

PROTOCOL (A)

SAMPLING PROTOCOL FOR PROKARYOTES

Collecting Prokaryotes on 0.22 µm pore size filters using Sterivex cartridges

The Standard Operating Procedure (SOP) for collecting marine bacterial communities is based on the protocol used at the Western Channel Observatory by Gilbert et. al., (2010), PLoS ONE 5(11); <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0015545>

1. **Collect minimum of 4-6 bacterial subsamples (sterivex filter -replicates)**, for which, at least the minimal reporting requirements are known. If possible - for each timezone - take the samples between 10am and 2pm, ideally at local noon.
 - a. 4-5 Sterivex filter should be shipped to HCMR (Greece) for DNA extraction
 - b. The remaining Sterivex filter should be stored at -80°C in your local freezer, as backup
2. **Isolate seawater using a Niskin bottle or 10% acid washed bucket from the surface (0-2m depth) of the water column.** Please make sure that you have enough water for the 5-6 subsamples. We would recommend collecting 20-40L of seawater.
3. Collect microbial community, by filtering the sampled seawater through **0.22µm Sterivex filter units*** available at (<http://www.millipore.com/catalogue/item/svgv010rs>). Please DO NOT perform a pre-filtration step. Instead, try to filter as much seawater as possible, up until the filter clogs. (Instructions on how to use Sterivex filters can be viewed at the video of the [MIRADA project at: http://amarallab.mbl.edu/mirada/mirada.html](http://amarallab.mbl.edu/mirada/mirada.html)).
4. **Filtration** using Sterivex filter should be done **using either a peristaltic pump or a hand pump** (e.g. 50mL sterile syringe). In either case, you will need a Luer-Lok adapter enabling a secure attachment to the Sterivex.

You could also use another pump type (e.g. vacuum pump), if you do so, please record such a deviation both to the logsheet and the online metadata form.
5. **After filtration**, please remove excess water, from inside the Sterivex filter, with the use of a syringe and by pumping air through the filter.
6. **Seal the Sterivex filter** by using a sticky tac, e.g. blu tack or similar. Please DO NOT USE PARAFILM, as it crumbles in very low temperatures, i.e. -80°C.

7. **Label your filters** according to the following type:

<OSD-ID>_<Month>_<Year>_<SiteName>_<ProtocolLabel>_<SampleNo>_<Depth>

e.g **OSD3_06_18_Helgoland_NPL022_1_1m**

8. **Protect you label from running.** For example seal the label on the filter with transparent adhesive tape (also known as Scotch tape, Sellotape or Tesafilm). Another option would be to use temperature resistant labels (e.g. known as Tough-Tags).
9. **Store your labeled filter in a FREEZABLE plastic bag.** Label the plastic bag according to (7), so in the end, your sample AND the plastic bag is correctly labeled.
10. **Freeze the plastic bag with the filters immediately in liquid nitrogen OR in a -80°C freezer.** For short-term storage a -20°C freezer can be used. For transport from sea to the land for a period of time shorter than one hour samples can be stored in sealed bag buried in ice.
11. Please record the following details (per filter) on the **logsheet**:
 - How much water you filtered
 - Time taken to filter the sample
 - Your observations about colour of the filter, after filtration.
12. Place your filters in **dry-ice** for shipping to HCMR (Greece), at the following address:

SHIPPING ADDRESS

Melanthia Stavroulaki
Institute of Marine Biology, Biotechnology and Aquaculture
Hellenic Centre for Marine Research
(Former USA Base) Gournes Padiados
71500 Heraklion Crete, Greece (Hellas)
Tel: +30-2810-337719, +30-2810-337840, +30-2810-337801

Relevant Metadata about the Sampling Protocol for Prokaryotes

List of “mandatory” and “optional” information for this sampling protocol, together with example values. These need to be written by hand for each sample in the SAMPLE section of the GOs Sampling Logsheets.

(Mandatory)	SAMPLE_Title	OSD3_06_18_Helgoland_NPL022_1_1m
(Mandatory)	SAMPLE_Depth (m)	1 (surface)
(Mandatory)	SAMPLE_Quantity	2 L
(Mandatory)	SAMPLE_Filtration_Time	30 min
(Mandatory)	SAMPLE_Container	Sterivex, 0.22 µm
(Mandatory)	SAMPLE_Content	Particulate matter
(Optional)	SAMPLE_Size-Fraction_Upper-Threshold	no pre-filtration
(Mandatory)	SAMPLE_Size-Fraction_Lower-Threshold	0.22 µm
(Optional)	SAMPLE_Treatment_Chemicals	none
(Mandatory)	SAMPLE_Treatment_Storage	Liquid Nitrogen or -80°C

Examples of CORRECT labeling of samples:

