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Version 2

Inhibitor-free DNA extraction from unsorted bulk samples V.2

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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 unsorted, homogenized bulk samples.  20 mL of homogenate can be processed in one extraction, but there is also a miniaturized version for  1 mL 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0751-100G**

EDTA disodium salt  EDTA disodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E5134-50G**

Sodium hydroxide  Sodium hydroxide - pellets **Fisher Scientific Catalog #S/4920/60**

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**

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

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**

Zirconia Beads, 2 mm diameter  Zirconia beads, 2 mm dia **BioSpec Products Catalog #11079124zx**

 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**

1.5 mL centrifuge tubes  Reaction tube, 1.5 ml, PP **Sarstedt Catalog #72.690.001**

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 sodium phosphate buffer **[M] 1 Molarity (M)** **pH 8**

- Add  142 g Sodium phosphate dibasic to a beaker
- Adjust the volume to  800 mL with ddH₂O
- Adjust pH to **pH 8** with Sodium phosphate monobasic
- Adjust volume to  1 L with ddH₂O
- 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 ddH₂O
- Adjust pH to **pH 8** with sodium hydroxide
- 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 $\text{pH } 8$ with HCl
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

500 mL sodium acetate stock solution 3 Molarity (M) $\text{pH } 5$

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

1 L Tris stock solution 1 Molarity (M) $\text{pH } 7.5$

- 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 with ddH₂O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution 1 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 with ddH₂O
- Sterilize by filtering and store at Room temperature

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

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

1 L Proteinase K storage buffer ($50 \text{ millimolar (mM)}$ Tris , $3 \text{ millimolar (mM)}$ CaCl₂ , $50 \% \text{ (v/v)}$ glycerol) $\text{pH } 7.8$

- Add 50 mL of 1 Molarity (M) Tris stock solution pH 7.5
- Add 333 mg calcium chloride
- Add 500 mL of glycerol 87%
- Adjust volume to 900 mL with ddH₂O
- Adjust pH to 7.8 with sodium hydroxide
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

Working solutions:

- 2 L universal lysis buffer (170 millimolar (mM) sodium phosphate ,
 110 millimolar (mM) guanidin thiocyanate , 10 millimolar (mM) sodium chloride ,
 0.5 % (v/v) sodium dodecylsulfat , 40 millimolar (mM) Tris , 20 millimolar (mM) EDTA) pH 8
- Add 26 g guanidin thiocyanate to a beaker and dissolve in 1 L ddH₂O (the buffer dissolves much quicker if preheated ddH₂O is used)
 - Add 340 mL of 1 Molarity (M) sodium phosphate buffer
 - Add 4 mL of 5 Molarity (M) NaCl stock solution
 - Add 80 mL of 1 Molarity (M) Tris stock solution pH 8
 - Add 80 mL of 0.5 Molarity (M) EDTA stock solution
 - Add 100 mL of 10 % (v/v) SDS stock solution
 - Adjust volume to 2 L
 - 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 ([M] 2.5 Mass Percent Guanidine hydrochloride , [M] 80 % (v/v) ethanol , [M] 120 millimolar (mM) sodium acetate) ⚡ 5

- Add 🧪 119.4 g guanidine hydrochloride to a beaker
- Add 🧪 400 mL ethanol
- Add 🧪 20 mL [M] 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 ([M] 10 millimolar (mM) Tris , [M] 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 ([M] 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

