BIOMARKS SAMPLING PROTOCOLS

BENTHOS

DNA/RNA & Rose Bengal stained Benthos

I- Introduction

The main objective of this part is to examine the diversity of benthic foraminifera based on traditional morphological approach and analysis of DNA sequences. We will analyse three types of samples obtained from surface sediment:

- Rose Bengal stained ethanol fixed samples;
- Total DNA extractions;
- Total RNA extractions.

We will use specific primers to PCR amplify a selected fragment of the SSU rDNA commonly used for identification of foraminifera. Moreover, we will use universal primers to analyse benthic community of other eukaryotes, based on V4 or V9 variable regions of the SSU. The sequences will be obtained using 454 pyrosequencing or Solexa methodology.

II – Major Peculiarities

Comparison of DNA sequence data with relative frequencies of foraminiferal species inferred from morphological study of fixed samples will allow evaluating the accuracy of DNA and RNA massive sequencing approaches for the assessment of foraminiferal species diversity.

III – Material and Instruments

- Multicorer or boxcorer are necessary to collect an undisturbed surface layer of the sediment.
- 50 ml cutted seringues for the sampling with boxcorer (optional)
- Sterile spoons
- 10 ml cryovials
- 50 ml Falcon tubes for samples fixed with Rose Bengal and formaldehyde
- 100 ml sterile polycarbonate bottles for living material
- Sieves 1000 um, 500µm, 125µm, 63 um, 32 um and eventually 20µm

IV – Chemicals and buffers

Liquid N2

The following kits will be necessary for DNA and RNA extractions:

- PowerMax Soil DNA Isolation Kit (10 preps) – ref. 12988-10
- PowerSoil RNA/DNA Isolation COMBO Kit – ref. 12866-COMBO
- Before sampling, add the lysis buffer to the beads tube in order to put directly the sediment sample on the boat.

Both kits are produced by MoBio and can be purchased in France from Ozyme SAS, 10 avenue Ampère, BP268, 78053 Saint-Quentin-en-Yvelines (tél. 01 34 60 24 24, fax: 01 34 60 92 12)

- In addition to the DNA/RNA kits, we will need rose Bengal solution in ethanol (1g per 1liter of 70% ethanol) for samples fixation and certain number of plastic containers for storage of fixed samples.

V – Material preparation

Surface sediment samples will be collected using sterile material (spoons, or syringes when the surface of sediment is an issue)

VI – Sampling and conditioning

- Take the corer and keep it straight to not mix the surface sediment with the deep-sediments;
- With a sterile spoon, take a sample of sediment at 1cm in surface and transfer it in the appropriate container;

Total fraction:

For each deployment, we are planning to collect:

- 1 PowerMax Soil DNA sample (5 g)
- 1 Powersoil RNA sample (2 g)
- 1 total sample (8 mL) flash-freezed in liquidN
- 1 fixed sediment samples (~ 5 mL)_add 2 volumes (~10 mL) of Rose Bengal solution in the Falcon tube. Shake slowly
- 1 live sample in 100mL bottle. Add filtered seawater. This sample will be dispatched in large fraction samples and live imaging sample.
- 1 sediment sample fixed with neutralized formaldehyde for microscopy and SEM

***Large fractions*: [63-500µm] – this fraction will be used for direct comparison of morphological assemblage with DNA/RNA data**

After the sampling, on the boat or in the lab (don't wait too long) :

- Slowly sieve the 100mL sample through 500 µm and 63 µm sieves. **Don't let dry the sediments, add filtered seawater!**
  It's better to work in a bucket to keep wet the sediments.

- Transfer the fraction [63-500] in 4 tubes (if you have enough material) :
  - 1 PowerMax Soil DNA sample (5 g) stored at +4°C ;
  - 1 Powersoil RNA sample (2 g) stored at +4°C ;
  - 1 total sample (8 mL) flash-freezed in liquidN
  - 1 fixed sediment samples (~ 5 mL) add 2 volumes (~10 mL) of Rose Bengale in the tube. Shake slowly. Store at room temperature.

VII – Storage and shipping conditions

- PowerMax Soil DNA sample : stored at +4°C, shipped with ice ;
- Powersoil RNA sample : stored at +4°C, shipped with ice ;
- Total sample : stored at -80°C, shipped on dry ice ;
- Fixed sediment samples : stored in the fridge in the lab, shipped with ice.

VIII – Chemicals and buffers preparation

DNA and RNA extractions will be processed in Roscoff laboratory. No preliminary preparation is necessary.

IX – Disposal of trials and waste

X - Bibliography

XI - Annexes

Pigments by HPLC

Benthos
Cut 3 cm of the upper layer from the corer and transfer sample to plastic box. Freeze.

