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Guanidine-based DNA extraction with silica-coated beads or silica spin columns V.2

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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

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-Seperator 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 clumn 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 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
- 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 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 [M] 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 ([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 ddH_2O
- Sterilize by filtering and store at Room temperature

Working solutions:

1 L GuHCl binding buffer ([M] 3 Molarity (M) Guanidine hydrochloride , [M] 10 millimolar (mM) Bis-Tris [M] 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

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 ([M] 10 millimolar (mM) Tris , [M] 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 ([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

Troubleshooting

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 GuHCl binding buffer and 20 µL silica beads working solution 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 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

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 µL GuHCl binding buffer with 200 µL 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.

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 µL 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 µL 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 µL GuHCl binding buffer with 200 µL 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 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

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 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

Note

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