Extracting Bacterial DNA from filters

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Abstract
Adapted from Tara Oceans extraction protocol (see citation).

Citation: Natalie Solonenko Extracting Bacterial DNA from filters. **protocols.io**
dx.doi.org/10.17504/protocols.io.qv8dw9w
Published: 11 Jun 2018

Protocol

Step 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

Step 2.
Incubate 45min at 37°C, shaking gently

Step 3.
Add SDS to 1% v/v (in this case, 100ul of 10% SDS solution was added to each sample)

Step 4.
Incubate 1hr at 55°C, shaking gently

Step 5.
Collect liquid lysate into a 2mL tube

Step 6.
Add 1mL phenol:chloroform:isoamyl alcohol and mix well

Step 7.
Centrifuge 5min at 8000g

Step 8.
Transfer aqueous phase to new 2mL tube

Step 9.
Repeat steps 6-8

Step 10.
Add 1mL chloroform and mix well

Step 11.
Centrifuge 5min at 8000g

Step 12.
Transfer aqueous phase to upper reservoir of a 4mL 100kDa Amicon concentrator

Step 13.
Centrifuge at 1000g until <200ul remains

Step 14.
Add 2mL sterile water to upper reservoir

Step 15.
Vortex for 20sec at 1500rpm

Step 16.
Centrifuge at 1000g until <200ul remains

Step 17.
Repeat steps 14-16

Step 18.
Collect sample from upper reservoir and transfer to 1.5mL tube

Step 19.
Check concentration with Qubit HS DNA assay