Formol

I- Introduction
Formalin fixed samples will be used to determine the diversity and abundance of an array of species using optical microscopy

II – Major Peculiarities
Formalin is relatively toxic but preserves the material in a more effective way and for a long time. The non-neutralised formalin works better with diatoms but dissolves coccoliths

III – Material and Instruments
Low quality dark glass bottles (500 mL, as low quality glass releases silicates and helps long lasting preservation for diatoms) with high-bound, resistant screw cap.

IV – Chemicals and buffers
Neutral Formaldehyde solution (e.g.) JTBaker 7040. This saturated aqueous solution of Formaldehyde (40%) is referred to as 100% formalin.

V – Material preparation
Samples should be fixed immediately. To avoid handling and measuring formalin on board, it is advisable to put the formalin in each bottle while preparing the material on land.

VI – Sampling and conditioning

VII – Storage and shipping conditions
Samples should be kept at 4°C in the dark.

VIII – Chemicals and buffers preparation
Calcium carbonate in excess (100g per liter of formalin) is normally used to neutralize formalin. To this end, calcium carbonate is added to formalin and the bottle is shaken gently. The excess calcium carbonate is allowed to settle before using the supernatant as fixative.

IX – Disposal of trials and waste
Formalin has to be disposed of among toxic liquids. The bottles can be washed and recycled for other samples.

X - Bibliography

XI - Annexes

Live Imaging

I- Introduction
Obtain taxonomic information based on cells motility/behavior and on cells that do not preserve well in usual fixatives.

II – Major Peculiarities
Time consuming. Need to be performed as soon as possible after sampling
It requires a lot of microscopic and taxonomical expertise, so not all scientists are capable to do so

III – Material and Instruments
Decontaminated plastic bottles.
Inverted microscope equipped with camera
Stereomicroscopes equipped with camera
Compound microscope with camera
Petridishes
Glass slides and cover slips
Pasteur pipettes

IV – Chemicals and buffers
None

V – Material preparation

VI – Sampling and conditioning

Fractions : [>500µm] ; [125µm-500µm] ; [63µm-125µm]

- On the boat, take a amount of sediments (~50mL of each corer) in a decontaminated bottle, dilute with filtered seawater if it is too concentrated. Stored in a bucked with seawater to keep the temperature.
- As soon as possible, slowly sieve all sample into 3 empacked 500 µm, 125 µm and 63 µm sieves. **Don't let dry the sediments, add filtered seawater!**

It's better to work in a bucket to keep wet the sediments.

- Transfer each fraction in a Falcon tube and store them in an incubator at the environmental temperature.
- Observe as soon as possible.

VII – Storage and shipping conditions
Hard drive or USB drive

VIII – Chemicals and buffers preparation
None
IX – Disposal of trials and waste
Remove glass material in an appropriate manner (see lab local rules)
X - Bibliography
XI - Annexes

Pesticides and metals

I- Introduction
e.g. Aims of the Analysis

II – Major Peculiarities

III – Material and Instruments

IV – Chemicals and buffers

V – Material preparation

VI – Sampling and conditioning
At least 50 g wet weight for the sediments

VII – Storage and shipping conditions

VIII – Chemicals and buffers preparation

IX – Disposal of trials and waste
Among other thing, please mention if it has to be recycled, treated.

X - Bibliography

XI – Annexes

Glutaraldehyde

I- Introduction
Glutaraldehyde fixed samples will be used to determine the diversity and abundance of ciliates using optical microscopy

II – Major Peculiarities
Glutaraldehyde is toxic but preserves the material in a more effective way and for a long time. Glutaraldehyde should be added on board as soon as possible.

III – Material and Instruments
50 ml conical tubes
50 ml or larger syringes
0.22 ul sterile filters that fit onto the syinges

IV – Chemicals and buffers
Glutaraldehyde 50%

V – Material preparation
Samples should be fixed immediately. To avoid handling and measuring glutaraldehyde on board, it is advisable to put the glutaraldehyde/sterile water in each tube before hand on land.
One ml of glutaraldehyde should be added to 24 ml of sterile seawater. 25 ml of sediment sample should be added to the glutaraldehyde/seawater.

VI – Sampling and conditioning
Four tubes, each containing 25 ml of sediment, should be collected from each sampling site.

VII – Storage and shipping conditions
Samples should be kept at 4°C.

VIII – Chemicals and buffers preparation

IX – Disposal of trials and waste
Glutaraldehyde has to be disposed of among toxic liquids.

X - Bibliography

XI - Annexes