**

- 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 Mass Percent** **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 $\text{pH } 7.5$ with HCl
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

$[M] 1 \text{ Molarity (M)}$ $\text{pH } 8.5$

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

1 L wash buffer stock solution ($[M] 50 \text{ millimolar (mM)}$ Tris) $\text{pH } 7.5$

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

Working solutions:

500 mL bead-beating solution ($[M] 180 \text{ millimolar (mM)}$ sodium phosphate , $[M] 120 \text{ millimolar (mM)}$ guanidinium thiocyanate) $\text{pH } 8$

- Add 12.8 g sodium phosphate dibasic to a beaker
- Add 7.1 g guanidinium thiocyanate
- Adjust volume to 490 mL with ddH₂O
- Adjust pH to $\text{pH } 8$ by adding sodium phosphate monobasic
- Adjust volume to 500 mL with ddH₂O
- Sterilize by filtering and store at Room temperature

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

- Add 200 mL of $[M] 10 \text{ Mass / \% volume SDS}$ stock solution to a beaker
- Add 15 mL of $[M] 5 \text{ Molarity (M)}$ sodium chloride stock solution
- Add 250 mL of $[M] 1 \text{ Molarity (M)}$ Tris stock solution $\text{pH } 8$
- Adjust volume to 500 mL with ddH₂O



- Sterilize by filtering and store at Room temperature

500 mL ammonium acetate buffer (130 millimolar (mM) ammonium acetate)

- Add 5 g ammonium acetate to a beaker
- Adjust volume to 500 mL with ddH₂O
- Sterilize by filtering and store at Room temperature

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

- Add 27.2 g aluminium ammonium sulfate dodecahydrate to a beaker
- Adjust volume to 500 mL with ddH₂O
- Sterilize by filtering and store at Room temperature

500 mL DNA binding buffer (2.5 Mass Percent Guanidine hydrochloride , 80 % (v/v) ethanol , 120 millimolar (mM) sodium acetate) 5

- Add 119.4 g guanidine hydrochloride to a beaker
- Add 400 mL ethanol
- Add 20 mL 3 Molarity (M) sodium acetate stock solution 5
- Adjust volume to 500 mL with ddH₂O
- Sterilize by filtering and store at Room temperature

1 L wash buffer (10 millimolar (mM) Tris , 80 % (v/v) Ethanol) 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 (10 millimolar (mM) Tris) 8.5

- Add 10 mL Tris stock solution 8.5 to a beaker
- Adjust volume to 1 L with ddH₂O
- 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

Make sure all buffers are prepared before starting.

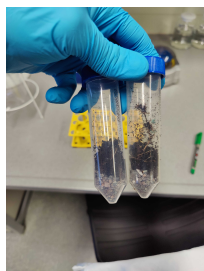
Protocol for up to 10 g of input material

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.

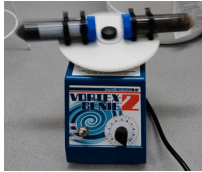


Note

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

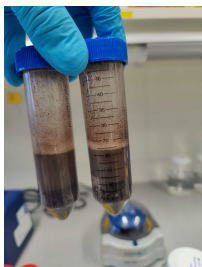


Note

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

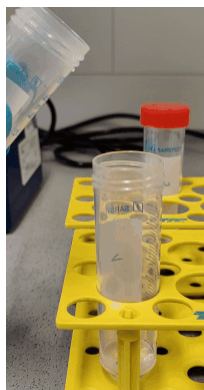
For the large volume protocol, the samples can be carefully poured instead of being pipetted.

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

10m

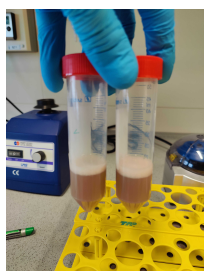
- 7  2500 x g, 20°C, 00:04:00 . Transfer the supernatant to a new tube avoiding the pellet. The solution may still be colored, depending on the input material.

4m



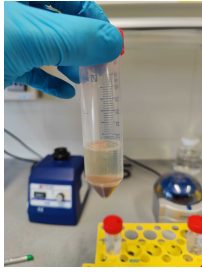
- 8 Add  4 mL of inhibitor removal buffer . A precipitate may form. Vortex shortly, incubate at  4 °C for  00:10:00 .

10m



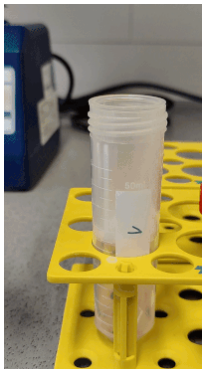
- 9  2500 x g, 20°C, 00:04:00 . The solution will clear up. Avoiding the pellet, transfer up to  15 mL to a new tube.

4m



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 to go for downstream analysis.

1m



Protocol for up to 500 mg of input material

50m

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

17 Add  500 mg of soil or sediment sample.

Note

The optimal input is dependent of the input material used. For soils rich in organic material 200-250 mg input might be enough for sufficient output. For sediments mainly consisting of sand up to 1 g input may be used. Test the upper limit suitable for your sample type by increasing the input in small steps. If too much input is used it usually results in meager yields.

18 Add  1380 µL bead-beating solution and  240 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  600 µL of 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 $^{\circ}\text{C}$ for 00:05:00 . 5m
- 24 10000 x g, 20 $^{\circ}\text{C}$, 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 $^{\circ}\text{C}$, 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 $^{\circ}\text{C}$, 00:00:30 to wash the column. Discard the flow-through. 30s
- 29 10000 x g, 20 $^{\circ}\text{C}$, 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 $^{\circ}\text{C}$, 00:01:00 to elute the DNA. DNA eluate should be completely colorless and ready to go for downstream analysis. 1m

Protocol references

The binding buffer from this protocol originates from the following protocol:

<https://www.nature.com/articles/s41587-020-0588-y>