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Extracting Bacterial DNA from filters

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Gene Flow in Subzero Br...



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Protocol status: Working

We use this protocol and it's working

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Abstract

Adapted from Tara Oceans extraction protocol (see citation).



- 1 Add 1mL lysis buffer to each filter in a 5mL snap cap tubea.
Lysis buffer:
 - i. 40mM EDTA
 - ii. 50mM Tris
 - iii. 0.75M sucrose
- 2 Incubate 45min at 37°C, shaking gently
- 3 Add SDS to 1% v/v (in this case, 100ul of 10% SDS solution was added to each sample)
- 4 Incubate 1hr at 55°C, shaking gently
- 5 Collect liquid lysate into a 2mL tube
- 6 Add 1mL phenol:chloroform:isoamyl alcohol and mix well
- 7 Centrifuge 5min at 8000g
- 8 Transfer aqueous phase to new 2mL tube
- 9 Repeat steps 6-8
- 10 Add 1mL chloroform and mix well
- 11 Centrifuge 5min at 8000g
- 12 Transfer aqueous phase to upper reservoir of a 4mL 100kDa Amicon concentrator
- 13 Centrifuge at 1000g until <200ul remains



- 14 Add 2mL sterile water to upper reservoir
- 15 Vortex for 20sec at 1500rpm
- 16 Centrifuge at 1000g until <200ul remains
- 17 Repeat steps 14-16
- 18 Collect sample from upper reservoir and transfer to 1.5mL tube
- 19 Check concentration with Qubit HS DNA assay