

BIOMARKS SAMPLING PROTOCOLS

GIRUS

I. Sediment (main Girus sample)

Sampling strategy

1 bottle (500ml) will be used to collect disturbed surface sediment.

From 500ml sediment, 70 ml will be collected for FISH, 50 ml for cultures and 380 ml for DNA.

If collected quantity of sediment is smaller (e.g. using multicorer), quantity for sediment for DNA should be reduced. In all cases, sterile materials should be used (spoons, or syringes when the surface of sediment is an issue)

Marine sediment metagenomic

I- Introduction

The main objective of this part is to examine the diversity of benthic Giruses based on the analysis of DNA sequences.

II – Material and Instruments

1 Bottle (500ml)

III – Sampling and conditioning

DNA samples should be stored frozen (-20°C) and shipped on ice.

FISH on marine sediments

I- Introduction

The main objective of this part is to detect benthic Girus host using specific in situ fluorescence

We will use specific probes to target infected cells in sediment

II – Material and Instruments

- Flacon (50ml)

III – Material preparation

Sediment including the water will be stored into two 50ml falcon filling until 35 ml

IV – Chemicals and buffers

Paraformaldehyde 10X

PBS 1X

Ethanol

V – at laboratory

Prepare 2 replicates.

- From sediment, fix 35 ml of SW with 14 ml of Paraformaldehyde (4% final conc.)

- Let it incubate for 3 hour in the dark.
- Centrifuge falcon 5min max speed
- remove the supernatant
- Rinse with ca 50 ml of PBS 1X
- Centrifuge falcon 5min max speed
- remove the supernatant
- Store in a mix of PBS/Ethanol (1:1) in a 50 ml falcon tube.

VI – Storage and shipping conditions

- Stored and shipped at RT

Cultures of marine Giruses from sediment

I- Introduction

Culture of marine Giruses from sediment

II – Major Peculiarities

Sediment (as much as possible) including the seawater will be kept in falcon at room temperature

III – Sampling and conditioning

The samples will be sent at room temperature

II. water column (when possible)

Surface metagenomic

I- Introduction

Analyze the diversity of marine Giruses

II – Major Peculiarities

50 ~100 L of sea water

III – Material and Instruments

Peristaltic pump (I/P 2 channels)

2 Filtration units 142 mm

Filters 150mm (GF/A, 1.6µm)

Filters 142mm (0.2µm Express Plus)

IV – Material preparation

Set up the pump, tubing and filter holders

V – Sampling and conditioning

The pumping is done first through a GF/A filter then through a 0.22µm Express Plus filter (use more than one filters if required). Make sure that the filter is centrally positioned and that the inlet & outlet tubes are securely fixed. The filter holders should be set up in series to optimise sample processing times. Gently turn on the pump until some water is in contact with the filter, and then turn up the flow rate. It may be necessary to remove trapped air by raising the bleed valve lever before a high flow rate can be maintained. Turn off the pump, and remove remaining water in the filter holder using manual vacuum pump, before removing the filter.

VI – Sampling and conditioning

Filters (GF/A and Express plus) were put it into a 50ml falcon tube directly using the first pair of tweezers and screw the cap down tightly (with no addition of liquid). These should then be stored frozen at -20°C and ship on ice.

Cultures of marine Giruses

I- Introduction

Culture of marine Giruses

II – Major Peculiarities

2L of sea water stem from Surface metagenomic giruses after the GF/A filtration

III – Material and Instruments

Filter membranes 47mm 0.22µm GTTP04700
47mm system filtration for non-fixed samples

IV – Sampling and conditioning

- Use Pre-filter surface seawater through GF/A filters (the one used for the above large filtration if possible) and collect in a bottle.
- Set the filters (0.22 GTTP04700) on the filter holders on available filtration ramps carefully avoiding air bubbles.
- Full the funnel (1L) with the sample (2L = replicates)
- Start the pump and stop it when all filtrations finish.
- Remove the membrane using **ethanol cleaned tweezers**

V – Sampling and conditioning

The filter membrane (GTTP04700) were put face up in a sterile petrislide and encircle the joint with parafilm (keep at 4 °C). Samples should be stored at 4°C and shipped at Room temperature