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Extracting Bacterial DNA from filters

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Manuscript citation:
Alberti, A. et al. Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition. Sci. Data 4:170093 doi: 10.1038/sdata.2017.93 (2017).

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