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

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ABSTRACT

This protocol describes how to extract DNA from samples lysed as described in

Sample preparation and lysis of homogenized malaise trap samples
by Dominik Buchner,
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PREVIEW RUN

using guanidine hydrochloride and ethanol-based buffer combined with silica-coated magnetic beads or silica spin columns. The spin column protocol can be used either with centrifugation or, alternatively, a vacuum manifold. Compared to approaches with magnetic beads, with silica spin column protocols higher yields are possible since the amount of lysate used can be increased. The bead-based protocol is an automation-friendly alternative.

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GUIDELINES

Follow general lab etiquette. Wear gloves to prevent contamination of samples. Clean the workspace before starting and after finishing 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 the supply situation.

Chemicals:

Guanidine hydrochloride **Fisher**

Guanidine hydrochloride **Scientific Catalog #10543325**

Bis-Tris **Carl**

Bis-Tris **Roth Catalog #9140.1**

Ethanol absolute 99.8% **Fisher**

Ethanol absolute **Scientific Catalog #11994041**

Phenol red indicator solution

Phenol red indicator solution **VWR international Ltd Catalog #HACH21132**

Hydrochloric acid fuming 37% **Sigma**

Hydrochloric acid fuming 37% **Aldrich Catalog #1003171011**

SeraSil-Mag 400 silica-coated beads

SeraSil-Mag 400 silica coated superparamagnetic beads **Sigma Aldrich Catalog #GE29357371**

Tris ultrapure

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

EDTA disodium salt **Sigma**

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

Sodium hydroxide - pellets **Fisher**

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

Labware:
50 mL Falcon tube

Easy Reader Conical Polypropylene Centrifuge Tube **Fisher Scientific Catalog #11512303**

125 mL Nalgene Wide-Mouth Bottle

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Thermo Scientific Nalgene Wide-Mouth LDPE Bottle with Closure **Fisher Scientific Catalog #10044180**

Neodyme

Large magnet magnet **Magnethandel Catalog #3935**

1.2 mL square-well plate

1.2 mL square-well storage plate **Thermo Fisher Scientific Catalog #AB1127**

MM-Seperator M96 **Carl**

96-well plate magnet **Roth Catalog #2141.1**

Hard-Shell 96-well PCR plate **BioRad**

Hard-Shell PCR Plate **Sciences Catalog #HSP9601**

EconoSpin mini spin column

EconoSpin mini spin clumn with lid **Epoch Life Science Catalog #1920-050**

1.5 mL microcentrifuge

1.5 mL Microcentrifuge tubes tube **Sarstedt Catalog #72,690,001**

EconoSpin 96-well filter plate

EconoSpin 96-well filter plate **Epoch Life Science Catalog #2020-001**

Stock solutions:

50 mL Bis-Tris stock solution [M]**1 Molarity (M)**

- Add **10.5 g Bis-Tris** to a **50 mL** Falcon tube
- Adjust volume to **50 mL** with ddH_2O
- Vortex to completely dissolve the Bis-Tris
- Store at **4 °C**

1 L Tris stock solution [M]**1 Molarity (M)** pH**8.5**

- Add **121.14 g Tris ultrapure 99.9%** to a beaker
- Adjust volume to **800 mL** with ddH_2O
- Adjust pH to pH**8.5** with HCl
- Adjust volume to **1 L** with ddH_2O
- 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 ddH_2O
- Adjust pH to pH**8** with HCl
- Adjust volume to **1 L** with ddH_2O

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- Sterilize by filtering and store at **Room temperature**

1 L Tris stock solution **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** with ddH_2O
- Sterilize by filtering and store at **Room temperature**

1 L EDTA stock solution **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 wash buffer stock solution (**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 ddH_2O
- Sterilize by filtering and store at **Room temperature**

Working solutions:

1 L GuHCl binding buffer (**3 Molarity (M) Guanidine hydrochloride** , **10 millimolar (mM) Bis-Tris** **90 % (v/v) Ethanol**) **pH 6**

- Add **286.6 g Guanidine hydrochloride** in a beaker
- Adjust volume to **900 mL with Ethanol absolute**
- Add **10 mL Bis-Tris stock solution**
- Adjust volume to **980 mL** with ddH_2O
- Add **4 mL Phenol red indicator solution**
- Dissolve the Guanidine hydrochloride by mixing on a magnetic stirrer
- Adjust to **pH 6** with HCl
- Adjust volume to **1 L** with ddH_2O
- Sterilize by filtering and store at **Room temperature**

1 L TE minimum buffer **pH 8**

- Add **10 mL Tris stock solution** **pH 8** to a beaker
- Add **200 µL EDTA stock solution** **pH 8**
- Adjust volume to **1 L** with ddH_2O
- Sterilize by filtering and store at **Room temperature**

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100 mL silica beads working solution

- Add **5 mL SeraSil-Mag 400 beads** to a clean **125 mL** Nalgene bottle
- Add **25 mL TE minimum buffer**
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for **00:05:00** to pellett the beads
- Discard the supernatant
- Add **25 mL TE minimum buffer**
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for **00:05:00** to pellett the beads
- Discard the supernatant
- Add **100 mL TE minimum buffer**
- Store at **Room temperature**

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

- Add **200 mL wash 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**) **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 STARTING

Make sure all buffers are prepared before starting.

1 To clear the lysates **11.000 x g, 20°C, 00:03:00** 3m

Bead-based protocol 2m

2 Prepare **240 µL GuHCl binding buffer** and **20 µL silica beads working solution** per sample in

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a **1.2 mL square well plate**

3 Add **100 µL of the cleared lysate**

The amount of lysate used in this protocol is flexible as long as it fits the plate used in the protocol. If the amount is to be changed the amount of binding buffer has to be adjusted accoringly as well to maintain a constant ratio of **lysate volume + 20 µL beads to binding buffer.**
The binding buffer volume can be calculated as follows:
binding buffer volume = 2 x (lysate volume + 20 µL beads)

4 **700 rpm, Room temperature , 00:05:00 to bind the DNA to the beads**

5 Place the plate on a magnet to pellet the beads for **00:02:00** 2m

Depending on the magnet and volume used separation times may vary and have to be adjusted accordingly.

6 Discard the supernatant by pipetting

7 Add **100 µL of wash buffer** to each sample

8 **1000 rpm, Room temperature , 00:01:00 to wash excess salt off the beads**

9 Place the plate on a magnet to pellet the beads for **00:01:00** 1m

10 Discard the supernatant by pipetting

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11 **go to step #7 and repeat once for a total of 2 washes**

12 Incubate the plate at **50 °C to dry off residuals of ethanol**

13 Add **100 µL elution buffer** to each sample

14 **1000 rpm, Room temperature , 00:05:00 to elute the DNA from the beads**

> Elution at **50 °C** or with pre-warmed elution buffer may increase the yield.

15 Place the plate on a magnet to pellet the beads for **00:02:00** 2m

16 Transfer **95 µL** of the DNA to a new PCR plate. Store at **-20 °C**

> Leaving **5 µL** of elution buffer is recommended to avoid carry-over of beads.

Spin column protocol (centrifugation) 1m

17 Combine **400 µL GuHCl binding buffer** with **200 µL of the cleared lysate** , vortex shortly

> The amount of lysate used in this protocol is flexible. The ratio of GuHCl binding buffer to lysate should remain 2:1.

18 Load all of the volume on a silica spin column and **11.000 x g, Room temperature, 00:01:00** to bind the DNA, discard the flow-through 1m

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If the binding buffer - lysate mixture exceeds the bed volume of the spin column it has to be loaded as often as needed to pass the complete volume through the spin column.

19 Add **600 µL of wash buffer** to the spin column and **11.000 x g, Room temperature, 00:00:30** 30s , discard the flow-through

The amount of wash buffer should be adjusted to the maximum volume that has been loaded on the column to bind the DNA to remove all remaining traces of salts.

20 **go to step #19** and repeat for a total of 2 washes

21 **11.000 x g, Room temperature, 00:01:00** to dry the silica membrane 1m

22 Discard the collection tube and place the spin column in a clean **1.5 mL** microcentrifuge tube

23 Add **100 µL of elution buffer** directly to the silica membrane

24 Incubate for **00:03:00** at **Room temperature** 3m

Yield might be increased by using elution buffer pre-warmed to **50 °C**

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25 **11.000 x g, Room temperature, 00:01:00** to elute the DNA. Discard the spin column, and store the eluted DNA at **-20 °C** 1m

Spin column protocol (vacuum manifold) 1m

26 Combine **400 µL GuHCl binding buffer** with **200 µL of the cleared lysate** , vortex shortly

The amount of lysate used in this protocol is flexible. The ratio of GuHCl binding buffer to lysate should remain 2:1.

27 Load all of the volume on a silica spin column or 96-well filter plate placed in a vacuum manifold. Apply vacuum until all of the volume has passed the column (**00:02:00**). Release the vacuum 2m

If the binding buffer - lysate mixture exceeds the bed volume of the spin column or filter plate it has to be loaded as often as needed to pass the complete volume through the spin column or filter plate. Times for application of vacuum may vary depending on the pump used. If a well clogs completely, carefully clean the membrane with a sterile pipette tip without piercing it.

28 Add **600 µL of wash buffer** to the spin column or filter plate. Apply vacuum until all of the buffer has passed the column (**00:01:00**). Release the vacuum 1m

The amount of wash buffer should be adjusted to the maximum volume that has been loaded on the column to bind the DNA to remove all remaining traces of salts.

29 **go to step #28** and repeat for a total of 2 washes

30 Apply vacuum for **00:10:00** to completely dry the silica membrane 10m

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More time might be needed if a weaker pump is used. If traces of wash buffer remain on the membrane it should be dried at **50 °C** for **00:05:00** on a heat block stacked inside of a 1.2 mL storage plate.

30.1 For spin columns:
go to step #23 and follow the protocol for centrifugation

30.2 For 96-well filter plates:
Place a suitable collection plate in the vacuum manifold

Depending on the elution volume different collection plates may be suitable. For large volumes a storage plate (1.2 mL or 2.2 mL) is recommended. For smaller volumes a 96-well PCR plate or a U-bottom assay plate is recommended.

30.3 Add **100 µL of elution buffer** directly to the silica membrane. Apply vacuum until all of the elution buffer has passed the column (**00:01:00**). Store eluted DNA at **-20 °C** 1m

Yield might be increased by using elution buffer pre-warmed to **50 °C**

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