BIOMARKS SAMPLING PROTOCOLS

MOLECULAR

DNA & RNA subsurface and DCM

I- Introduction
Analyze the diversity of marine protists across different size fractions.

II – Major Peculiarities
For each depth and size fraction:
2x100L for metagenomics purposes
8x10-20L for PCR based approaches

Timing:
For a particular depth, once the 100 L filtration are started, you can set up and start the 4x20L filtration and run both in parallel.

2 persons full day on it.
Number of replicates are indicative, do as many as you can.

III – Material and Instruments
Peristaltic pump (I/P 2 channels) Hiro's type
Peristaltic pump (L/S 4 channels) Silvia's type
Filtration units 142 mm
In line Filtration units 47 mm

IV – Chemicals and buffers
RNA Later

V – Material preparation
Set up the pump, tubing and filter holders

VI – Sampling and conditioning

Collect SW with Niskin bottles, sieve it onto 20µm before to fill up 2x50L carboys (or 1x100L). Filter the <20µm SW onto a 3 µm polycarbonate filter 142mm followed by a 0.8 µm polycarbonate filter 142mm (in line setup of the filter holders). Use the two channels of the pump, one coming from each 50L carboys that merge to pass through the filters. Once filtration is done, collect the filter with clean tweezers, transfer the two filters in separate 15ml falcon tubes and cover the filter with RNA Later (ca 5 ml). Label the tubes accordingly to the size fractions: S100RNA3-20µm and S100RNA0.8-3µm. Flash freeze the tubes in liquid nitrogen.

Repeat the procedure described above and label the tubes S100DNA3-20µm or S100DNA0.8-3µm.

Collect SW with Niskin bottles, sieve it onto 20µm before to fill up 4x20L carboys. Filter the <20µm SW onto a 3 µm polycarbonate filter 47mm followed by a 0.8 µm polycarbonate filter 47mm (in line setup of the filter holders). Use the four channels of the pump separately, with four independent set up. Once filtration is done, collect the filter with clean tweezers, transfer the two filters in separate 2ml cryotubes and cover the filters with RNA Later (ca 1 ml). If required to be able to filter larger volume you can change the 3µm filter and pool them in one single cryotube. The

0.8µm filter should handle 20L without problems. Label the tubes accordingly to the size fractions: S100RNA3-20µm (1 to 4) and S100RNA0.8-3µm (1 to 4). Flash freeze the tubes in liquid nitrogen.

Repeat the procedure described above and label the tubes S100DNA3-20µm (1 to 4) or S100DNA0.8-3µm (1 to 4).

Repeat the whole procedures for SW from DCM *(replace the S by D in the labels)*

From 20µm plankton nets (see Tara protocols)
1- Horizontal net tow
2- Oblique net tow

VII – Storage and shipping conditions
After having flash freezed the tubes in liquid nitrogen and store the tube whatever possible at -80°C or at -20°C.
Ship on dry ice.

VIII – Chemicals and buffers preparation
None

IX – Disposal of trials and waste
None

X - Bibliography

XI - Annexes

Extracellular DNA

I- Introduction
Estimate the quantity of extracellular DNA in sea water and analyze its diversity through molecular markers. This parameter potentially set the baseline for diversity studies of environmental protistan communities.

II – Major Peculiarities
Work only on 0.2 µm filtered SW

III – Material and Instruments
4 x 20 L carboys (2 of which are contaminated with CTAB)
4 x 1 L Bottles (2 of which are contaminated with CTAB)
0.2 µm 142 mm PC filters
0.2 µm 47 mm PC filters
2 x 47 mm filtration units (1 of which are contaminated with CTAB)
2 x 142 mm filtration units (1 of which are contaminated with CTAB)
50 ml and 15 ml falcon tubes
2 ml Eppendorfs
DNA & RNA free (flamed) tweezers to fold the filters

IV – Chemicals and buffers
CTAB

V – Material preparation

VI – Sampling and conditioning
For the entire procedure: Collect 100L from the SW filtered onto 0.6µm for DNA and RNA ("waste" water of the previous filtrations). Filter those 100L dispensed in 20L carboys onto 0.2µm pore size filter 142 mm.

** For molecular Biology*
- Collect 20L of seawater samples previously filtered onto 0.2 µm pore-size filters.
- Add to 20 L of sample 400 ml of 0.5% CTAB solution pH 8.0 to precipitate the extracellular DNA. Mix and let sit for 1 h at room temperature
- Filtrate the CTAB-treated seawater through 0.2 µm, 142 mm, polycarbonate (PC) filters by vacuum (or peristaltic depending of material available). The CTAB precipitate should be captured on the PC filter.
- Remove the filter and place into a 15 ml sterile tube.

** For quantification*
- Collect 1 L of seawater samples previously filtered onto 0.2 µm pore-size filters.
- Add to 1 L of sample 20 ml of 0.5% CTAB solution pH 8.0 to precipitate the extracellular DNA. Mix and let sit for 1 h at room temperature.
- Filtrate the CTAB-treated seawater through 0.2 µm, 47 mm, polycarbonate (PC) filters by vacuum. The CTAB precipitate should be captured on the PC filter.
- Remove the filter and place into a 2 ml Eppendorf sterile tube.

** For contribution of Viral DNA to Total DNA pool*

- Collect 35-40 ml of seawater previously filtered onto 0.2 µm pore-size filter into a 50 ml Falcon sterile tube.

**** Prepare 3 replicates (at least 2 if not enough time) for each type of analysis, i.e.*
3 x 142 mm filters in 15 ml falcons
3 x 47 mm filters in 2 ml eppendorfs
3 x 50 ml falcons filled with 35 ml of filtered SW

VII – Storage and shipping conditions

Store samples (i.e. tubes) at -20°C and ship on dry ice.

VIII – Chemicals and buffers preparation

Preparation of CTAB solution pH 8.0

Cetyltrimethylammonium bromide (CTAB)	5 gr.
TRIS (FW =121.1)	60.55 gr.
EDTA (FW=372.2)	18.60 gr.
Q-water	1 liter qsp

Store at 4°C

IX – Disposal of trials and waste

X - Bibliography

XI - Annexes