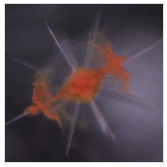

Version 2

Jan 12, 2021

Isolation of clean single-cell samples for physiological or molecular experiments (Radiolaria, Acantharia) V.2

Joost Mansour[1], Fabrice Not[1]

[1]Sorbonne University, CNRS, UMR7144 Adaptation and Diversity in Marine Environment (AD2M) laboratory, Ecology of Marine Plankton team, Station Biologique de Roscoff, Place Georges Teissier, 29680 Roscoff, France

1 *Works for me* dx.doi.org/10.17504/protocols.io.bqvrmw56

Ecology of Marine Plankton (ECOMAP) team - Roscoff | Symbiosis Model Systems

Joost Mansour

ABSTRACT

Many marine protists are not culturable and therefore challenging to study, nonetheless, they are essential in all marine ecosystems. The development of single-cell techniques is allowing for more marine protists to be studied. Thereby Cultivation-independent studies are essential for the study of organisms sensitive to the sampling procedure, such as Radiolaria.

This method describes the means to acquire clean isolated single-cells from plankton nets for physiological and/or transcriptomic analysis. The isolation of cells in this protocol is followed by preparing the cells for RNA extraction (protocol: Single-cell total RNA extraction from marine protists (e.g. Acantharia, *Strombidium* cf *basimorphum*, and *Prymnesium parvum*)).

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KEYWORDS

RNA-seq, Radiolaria, cell isolation, Acantharia, protist, plankton, sampling, Single-cell, RNA, protist, plankton

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GUIDELINES

This work is conducted under field conditions, where facilities are often not as good as in terrestrial-bound laboratories. Thus, sample preparations are typically not conducted working in a controlled air-stream, under a hood, nor has the work area necessarily been treated with RNAse inhibitors (or similar). Aseptic techniques and gloves, to minimize further contamination, have been used though. Nonetheless, this procedure works even for follow-up RNA extraction studies.

MATERIALS TEXT

Petri

- Dish **P212121 Catalog #LI-PD01100** In 2 steps (glass Petri dishes or 6 well plates can serve the same function, though at least one larger Petri dish is recommended)

PCR Tubes & Caps, RNase-free, 0.2 mL (8-strip format) **Thermo**

- **Fisher Catalog #AM12230** (x #samples)
- Micropipette and 100 µl filter pipet-tips
- Ice **Contributed by users**
- Liquid nitrogen in dewar **Contributed by users** (optional, but preferred)

Borosilicate Glass Disposable Pasteur Pipettes, Plain End, 145mm **Thermo**

- **Fisher Catalog #CP2555402**
- Bunsen burner **Contributed by users** (a portable Spirit burner can also do the job)
- Tubing to fit the glass Pasteur pipet
- Filter mouthpiece

Stericup Quick Release-GP Sterile Vacuum Filtration System **Millipore**

- **Sigma Catalog #S2GPU02RE** (or other 0.2 µm-pore-size filtering method)
- Ethanol **Contributed by users** for cleaning

RNAqueous™-Micro Total RNA Isolation Kit **Thermo**

- Lysis buffer from **Fisher Catalog #AM1931**

Stereo microscope EZ4, Leica 10447197
Leica EZ4 educational stereomicroscope.

(or another preferred stereo microscope that allows for micro handling in a Petri dish under it).

SAFETY WARNINGS

Great care must be taken in the sampling method, for Radiolaria are fragile.
For endosymbiotic uncultivatable plankton, sampling using slow horizontal plankton net tows are proven to be the best compromise between ease of sampling and retaining cell quality.

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Clean single-cells can be acquired by this protocol for any plankton. The sample preparation for RNA extraction is though only verified for Acantharia, Strombidium cf basimorphum, and Prymnesium parvum.

ABSTRACT

Many marine protists are not culturable and therefore challenging to study, nonetheless, they are essential in all marine ecosystems. The development of single-cell techniques is allowing for more marine protists to be studied. Thereby Cultivation-independent studies are essential for the study of organisms sensitive to the sampling procedure, such as Radiolaria.

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BEFORE STARTING

Collect your favorite marine protists with a plankton net of choice.
We use a 150 µm-mesh-size plankton net for Acantharia.

