Confocal Microscopy imaging for Opaline Silica Single Cell Skeletons (Polycystines Radiolaria)

Miguel Mendez Sandin[1]

[1]Station Blologique de Roscoff

Oct 10, 2018

Working

dx.doi.org/10.17504/protocols.io.t5qeq5w

Roscoff Plankton Group

Miguel Mendez Sandin
Station Blologique de Roscoff

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Recovering skeletons

1 After DNA extraction, recover skeleton from the eluted pellet under binoculars or inverted microscope.

Note: During DNA extraction: dilute waste from the extraction procedure (i.e. pellet debris, containing the skeleton) in milliQ water and store skeletons at -20°C.

Rinsing

2 Rinse skeleton several times in milli-Q water to decrease the concentration of SDS and other lysis and DNA precipitation reagents.

Cleaning

3 Transfer skeleton into 1.5 ml Eppendorf tubes containing 50 µl of hydrogen peroxide (H_2O_2).

4 Heat at 70°C for 10 min to remove residual organic matter.

Diluting

5 Add 1 ml of milli-Q water.

Rinsing

6 Handpick skeleton under binoculars or inverted microscope and repeat several rinsing steps.

Staining

Transferred into 1.5 ml Eppendorf tubes containing 25µl of fluorescent staining mix (Colin et al., 2017).

7 Colin, S., Coelho, L.P., Sunagawa, S., Bowler, C., Karsenti, E., Bork, P., Pepperkok, R., de Vargas, C., 2017. Quantitative 3D-imaging for cell biology and ecology of environmental microbial eukaryotes. Elife 6, 1–15. doi:10.7554/eLife.26066

8 Let the tubes for two hours for skeleton staining at room temperature and dark conditions.

Diluting

9 Add 1.5 ml of milli-Q water to dilute the dye.

Rinsing

10 Handpick the skeleton and repeat 4-6 rinsing steps into milli-Q water to properly clean the sample.

Preparing for imaging

11 Transfer skeleton into a micro well plate.

12 Dry at 70 °C for 2 hours.

Imaging

13 Skeleton ready to be imaged