

DNA extraction from Sterivex filters
Byron Crump, October 30, 2007

DNA extraction and purification: Field samples will contain 1 or 2 ml of DNA Extraction Buffer (DEB: 100 mM Tris buffer (pH 8), 100 mM NaEDTA (pH 8), 100mM phosphate buffer (pH 8), 1.5 M NaCl, 1% CTAB) and they were frozen at -80°C .

Transfer buffer and filter to microcentrifuge tubes. Defrost filter. Working over a sterile polypropylene cutting board (or fresh square of sterile aluminum foil) in a laminar flow hood (if available), squeeze cartridge with pliers near outport end to crack open. Pour off buffer into 1 (or distribute into 2) sterile 2 ml microcentrifuge tubes (the kind with an o-ring in the lid, I like Fisher cat # 05-538-69C, Corning* Brand Microcentrifuge Tubes, 500/cs). The buffer may also be drawn out of the inport with a sterile syringe prior to cracking if preferred. Cut filter off barrel with sterile Exacto knife (or razor blade), fold filter so that sample is on the inside, cut filter into small pieces (4-8), and distribute into microcentrifuge tubes with sterile forceps. (Forceps and Exacto knife should be ethanol flamed between samples.)

Add Proteinase-K & Lysozyme and Freeze-Thaw: Add 20 ul proteinase-K (1% in water or 10 mg/ml) and 20 ul of lysozyme (10% in water or 100 mg/ml) to each microcentrifuge tube, freeze tubes at -80°C for 15 minutes and thaw at 37°C for 5 minutes three times. [Final concentrations of enzymes ~ 0.2 mg/ml proteinase K, and 2 mg/ml lysozyme (assuming 1 ml of sample per tube)]. You may also combine the proteinase-K and lysozyme and add a single 40 ul aliquot.

-----Safe stopping point-----

37°C incubation: Make sure tubes are closed tight. Incubate tubes for 30 min at 37°C in a water bath.

SDS & 65°C incubation: Add 50 ul filter-sterilized SDS (20% sln. in water) to each microcentrifuge tube, invert several times to mix. Make sure tubes are closed tight. Incubate for 2 h (1 h minimum) at 65°C in water bath.

Phenol-Chloroform extraction: Working in a fume hood, fill microcentrifuge tubes the rest of the way (\sim level with the base of the cap) with phenol:chloroform:isoamyl alcohol (25:24:1, pH 8.0). Vortex. Centrifuge in microcentrifuge at low speed (3000 rpm) for 5 min. Transfer top layer to new microcentrifuge tube and repeat (total 2 washes). Then transfer top layer to new microcentrifuge tube (transfer 1.25 mL maximum to ensure that there is enough room for the following step). Note that if you used Sterivex-GP filters the filter material will melt in the phenol:chloroform:isoamyl alcohol and form a layer at the aqueous:organic interface.

Precipitation: Estimate buffer volume from hash marks on side of microcentrifuge tubes, and add 0.6 volumes of room temperature 100% Isopropanol. Swirl or invert gently to mix. Incubate at room temperature for 2 h to overnight (1 h minimum).

Pellet: Centrifuge in microcentrifuge at top speed (13000 rpm) for 30 min. Carefully aspirate, pipette, or pour off buffer+isopropanol (I pour into a 15ml 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 microcentrifuge tubes, 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 roto-evaporator (~ 15 minutes, flick tube to see if it's completely dry). Pellets may detach from sides of tube, especially after second rinse.

Resuspend: Add 250 ul EB (10mM tris, pH 8.5 or some other buffer) or autoclaved-UV sterile ultra-pure water (most recently we've been using Fisher Brand sterile water that has been placed under UV light for 10 minutes) to microcentrifuge tubes, mix around (flick tube and shake down) to get all possible DNA wet, and then set tubes in a rack (If samples were split into 2 tubes resuspend in 125 ul of water). Allow pellet to dissolve for 1-2 hours in the refrigerator.

-----Safe stopping point---freeze samples at -80°C until the following day-----**OR**-----

Combine DNA from pairs of microcentrifuge tubes if applicable (this will become the working tube), then aliquot 150 ul DNA into a sterile screw-cap microcentrifuge tube (this is the archive tube) and freeze at -80°C. (Archive tube gets 150 ul, working tube gets 100 ul.)

NOTES: After final freeze-thaw do not put DEB buffer on ice or in the fridge because the SDS will precipitate. Phenol-chloroform extraction and precipitation takes ~4 hours for 24 samples. If >70% EtOH is used to rinse pellets, salts will ppt in solution. Rewash pellets with 70% EtOH. Can do extraction in 1 tube if sterivex contains only 1 ml of DNA extraction buffer.

Adapted from...Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316-322

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