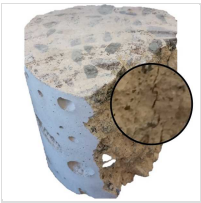

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DNA extraction from concrete V.1

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protocol .

CivilMicroLab

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This is a protocol for extracting DNA from concrete, based on the protocol developed by L. S. Weyrich, et al. for extraction of DNA from ancient calcified dental plaque. We have scaled it up for larger sample sizes and made some additional modifications for the chemistry of concrete. DNA extracted using this method is suitable for metagenomic sequencing by Illumina MiSeq and NextSeq, as well as amplicon sequencing. This protocol should yield 10 ng to 5 µg DNA per 10 g of concrete, depending on the age and integrity of the sample.

Reference: L. S. Weyrich et al., Laboratory contamination over time during low-biomass sample analysis. *Mol. Ecol. Resour.* **19**, 982–996 (2019).

Anders Kiledal, Julia A Maresca 2021. DNA extraction from concrete. **protocols.io** https://protocols.io/view/dna-extraction-from-concrete-bzwgp7bw

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protocol ,

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Concrete is a *very* low biomass system, and thus susceptible to laboratory and reagent contamination. A negative control sample -- either extracting DNA with no powder, or using sterilized glass beads as the "sample" -- is strongly recommended. We also strongly recommend doing as much of this as possible inside a PCR or laminar flow hood to minimize laboratory contamination.

SOLUTIONS

1. **QG buffer** (store in dark)
   - Qiagen version: 5.5 M guanidine thiocyanate (GuSCN); 20 mM Tris HCl pH 6.6; cresol red pH indicator.
   - Modified [QGmod]: 5 M guanidine thiocyanate, 18.1 mM Tris HCL, 1.3% Triton X-100, 25 mM NaCl in ddH_2O.

2. Silica suspension

*Makes enough for many extractions (doesn't have to be made fresh). Store at room temperature or –20°C.

- Mix 6 g of silicon dioxide powder (Sigma-Aldrich) in 50 mL DNA-free H_2O
- Leave 1 hr to allow the largest particles to settle out.
- Transfer top 40 mL of suspension to new tube; discard large particles.
- Allow to settle overnight
- Remove top 30 mL of suspension and discard, leaving ~10 mL of medium-sized silica suspension.

3. 0.5 M EDTA

4. 20% SDS

5. 20 mg/mL Proteinase K dissolved in ddH_20

6. 1 mg/mL yeast RNA dissolved in ddH_20

7. Glacial acetic acid

8. 10 mM Tris, pH 8.0

9. 80% ethanol

MATERIALS

- 50 mL Falcon tubes (2 per sample)
- 1.5 mL microcentrifuge tubes (1 per sample)
- 25-50 mL serological pipets
- micropipets and tips

EQUIPMENT

- Rock grinder
- PCR hood or similar
- Centrifuge
- Microcentrifuge
- Vortexer
- Platform shaker
- Water bath or incubator at 60°C

- Guanidine thiocyanate is highly toxic (MSDS): Gloves, eye protection, and a dust

mask are recommended while working with this solution.
- Silicon dioxide is a potential hazard when inhaled, and a dust mask is recommended while working with this as well.

- Prepare solutions
- Sterilize work area
- Clean rock grinder

Sample Grinding

1 Wash sample holder and puck of the ring and puck mill with soap and hot water. Rinse thoroughly and dry.

2 Rinse sample holder and puck 2x with 95% ethanol.

3 Sterilize sample holder and puck under germicidal UV light for 40 min. 40m

4 Roughly break ~10 g concrete with a hammer into pieces that are small enough to fit into a ring and puck mill or equivalent grinder 10m

5 Grind samples to powder (usually requires 1-5 minutes of grinding). The ground powder can be stored at -20°C (or -80°C) prior to DNA extraction. 5m

Sample Pre-Treatment (Day 1)

6 In a 50 mL conical tube, place 10 g pulverized concrete, 5 mL 0.5 M EDTA, 150 µL Proteinase K (20 mg/mL), 138 µL 20% SDS, and 0.2 mL glacial acetic acid. Incubate at 55°C overnight with gentle rotation. 12h

DNA Extraction (Days 2 and 3) 14h 20m

7 Some samples may absorb a large volume of the pre-treatment solution, leaving very little supernatant. If this happens, add 5-10 mL of sterile water before vortexing.

8 Vortex at maximum speed on a platform vortexer for 10 min (this is similar to bead beating, but using the material itself instead of beads). 10m

9 Centrifuge 3 min, 5000 RPM 3m

10 Transfer supernatant to new 50 mL conical tube 1m

11 Add 30 mL QGmod. If solution is purple (this indicates that the pH > 7.5), adjust pH with acetic acid until solution is yellow (neutral or acidic). Add 125 µl silica suspension (well mixed) and 5 µl yeast RNA (1 mg/mL, acts as a carrier molecule). Mix well and allow to bind overnight at room temperature with gentle rotation. 12h

12 Centrifuge 5 min at 5000 RPM to pellet silica. 5m

13 Decant supernatant (pour off, centrifuge the tube briefly, and carefully pipette off what remains to prevent carryover of Buffer QG. Discard supernatant.) 3m

14 Resuspend pellet in 10 mL 80% EtOH. (To prevent clogging the pipet tips, wait ~5 min after adding ethanol before trying to resuspend. It may be necessary to scrape the tube over a tube rack or bang it on the bench to resuspend the silica particles. The solution can also be stirred with the tip. Try to break up any large clumps before attempting to draw the solution into the pipet tip.) 10m

15 Centrifuge 30 min, 5000 RPM, 4°C 30m

16 Decant supernatant (pour off, pulse in centrifuge, carefully pipette off what remains) 3m

17 Resuspend silica pellet in 1 mL 80% ethanol and transfer solution to 1.5 mL microcentrifuge tube 5m

18 Centrifuge 3 min at 13000 RPM 3m

19 Decant or pipette off supernatant 1m

20 Air dry silica with bound DNA. This usually takes ~1 hour at room temperature in a PCR hood. 1h

21 Resuspend in 50 µL 10 mM Tris, pre-warmed to 60°C, then elute for 5 min at 60°C with gentle rotation. Centrifuge 3 min at 13000 RPM and transfer supernatant (with DNA) to fresh tube. Repeat and combine eluates. 6m

22 A minuscule amount of silica may remain but should not interfere with downstream applications. Any remaining silica can be pelleted to reduce future transfer.

23 Quantify with Qubit and store at -20°C.