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# Rapid DNA Extraction from sediment using spin columns (teaching)

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**Protocol status:** Working
**We use this protocol occasionally for teaching and it's working well.**

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## Abstract

An extraction method for the rapid extraction of DNA from sediment material, using a Lysis Buffer from Rohland et al. (2018) and DNA binding and purification components of a Qiagen Soil DNA extraction kit.

## DNA Lysis and Isolation

1 ***Lysis***

Note

**General advice**

- Use best practise pipetting techniques (e.g., wear gloves, avoid contamination)

1.1 Add 500 µL lysis buffer to your sample (your tutors will do this 24 hrs prior to the prac).

0,25 g sediment

| | A | B |
|---|---|---|
| | **Lysis Buffer Components** | **Vol. (uL)** |
| | 0.5M EDTA (pH 8) | 450 |
| | H20 | 37.25 |
| | Tween 20 | 0.25 |
| | Proteinase K | 12.5 |
| | Total | *500* |

1.2 Vortex briefly to mix sediments with lysis buffer (your tutors will do this 24 hrs prior to the prac).

1.3 Incubate over night on rotator (your tutors will do this 24 hrs prior to the prac).

10 rpm, Room temperature 17 hrs

1.4 Wipe the outside of the tubes with 80 % ethanol.

Note

- wipe especially along the rim of the tubes, as they are the most prone to contamination
- label your tubes clearly again, in case the labeling has been removed from the ethanol

1.5 Centrifuge the tubes for 2 minutes at 16,400 g to separate the lysate from undigested sample material.

Note

Before starting step 1.4:

- clean the centrifuge rotor surface with a bit of ethanol
- ensure the centrifuge is balanced (i.e., an even number of tubes, each tube contains the same volume of liquid, tubes are arranged opposite of each other)
- ensure the lid is on the rotor before closing the centrifuge and turning it on

1.6 Transfer the lysate to a fresh 1.7 mL tube. Be careful not to transfer any sediment.

Note

- it's useful to place the pipette over the lowest point of the pellet while you're taking up the supernatant, do not touch the pellet
- pipette slowly and continuously

## Bind DNA

2 Add 1200 µL of Solution **C4** (50 - 70 % guanidinhydrochlorid + 1 - 10 % isopropylalcohol) to the supernatant and vortex for 5 s.

2.1 ***Loading procedure (3 x) of the spin column***

2.2 Load the **first 675 µL** of the supernatant onto an Spin Column and centrifuge.
10000 x g , room temperature, 1 min.
Discard the flow-through.

2.3 Load the **second 675 µL** of the supernatant onto an Spin Column and centrifuge.
10000 x g , room temperature, 1 min.
Discard the flow-through.

2.4 Load the **rest** of the supernatant onto an Spin Column and centrifuge.
10000 x g , room temperature, 1 min.
Discard the flow-through.

## Wash

3 ***Removing any contaminants / residues from the filter membrane***

3.1 Add 500 µL of Solution **C5** (= 70 % ethanol) to the spin column.

3.2 Centrifuge the spin column and discard the flow-through.
10000 x g , 30 s, room temperature

3.3 Centrifuge the spin column again and discard the flow-through with the collection tube.
10000 x g , 1 min, room temperature

3.4 Carefully place the spin column in a clean 1.7 mL tube. Avoid splashing any Solution C5 onto the spin column.

Note

- don't touch the spin column itself (risk of contamination), only the rim (top of column where the lid is)

## Elute

4 ***Elute the bound DNA from the filter membrane.***

4.1 Add 25 µL of Elution Buffer (EB / **C6** buffer) to the center of the white filter membrane, let sit for 2 minutes.

Note

- It's important to double check whether the membrane filter has fully soaked up all of the 25 µL of **C6** buffer. Aim to get the elution buffer into the centre of the filter membrane, but avoid touching the filter membrane with the pipette tip!
- Stick to the incubation time. Incubating for a bit longer is okay, but you shouldn't incubate for a shorter period of time.

4.2 Centrifuge 10000 x g , 30 s
and discard the Spin Column but **keep the tube** containing your extracted DNA!

4.3 Combine the replicate sediment DNA extracts in one tube.
Leave the control extract in a separate tube.

Expected result

- *1 x tube* with ~ 75 µL of transparent DNA extract --> ready for downstream applications
- *1 x tube* with the extraction control

## Storage

5 Store your DNA extract and control (clearly labeled in a rack or cryo box) in the freezer at -20 °C until the next prac.