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Isolate prokaryotes from sponge tissue (SAP)

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hostmicrobeprotocols



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ABSTRACT

Protocol to create a fixed suspension of sponge associated prokaryotes (SAP) from sponge tissue. This can serve as the basis for Fluorescence *in situ* hybridisation and/or cell sorting.

This protocol was tested for different sponge species but might also be adapted to other organisms (let others know)

The protocol was modified from :

Fieseler L, Horn M, Wagner M, Hentschel U. (2006) Discovery of the novel candidate phylum "Potibactetia" in marine sponges (vol 70, pg 3724, 2004). Applied and Environmental Microbiology;72(8):5677-.

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GUIDELINES

Keep sample on ice during preparation.

STEPS MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Corning® 40µm Cell Strainer	431750	Corning

Dissect and homogenise tissue

- 1 Mince sponge tissue in ice cold CMASW using a razor blade within a petry dish on ice



Tissue can be either fresh or thawed from -20°C samples

Process about the tissue volume that would fit in a 1.5 ml eppendorf tube

- 2 Squeeze remaining pieces using 15 ml falcon tube lid **0 °C on ice**

- 3 Transfer to fresh 50ml Falcon tube and add up to **20 ml CMASW**

4 Incubate ⌚ 00:30:00 on ice

5 Vortex strongly ⌚ 00:05:00 at 🌡 21 °C

Purify

6 Filter through 40µm cell strainer into a fresh 50 ml Falcon tube



Corning® 40µm Cell Strainer
by Corning
Catalog #: 431750

7 Transfer to fresh 50 ml falcon tube and add up to 🧴 50 ml CMASW

8 Spin down at 600g to pellet cells for ⌚ 00:10:00 at 🌡 4 °C

9 Transfer supernatant to fresh 50ml falcon tube and add up to 🧴 50 ml CMASW



The supernatant should contain the bacterial fraction

10 Spin down at 1600g to pellet cells for ⌚ 00:10:00 at 🌡 4 °C and resuspend in 🧴 50 ml CMASW



11 Repeat step 10 until solution becomes clear



In case of sponge tissue this gets rid of secondary metabolites that inhibit downstream applications.

Fix cells


12 If solution is clear resuspend pellet into 1ml ice cold [M] 1 Mass Percent PFA in CMASW and transfer to fresh 2ml tube




4% PFA for fixation
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PREVIEW

RUN

12.1  use mask be careful with PFA

pour 2 g of paraformaldehyde (PFA) powder in 50 ml phosphate buffered saline (PBS; 130 mM NaCl, 10 mM Na₂HPO₄/NaH₂PO₄, pH 7.4)

 adjust to amount actually required


12.2 heat to approx. 60° C (must not boil!), until suspension is clear (approx. 1/2 h); if not add some drops of 1N NaOH

30m

12.3 check pH and adjust to pH 7.0

12.4 filter through 0.2 µm filter and place on ice

13 Fix overnight  4 °C in fridge


 Fixation time and fixative concentration might be optimised for different cell types
find basic guidelines here: [Silva protocols](#)

14 Pellet cells by centrifugation ( 00:10:00 at 4000 x g) discharge supernatant

15 Thoroughly resuspend fixed cells in 500 µl PBS

16 Repeat step 5 and 6

17 Add 500 µl absolute ethanol and resuspend cells thoroughly

18 At this stage the cell suspension can be stored at  -20 °C for several months



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