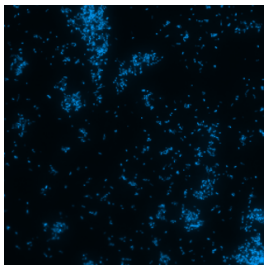
Jun 22, 2019

Isolate prokaryotes from sponge tissue (SAP)



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Manuscript citation:

Protocol status: Working
We used this protocol for different purposes and it is working

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Protocol Integer ID: 17397

Keywords: cell purification, targeted enrichment

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.

Materials

STEP MATERIALS

Corning® 40µm Cell Strainer **Corning Catalog #**431750

Protocol materials

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Before start

- Prepare CMASW:

400mM NaCl, 27.6mM Na_2SO_4, 2.3mM $NaHCO_3$, 8.9mM KCl, 0.8mM KBr, 0.4 mM H_3BO_3, 0.15mM $SrCl_2$, 0.07mM NaF in MilliQ and autoclave. >>(This likely will also work with NaCl only but adjust salinity)

Dissect and homogenise tissue

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

Note

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 **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

Note

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

Note

In case of sponge tissue this gets rid of secondary metbolites 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

Protocol

NAME

4% PFA for fixation

CREATED BY

Martin Thomas Jahn

PREVIEW

12.1 **Safety information**

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 Na 2 HPO 4 /NaH 2 PO 4 , pH 7.4)

Note

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

Note

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