Worden Lab
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DNA Extraction – from Filters

We use this method to extract DNA for quantifying natural populations using qPCR or barcoded ribosomal RNA sequencing. It is used on natural seawater samples that have been filtered onto a supor filter and frozen either at -80oC or in liquid nitrogen until extraction (we do not put any buffer on filters prior to freezing). We found that this protocol had the best yields and improved representation of organisms like *Bathycoccus* that previously appeared to be missing from our samples (using other extraction methods). This protocol originally came to us from the Zehr lab. It is based on the Qiagen DNeasy Plant kit, with slight modifications to incubation times and column steps. In addition a mechanical lysis step has been added.

Note that DNA will be eluted from a spin column in step 24. Qiagen recommends elution with buffer AE supplied in their kit. We use TE, pH 8 for DNA extractions used for qPCR (well actually for any use). It is important to perform inhibition tests when using these extracts in qPCR assays.

Preparation

1. Mix two sizes of glass beads (0.1 and 0.5 mm) in a 1:1 ratio in a 60 ml syringe, aliquot roughly 100 µl into 2 ml screw cap tubes and autoclave.
   *Note: keep the cap loose while autoclaving
2. Turn on water bath, set temp to 65°C.
3. Turn on incubator, set temp to 55°C
4. Before step 23 (in DNeasy kit) pre-heat TE (or buffer AE) to 65 °C in water bath or heat block.
5. Set up an ice bucket.

Freeze Fracture

6. Fill the liquid nitrogen container
7. Transfer the filter into 2 ml screw cap tubes with glass beads.
   *Note: use 95% or 70% ethanol to wipe tweezers.
8. Add 400 µl AP1 (from Qiagen kit). Incubate tubes in liquid nitrogen until frozen solid and then transfer to water bath (65°C). Repeat this freeze-thaw step 3 times.
   *Note: make sure the top/cap of the tubes is NOT immersed in the water bath by arraying the tubes in a foam floater and transferring the entire floater back and forth between nitrogen and water bath. This helps prevent sample contamination.

Bead Beat

9. Transfer tubes directly from the last thaw step to bead beater. Bead beat samples for 2 min.
   *Note: blot off water from the tubes carefully with a kimwipe.
   *Note: Make sure tube holder is tightly screwed onto the bead beater and machine doesn't move off the bench due to vibration.
10. Remove from bead beater and pulse-centrifuge tubes to reduce foam.

Proteinase-K Treatment

11. Add 45 µl of proteinase-K (see below for product info; Worden lab this is in Sebastian's drawer) to each tube and invert to mix well. Put tubes in a whirlpack or zip lock bag.
12. Put bags into incubator (55°C) and incubate for one hour with moderate mixing (we use a rotisserie, but a gentle shaker should also work).
    *Note: After this step, turn the temperature up to 65°C

Rest of (Modified) Qiagen Plant kit protocol

13. Remove tubes from incubator. Add 4 µl of RNase A stock solution (Qiagen) to each tube and vortex vigorously.
14. Incubate tubes at 65°C for 10 min. Vortex tubes 2 or 3 times during incubation.
15. With sterile needles or tips remove filters
    *Note: Try to squeeze liquid out of the filters as much as possible. Needles need to go into a red sharps box.
16. Add 130 µl P3 to lysate and vortex. Incubate tubes on ice for 10 min.
17. Centrifuge at 20,000 x *g* for 5 min. to pellet precipitates and beads.
18. Apply supernatant from above to QIAshredder Mini Spin Column (lilac) placed in a 2 ml collection tube and centrifuge at 20,000 x *g* for 2 min.
19. Transfer flow-through from above to new 2 ml tube. If a debris pellet has formed, avoid it while transferring. Steps 17 and 18 may need to be done twice if volume from step 16 is larger than 650 µl. Flow-through from repeat should be added to same 2 ml tube as the flow-through from the first spin.
20. Add 1.5 volumes of Buffer AW1 mix (as outlined in Qiagen kit) to the cleared lysate. Mix by pipetting.
    *Note: be sure ethanol has been added to the buffer before adding to lysate!
21. Apply 650 µl of the mixture from above to DNeasy Mini Spin (white) column sitting in a 2 ml collection tube. Centrifuge for 1 min. at 6,000 x *g*. Discard flow-through. Repeat with remaining sample using same collection tube until all sample has been run through the column
22. Place DNeasy spin column in a new collection tube from kit, and add 500 µl of Buffer AW2 to the DNeasy Spin Column and centrifuge 1 min. at 6,000 x *g*. Discard flow-through and reuse collection tube in next step.
23. Add 500 µl Buffer AW2 to the DNeasy Spin Column and centrifuge 2 min. at 20,000 x *g* to dry the membrane.
24. Transfer the spin column to a sterile 1.5 ml microcentrifuge tube, being careful not to allow bottom of column to come in contact with liquid in

collection tube. Apply 25 µl of pre-heated TE (or buffer AE) directly to the DNeasy Spin Column membrane. Incubate at room temp for 5 min. Centrifuge for 5 min. at 6,000 x *g* to elute. Repeat with a second 25 µl aliquot of TE (or buffer AE) so that final total volume is 50 µl.

25. Aliquot and store DNA extract at -80°C for environmental DNA (-20°C sufficient for cultures)

Ordering info

REAGENT/PRODUCT	VENDOR	CATALOG NO
2 ml Vial w/Cap (sterile)	Biospec Products	522S
0.1 mm glass beads	Biospec Products	11079101
0.5 mm glass beads	Biospec Products	11079105
Dneasy Plant Mini Kit (250)	Qiagen	69106
Proteinase K (200 mg / 10 mL)	Qiagen	19133
BD needle 21 Gauge 1.5 inch	Fisher	14 826 5B
Supor-200, 0.2 µm pore size, 47 mm, membrane filter	Pall	60300