DNA Extraction of community DNA from the Baltic Sea (Bertilsson &Riemann)

For DNA extraction, 2-3 litres of water was pre-filtered through 47 mm, 3.0 μm pore-sized Isopore membrane filters (Millipore; to prevent filter clogging <1 litre was filtered per filter) and then through a 0.22 μm Sterivex capsule filter (Millipore) via peristaltic pump (~100 ml min^{-1}). Sterivex filters were frozen at –20°C until extraction. DNA was extracted from the filters using an enzyme/phenol-chloroform protocol as described in Riemann et al. (2000), but with a 30 min lysozyme digestion (5 mg ml^{-1}, final) at 37°C and an overnight proteinase K (100 μg ml^{-1} final) digestion at 55°C (Boström et al. 2004). DNA was re-suspended in TE and quantified using PicoGreen (Molecular probes).

Solutions Required

•*Lysis buffer*
  750 mM sucrose
  400 mM NaCl
  50 mM tris-HCl (pH 7.6)
  20 mM EDTA
•*Lysozyme* (5 mg/ml)—Made fresh each time
•*SDS* (10%)
•*Proteinase K* (20 mg/ml)—Fresh or frozen aliquots (<6 months)
•*NaAcetate* (3.0 M, pH. 5.2)
•*Ethanol* (100% and 70%)
•*Phenol:Chloroform:Isoamyl alcohol* (25:24:1)
•*Chloroform:Isoamyl alcohol* (24:1)

1. Add 1.8 ml lysis buffer containing lysozyme (fresh) (5 mg/ml) to the capsule, seal with parafilm and incubate at 37°C for 30 min.
2. Add 180 μl Proteinase K (20 mg/ml) + 100 μl 10% SDS. Be sure to mix thoroughly by numerous inversions and shaking. Incubate at 55°C overnight.
3. Pull extracts into syringe. Rinse filters with 1 ml 1XTE, shake, divide extracts equally between 3 x 2 ml Eppendorf tubes. A total of ≈1 ml in each.
4. Add 166 μl 3.0 M NaAcetate and 0.66 ml ice-cold isopropanol. Incubate at -20°C >1hr
5. Spin at 15,000 g for 30 min. Remove supernantant and resuspend in 3 x 133 μl 1XTE; 37°C for 30 min to 1 hr. Pool in one tube.

6. Extract twice with 500 μl phenol/chloroform/isoamylalcohol (25:24:1) and once with 600 μl chloroform/isoamylalcohol (24:1).
7. Add 1/10 vol NaAcetate + 2.5 vol 100% ethanol. Put in freezer as in Step 4.
8. Spin 30 min, 15,000 rpm, 4°C. Remove supernatant. Add 0.5 ml 70% ice-cold ethanol, spin again 10 min.
9. Dry carefully in SpeedVac and resuspend in 50 μl 1X TE.

References

Boström KH, Simu K, Hagström Å, Riemann L (2004) Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. Limnol. Oceanogr. Methods 2:365-373

Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microbiol. 66:578-587