Preparations 30m

1 Prepare filter-sterilised seawater (FSW, 0.2 µm-pore-size)

2 Prepare your Pasteur pipet isolation tool by elongating the pipet tip under a flame. Carefully break the tip of the pipet to the desired length (and width, longer is thinner).
Attach the tubing and optionally a filter on the other end.

Elongated (and bend) Pasteur pipet with tubbing and mouthpiece filter

Single-cell isolation 4h

3 Acquire a sub-sample from a concentrated plankton net sample in a
Petri Dish **P212121 Catalog #LI-PD01100**

3.1 Fill another Petri dish with FSW

4 Working under a

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Stereo microscope EZ4, Leica 10447197
Leica EZ4 educational stereomicroscope.

Isolate healthy cells from the sub-sample into the Petri Dish **P212121 Catalog #LI-PD01100** with clean FSW using a micropipette or modified Pasteur pipette.

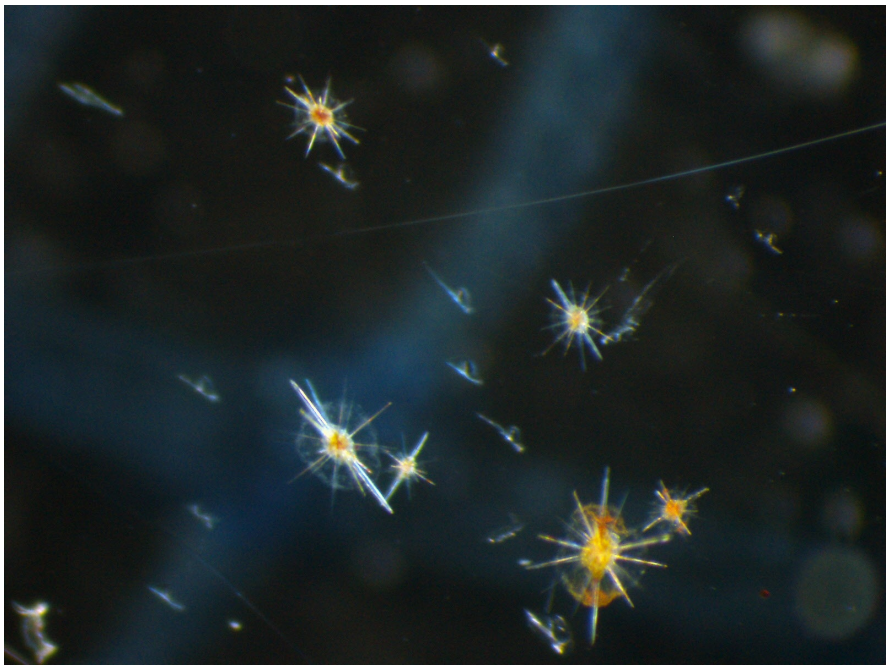
Dedicate no more than 20 Acantharia cells to a single Petri dish.

5 Incubate cells in FSW for **01:00:00** . 1h

5.1 Repeat the isolating procedure with a transfer to fresh FSW and **01:00:00** incubation a total of **three times**. 2h

This procedure allows for self-cleaning of particles attached to the cells and dilution to achieve effective extinction of any contaminating organisms accidentally taken with during isolation.

6 These Clean cells can be used for physiological experiments before proceeding to the next step.



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Some isolated Acantharia

Isolation for RNA extraction 30m

7 Prepare small plastic zip-locked bags for the samples along with a sample label (pencil on water-proof paper).

8 Prepare 100 µL lysis buffer (from RNAqueous kit) in 0.2 mL PCR tubes **On ice** , label tubes the same as the plastic bags.

Where possible continue to work on ice

9 Transfer one cell per lysis buffer-filled tube and put it in a dedicated small plastic bag (both labeled the same).
It is preferred to use a micropipette and 100 µl filter tips. Setting the volume to the minimum allows isolation of the cells with very little residue and a consistent amount of FSW.

Immediately freeze in liquid nitrogen.

Disregarding freezing in liquid nitrogen, and proceeding immediately to storage at **-80 °C** has proven to suffice. Though it is still preferred to freeze in liquid nitrogen when possible, especially for physiological experiments.

10 Store quickly at **-80 °C**

RNA extraction

11 Follow the protocol for RNA extraction.

Single-cell total RNA extraction from marine protists (e.g. Acantharia, *Strombidium* cf *basimorphum*, and *Prymnesium parvum*).

https://www.protocols.io/private/EDB0E27A323211EB952C0A58A9FEAC2A

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