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Guanidine-based DNA extraction with silica-coated beads or silica spin columns V.3

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We use this protocol and it's working

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Abstract

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

Protocol

NAME

Sample preparation and lysis of homogenized malaise trap samples

CREATED BY

Dominik Buchner

Preview

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.

Guidelines

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

Guanidine hydrochloride  Guanidine hydrochloride **Fisher Scientific Catalog #10543325**

Bis-Tris  Bis-Tris **Carl Roth Catalog #9140.1**

Ethanol absolute  Ethanol absolute 99.8% **Fisher Scientific Catalog #11994041**

Phenol red indicator solution

 Phenol red indicator solution **VWR International (Avantor) Catalog #HACH21132**

Hydrochloric acid fuming 37%

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

SeraSil-Mag 400 silica-coated beads

 SeraSil-Mag 400 silica coated superparamagnetic beads **Merck MilliporeSigma (Sigma-Aldrich) Catalog #GE29357371**

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

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

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

Sodium acetate  Sodium acetate **Carl Roth Catalog #6773.2**

Labware:

50 mL Falcon tube

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

125 mL Nalgene Wide-Mouth Bottle

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

Large magnet  Neodyme magnet **Magnethandel Catalog #3935**

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

96-well plate magnet  MM-Separator M96 **Carl Roth Catalog #2141.1**

Hard-Shell PCR Plate  Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #HSP9601**

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

1.5 mL Microcentrifuge tubes  1.5 mL microcentrifuge 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 Mass Percent

- Add 🧪 10.5 g Bis-Tris to a 🧪 50 mL Falcon tube
- Adjust volume to 🧪 50 mL with ddH₂O
- Vortex to completely dissolve the Bis-Tris
- Store at 🌡️ 4 °C

🧪 1 L Tris stock solution [M] 1 Mass Percent 📏 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 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

🧪 1 L Tris stock solution [M] 1 Mass Percent 📏 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 ddH₂O
- Sterilize by filtering and store at 🌡️ Room temperature

🧪 1 L EDTA stock solution [M] 0.5 Mass Percent 📏 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 wash buffer stock solution (50 millimolar (mM) Tris) 7.5

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

500 mL sodium acetate stock solution 3 Molarity (M) 5

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

Working solutions:

1 L DNA binding buffer (2.5 Mass Percent Guanidine hydrochloride , 80 % (v/v) Ethanol , 120 millimolar (mM) sodium acetate) 5

- Add 238.8 g Guanidine hydrochloride in a beaker
- Adjust volume to 800 mL with Ethanol absolute
- Add 40 mL sodium acetate stock solution 5
- Adjust volume to 1000 mL with ddH₂O
- Dissolve the Guanidine hydrochloride by mixing on a magnetic stirrer
- Sterilize by filtering and store at Room temperature

1 L TE minimum buffer 8

- Add 10 mL Tris stock solution 8 to a beaker
- Add 200 µL EDTA stock solution 8
- Adjust volume to 1 L 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 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 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.



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

3m

Bead-based protocol

2m

2 Prepare  240 µL DNA binding buffer and  20 µL serasil 400 beads per sample in a  1.2 mL square well plate

3 Add  100 µL of the cleared lysate

Note

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 accordingly 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

Note

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

11  and repeat once for a total of 2 washes

12 Incubate the plate at 50 °C to dry off residuals of ethanol for 00:05:00

5m

13 Add 100 µL elution buffer to each sample

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

Note

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

Note

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

Spin column protocol (centrifugation)

1m



- 17 Combine  400 μ L DNA binding buffer with  200 μ L of the cleared lysate , vortex shortly

Note

The amount of lysate used in this protocol is flexible. The ratio of DNA 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

Note

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 μ L of wash buffer to the spin column and  11.000 x g, Room temperature, 00:00:30 , discard the flow-through

30s

Note

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  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 μ L of elution buffer directly to the silica membrane

24 Incubate for  00:03:00 at  Room temperature

3m

Note

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

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 μ L DNA binding buffer with  200 μ L of the cleared lysate , vortex shortly

Note

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

**Note**

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 μ L 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

Note

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  and repeat for a total of 2 washes

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

10m

Note

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:
 and follow the protocol for centrifugation

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

**Note**

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 μ L 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

Note

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