DNA Extraction from Filtered Vent/Crustal Fluids or Seawater

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Abstract

This is a DNA extraction protocol adapted from Byron Crump's lab, which was developed from both Zhou et al. 1996 and Crump et al. 2003. It is intended to be used with 47 mm 0.22 um PES filters or a 0.22 uM Sterivex filter, both preserved with RNALater after sample collection.

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Protocol

Step 1.

Assumes a 47mm flat filter preserved in RNAlater in 50ml tube or Sterivex filter. Wipe bench top, pipets, racks, with Nucleo-clean, 70% ethanol Make sure you have autoclaved Swinnex 25mm filter holders and sterile 1X PBS. Note: If only extracting half the 47mm flat filter save the remaining half in the 50ml tube with half the RNAlater. If using half of a Sterivex, store remaining filter half in 2ml tube with fresh, filter-sterilized RNAlater.

Step 2.
Cut filter into small pieces on sterile piece of foil using a clean sterile razor blade (autoclaved/ethanol flamed) and forceps (ethanol flamed).

Step 3.
If extracting from a Sterivex, use pliers (ethanol-flamed) to crack cartridge, remove filter with razor blade, and cut into 6-8 pieces. Pour RNAlater from cartridge into 2ml tube to save for Step 4.

Step 4.
Place filter in new 50ml tube with 10mL of 1x PBS (autoclaved, 0.2um filtered).

Step 5.
Rinse filter using forceps, then place in clean 2mL tube. Add 1mL of DEB to 2mL tube, place on ice.

Step 6.
Pour RNAlater from 50ml filter tube into tube with 1x PBS rinse water and filter this solution through a 25mm 0.2um filter using a 60mL syringe. If only extracting half the filter, only pour off half the RNAlater.

Step 7.
Rinse 25mm 0.2um filter with 10mL 1x PBS. Push air through filter to remove any liquid.

Step 8.
Cut 25mm filter into pieces and add to 2mL tube with filter and DEB.

Step 9.

Add enzymes: Add 20 ul proteinase-K (10 mg/ml) and 40 ul of lysozyme (50 mg/ml) to 2mL tube, freeze tube at -80ºC for 15 minutes and thaw at 37ºC for 5 minutes three times. Can freeze at -80ºC until following day.

Step 10.
37ºC incubation: Incubate tube for 30 min at 37ºC in a water bath.

Step 11.
SDS & 65ºC incubation: Add 50 ul filter-sterilized SDS (20% sln. in water) to each 2mL tube, invert several times to mix. Incubate for 2 hours in 65ºC in water bath.10. Phenol-Chloroform extraction: Working in a fume hood, fill 2mL tube the rest of the way (level with the base of the cap) with phenol:chloroform:isoamyl alcohol (25:24:1, pH 8.0). Vortex. Centrifuge at low speed (3000 rpm) for 5 min. Transfer top layer to new 2mL tube and repeat (total 2 washes).

Step 12.
Precipitation: Estimate buffer volume from hash marks on side of 2mL tube, and add 0.6 volumes of room temperature 100% Isopropanol. Swirl or invert gently to mix. Incubate at room temperature for 2 hours to overnight in the dark.

Step 13.
Pellet: Centrifuge tube at top speed (13000 rpm) for 30 min. Carefully pour off buffer+isopropanol (I pour into a 50ml falcon tube and look to see if the pellet poured off. If the pellet is invisible then it usually won’t pour off). Add 1 ml 70% EtOH to 2mL tube, invert several times, and centrifuge at 13000 rpm for 10 min. Pour off EtOH. Repeat for a total of 2 rinses. Dry down pellet in Speed Vac for 15 minutes (flick tube to see if it’s completely dry). Pellets may detach from sides of tube, especially after second rinse.

Step 14.
Elution: Add 90-250 ul Nulcease-free sterile water or 1x TE buffer to 2mL tube, mix around (flick tube and shake down) to get all possible DNA wet. Allow pellet to dissolve for 1-2 hours in the refrigerator.

Step 15.
Prepare working and archive stocks and store at -20ºC / -80ºC.

Step 16.
Picogreen to determine concentration of DNA.