BIOMARKS SAMPLING PROTOCOLS

BENTHOS

DNA & RNA Benthos

I- Introduction

e.g. Aims of the Analysis

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.

IV – Chemicals and buffers

Including grade, brand, ref number etc…

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

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 ethanol and rose Bengal 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, syringes)

VI – Sampling and conditioning

For each deployment, we are planning to collect:

- 1 PowerMax Soil DNA sample (5 g)

- 1 Powersoil RNA sample (2 g)
- 3 fixed sediment samples (~ 5 g)

VII – Storage and shipping conditions

DNA and RNA samples should be stored frozen and shipped on dry ice. The fixed samples can be stored at room temperature.

VIII – Chemicals and buffers preparation

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

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

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
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
From each size fraction considered. Collect a small amount of sample, dilute if too concentrated, place it in a Petridishe or on a slide. Set the microscope and record 10 random fields for 2 min each.

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