extraction of RNA from sediments

MacGregor BJ, Moser DP, Alm EW, Nealson KH, Stahl DA (1997) Crenarchaeota in Lake Michigan sediments. *Applied and Environmental Microbiology* **63**(3): 1178-1181.

Biddle JF, Lipp JS, Lever MA, Lloyd KG, Sørensen KB, Anderson R, Fredricks HF, Elvert M, Kelly TJ, Schrag DP, Sogin ML, Brenchley JE, Teske A, House CH, Hinrichs K-U (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences of the USA* **103**(10): 3846-3851.

prepare vials:

glassware (for reagents), zirkonia beads (0.1 mm): bake overnight at 160 °C
screw-cap tubes (2 ml), PCR tubes (0.2 ml): from new bag, double-autoclave
Falcon tubes (15 ml): rinse inside with RNaseZap, then twice with DEPC-treated water
pipette tips: from new bag, double-autoclave, 20 min under UV light

prepare reagents:

- DEPC treated water:
  DI water + 0.1 % **die**thyl**p**yro**c**arbonate, leave overnight at room temperature
  autoclave longer than 1 h
- extraction buffer (5×):
  in DEPC treated water, dissolve 250 mM NaAc, 5 mM Na_2EDTA, 2 % SDS
  adjust pH to 5
- extraction buffer (2×): dilute extraction buffer (5×) by factor 2/5
- NH_4Ac solution: 7.5 M in DEPC treated water
- ethanol (80%): dilute ethanol (100%) with DEPC treated water
- phenol (pH 4.3): find in POST 718 fridge, warm to room temperature before opening
- chloroform: find in POST 718 hood cabinet
- isopropanol: find in POST 718 flammables cabinet

make sure the following kits for immediate downstream applications are in the lab:

- RNeasy Mini Kit (Qiagen), stored in lab drawer
- iScript cDNA Synthesis Kit (BioRad), stored in the large -20 °C freezer

label all containers

extract 4 portions of sample (procedure described below is for 1 portion until step 18)
process these 4 portions in parallel, but never process more than 1 sample at a time

keep everything on ice and super-clean!
wear gloves, keep vials closed whenever possible

time yourself to get an idea how long one extraction takes (helps in future scheduling)

extraction procedure:

1. screw-cap tube (2 ml) with:
   - 0.8 g beads
   - ca. 0.5 – 0.7 g sediment (0.3 – 0.4 cm^3)
   - 500 µl phenol
   - 400 µl extraction buffer (5×)
2. shake on bead beater (lowest setting: 25) for 50 s
3. centrifuge (15000 **g**) for 90 s → aqueous and phenolic phase separate
4. microcentrifuge tube (2 ml) with:
   - aqueous phase of extract (strictly avoid interphase)
   - 500 µl phenol
5. to the remaining contents of the screw-cap tube add:
   - 200 µl extraction buffer (2×)
6. shake on bead beater (second-lowest setting: 42) for 50 s
7. same as steps 3 and 4 (add aqueous phase to same microcentrifuge tube)
8. same as step 5
9. shake on bead beater (second-highest setting: 46) for 50 s
10. same as step 7
11. same as step 5
12. shake on bead beater (highest setting: 48) for 50 s
13. same as step 7

→ result: tube with 500 µl phenol + (400 + 200 +200 +200) µl aqueous extract

14. vortex this tube, centrifuge (15000 **g**) for 1 min
15. new microcentrifuge tube (2 ml) with:
    - aqueous phase of step 14 (strictly avoiding interphase)
    - 500 µl phenol/chloroform (1:1)
16. same as step 14
17. new microcentrifuge tube (2 ml) with:
    - aqueous phase of step 16 (strictly avoiding interphase)
    - 500 µl chloroform
18. same as step 14
19. Falcon tube (15 ml) with:
    - aqueous phase of step 18 [here combine extracts of all sediment portions]
    - ½ volume NH_4Ac solution
    - 1 volume isopropanol
20. mix well, put portions of 2 ml in new microcentrifuge tubes
21. incubate at -20 °C for 30 min to 2 h (or overnight) → RNA precipitates invisibly
22. centrifuge (15000 **g**, 30 min, 2°C), discard supernatant
23. add 1.5 ml ethanol (80 %), vortex until pellet is suspended, transfer into next tube, suspend pellet in there, transfer... until all RNA pellets are combined in the same 1.5 ml ethanol in the same tube
24. centrifuge (15000 **g**, 30 min, 2°C), discard supernatant, air-dry precipitate
25. dissolve precipitate in appropriate volume of DEPC-treated water (e.g. 15 µl)

26. place 10 μl in PCR tube, store at -80 °C
27. use up to 5 μl for GeneSpec
28. use the remaining 15 μl of purified RNA extract to construct cDNA (BioRad kit, follow directions)
29. store cDNA at -80 °C (yield will be good for 10 PCR reactions)