Extracting Bacterial DNA from filters

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ABSTRACT

Adapted from Tara Oceans extraction protocol (see citation).

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MANUSCRIPT CITATION:

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Add 1mL lysis buffer to each filter in a 5mL snap cap tube.  
Lysis buffer:  
i. 40mM EDTA  
ii. 50mM Tris  
iii. 0.75M sucrose

Incubate 45min at 37°C, shaking gently

Add SDS to 1% v/v (in this case, 100ul of 10% SDS solution was added to each sample)

Incubate 1hr at 55°C, shaking gently

Collect liquid lysate into a 2mL tube

Add 1mL phenol:chloroform:isoamyl alcohol and mix well

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