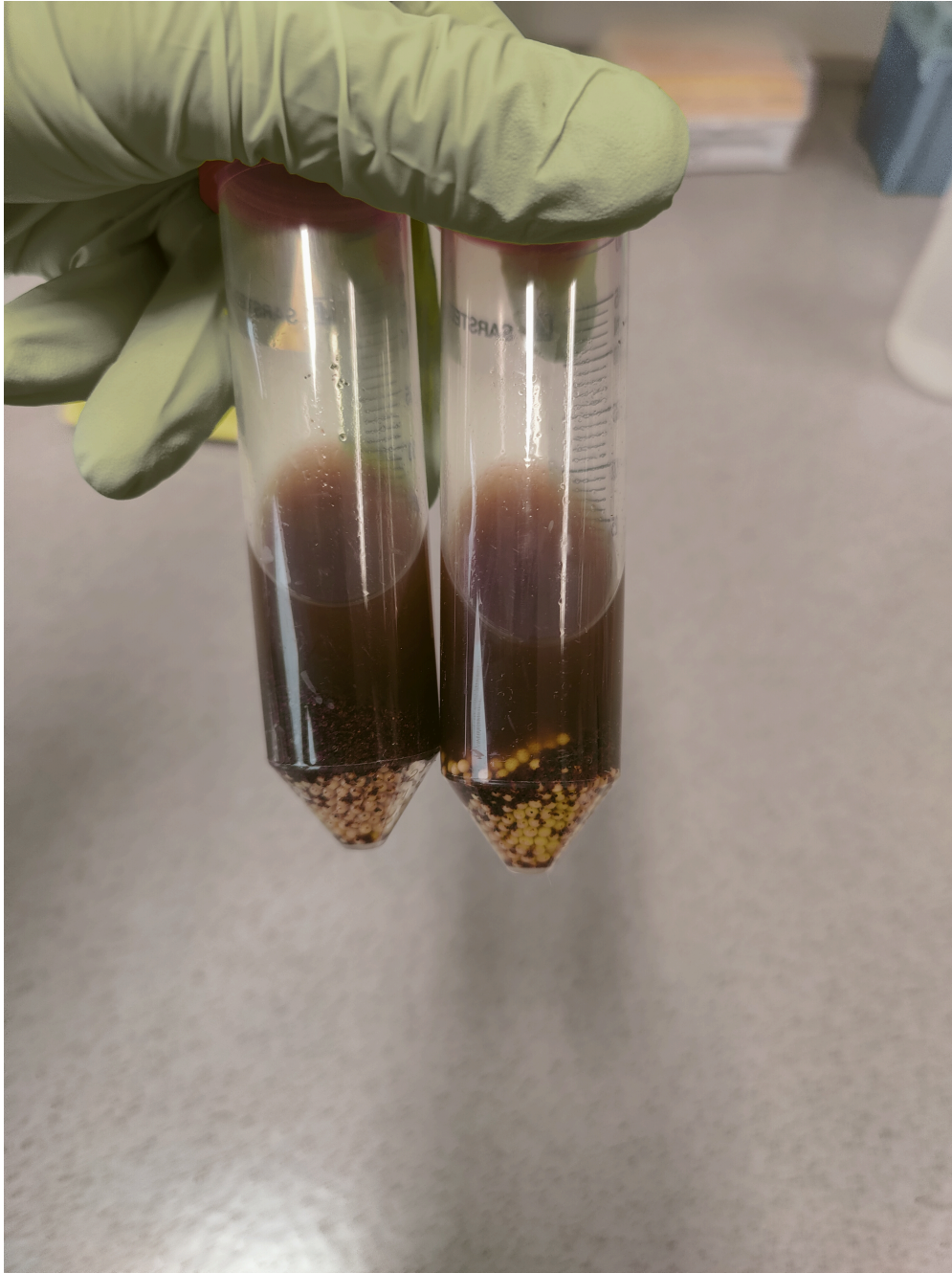
Make sure all buffers are prepared before starting.



Protocol for up to 20 mL of input material

43m 30s

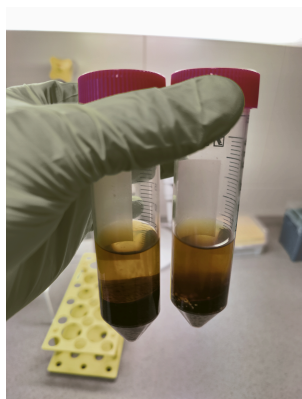
- 1 Prepare one 50 mL centrifuge tube per sample with 7.5 g of garnet beads (0.3 mm diameter) and 7.5 g of zirconia beads (2 mm diameter).
- 2 Add up to  20 mL bulk sample in ethanol to the tube.



Tube with up to 20 mL of bulk sample in ethanol

- 3  4000 x g, Room temperature, 00:03:00 to pellet the material. Decant as much ethanol as possible.

3m



After centrifugation the material is pelleted



After decanting the ethanol.

- 4 Add  16.2 mL universal lysis buffer and  1.5 mL of Proteinase K stock solution
Vortex shortly.

Note

The universal lysis buffer tends to precipitate at room temperature. Heat the buffer to ~

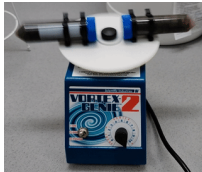
 70 °C

until it is clear again.

- 5 Place the samples on a Vortex adapter (e.g. Qiagen) and vortex at maximum speed for

 00:10:00 .

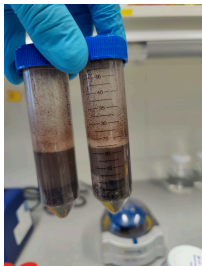
10m



- 6  1400 rpm, 56°C, 00:30:00 to fully lyse the material.

- 7  2500 x g, 20°C, 00:03:00 . Transfer the supernatant to a new tube.

3m



Note

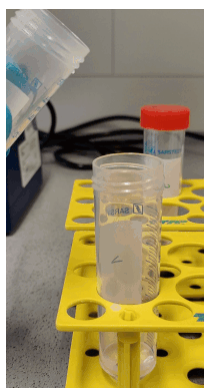
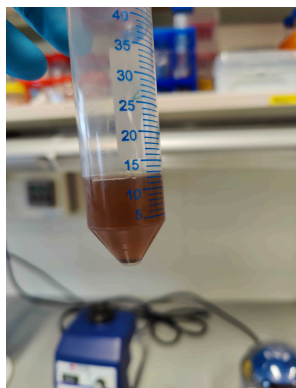
For the large volume protocol, the samples can be carefully poured instead of being pipetted.

8 Add 5 mL ammonium acetate buffer , vortex shortly, and incubate at 4 °C for 00:05:00 .

5m

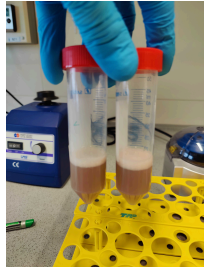
9 2500 x g, 20°C, 00:04:00 . Transfer the supernatant to a new tube avoiding the pellet. The solution may still be colored, depending on the input material.

4m



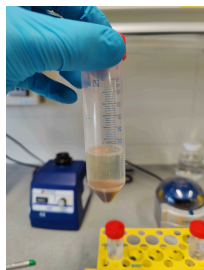
10 Add 4 mL of inhibitor removal buffer . A precipitate may form. Vortex shortly, incubate at 4 °C for 00:05:00 .

5m



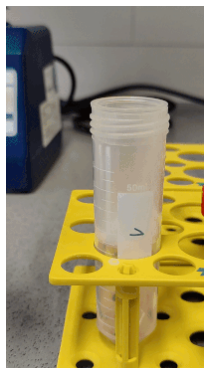
- 11  2500 x g, 20°C, 00:04:00 . The solution will clear up. Avoiding the pellet, transfer up to  15 mL to a new tube.

4m



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

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



- 14  2500 x g, 20°C, 00:00:30 . Discard the flow-through. Repeat once to bind the complete sample volume. 30s
- 15 Add  10 mL wash buffer .  2500 x g, 20°C, 00:05:00 to wash and dry the column. 5m
- 16 Transfer the column to a new tube. Add  1 mL elution buffer . Incubate for  00:03:00 at  Room temperature . 3m
- 17  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 1 mL of input material

56m

- 18 Prepare one 2 mL centrifuge tube per sample with 750 mg of garnet beads (0.3 mm diameter) and 750 mg of zirconia beads (2 mm diameter).
- 19 Add  1000 μ L of unsorted bulk sample. 3m
-  4000 x g, 20°C, 00:03:00 and remove as much of the ethanol as possible without disturbing the pellet.



- 20 Add  900 μ L universal lysis buffer and  100 μ L Proteinase K . Vortex shortly.



- 21 Place the samples in a bead-beater (e.g. BioSpec Products Mini Bead-beater) and bead-beat at maximum speed for 00:02:00 . If no bead-beater is available this step can be skipped, but the yield might decrease.

2m

*

- 22 Place the samples on a ThermoMixer and incubate for at least 00:30:00 at 56 °C and at maximum RPM

30m



- 23 10000 x g, 20°C, 00:03:00 . Transfer 600 µL of the supernatant to a new tube.

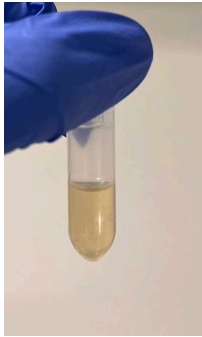
3m

Note

If you want to extract technical replicates, this is the right time to split your samples into 2x 300 µL .

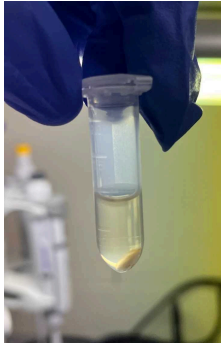
- 24 Add  250 μ L ammonium acetate buffer , vortex shortly, and incubate at  4 °C for  00:05:00 .

5m



- 25  10000 x g, 20°C, 00:01:00 . Transfer the supernatant to a new tube.

1m



- 26 Add  200 μ L of inhibitor removal buffer . A precipitate may form. Vortex shortly, incubate at  4 °C for  00:05:00 .

5m



- 27  10000 x g, 20°C, 00:01:00 . Transfer  600 µL of the supernatant to a new tube.

1m



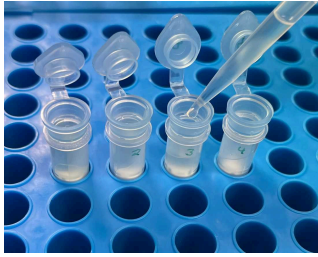
- 28 Add  1200 µL DNA binding buffer . Vortex to mix.



Note

Depending on the amount of lysate and / or technical replication the amount of binding buffer differs. General rule: The amount of binding buffer should be at least twice the amount of lysate / sample material.

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



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

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

32 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

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

Note

The amount of elution buffer is variable and can range from 30 to 200 µL depending if concentrated sample or more volume is required.

34 10000 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



