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Isolate prokaryotes from sponge tissue (SAP)

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ABSTRACT

Protocoll 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-.

PMID:15184179 DOI:10.1128/AEM.70.6.3724-3732.2004

GUIDELINES

Keep sample on ice during preparation.

STEPS MATERIALS

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

Dissect and homogenise tissue

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

- Squeeze remaining pieces using 15 ml falcon tube lid 80 °C on ice
- 3 Transfer to fresh 50ml Falcon tube and add up to **■20 ml CMASW**

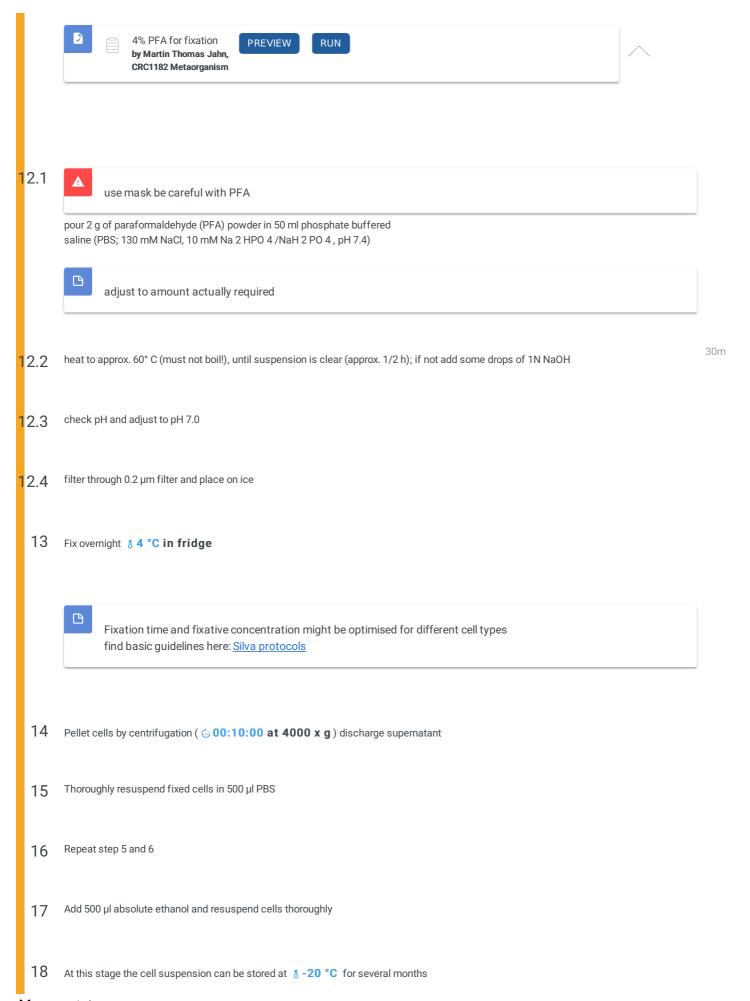
- Incubate © 00:30:00 on ice Vortex strongly © 00:05:00 at § 21 °C Purify 6 Filter through 40µm cell strainer into a fresh 50 ml Falcon tube Corning® 40µm Cell Strainer by Corning Catalog #: 431750 Transfer to fresh 50 ml falcon tube and add up to 30 ml CMASW Spin down at 600g to pellet cells for © 00:10:00 at 8 4 °C 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 metbolites that inhibit downstream applications.
- If solution is clear resuspend pellet into 1ml ice cold [M]1 Mass Percent PFA in CMASW and transfer to fresh 2ml tube

Fix cells

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