Version 3
Oct 10, 2018
Working

# Scanning Electron Microscopy imaging for Opaline Silica Single Cell Skeletons (Polycystines Radiolaria) Version 3

Miguel Mendez Sandin[1]
[1]Station Biologique de Roscoff

dx.doi.org/10.17504/protocols.io.ug9etz6

Roscoff Plankton Group

Miguel Mendez Sandin
Station Biologique 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.

Protocol adapted from Biard et al. (2015).
Biard, T., Pillet, L., Decelle, J., Poirier, C., Suzuki, N., Not, F., 2015. Towards an Integrative Morpho-molecular Classification of the Collodaria (Polycystinea, Radiolaria). Protist 166, 374–388. doi:10.1016/j.protis.2015.05.002

## 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.

## Preparing for imaging

7 Transfer skeleton in a glued SEM pin stub mount with the less water as possible.

Note: no polycarbonate membrane, place the skeleton directly in the glue of the pin stub.

## Drying

8 Let dry 30 minutes / 1 hour.

## Imaging

9 Skeleton ready to be imaged.