

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  $\mu\text{m}$ , 500 $\mu\text{m}$ , 125 $\mu\text{m}$ , 63  $\mu\text{m}$ , 32  $\mu\text{m}$  and eventually 20 $\mu\text{m}$

### **IV – Chemicals and buffers**

Liquid N<sub>2</sub>

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 : [ $>500\mu\text{m}$ ] ; [ $125\mu\text{m}-500\mu\text{m}$ ] ; [ $63\mu\text{m}-125\mu\text{m}$ ]**

- 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 bucket with seawater to keep the temperature.
- As soon as possible, slowly sieve all sample into 3 empacked 500  $\mu\text{m}$ , 125  $\mu\text{m}$  and 63  $\mu\text{m}$  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 syringes

## **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**