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Working

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

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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

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

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