

COLLECTION STANDARD OPERATING PROCEDURE

eDNA

I. Sample collection

- The collection of samples for eDNA should be done as soon as possible, either directly onboard or immediately after the return to the lab; in this latter case seawater should be kept in the shade and at cool temperature.
- Samples for eDNA must be collected from the same Niskin used for the sampling of chemical parameters and pigments (e.g., POC, DOC, and chlorophyll *a*).
- Seawater is collected in 10 L bottles (pre-washed with HCl 10% and rinsed with DDW).
- Gently homogenize the bottle and pour the seawater into a 1L cylinder.
- Filter three replicates for each sampling depth on nitrocellulose filters (47 mm in diameter and 0.45 μm porosity).
- Samples are filtered using an inline custom-built filtration system equipped with 47 mm filter holders and connected to a vacuum pump. Before and after filtering, the filter holder must be cleaned with alcohol (and let the alcohol dry off, otherwise eDNA degrades while filtering).
- After filtration, take the filter with tweezers and put it on a sterile Petri dish, fold the filter in half and cut it into two parts with a sterile (or previously cleaned with alcohol) razor blade.
- Put each half-filter into different 1.2 mL cryovials (2 vials in total, 1 half-filter in 1 vial). The two vials must be coded as **NRED + SAMPLE NUMBER**. The two vials, including a half-filter each, bear the same code, one of them will be processed for eDNA extraction, the second one is a back-up.
- Repeat operations from step 4 to step 7 until you have 3 replicates (each coming from a 1L volume of seawater) and 6 vials in total.
- Stock vials at $-80\text{ }^{\circ}\text{C}$.
- Carefully wash the filtration apparatus with distilled water and then with alcohol.

II. List of material, equipment and supplies

- Pre-washed 10L bottle
- Nitrocellulose membrane filters (GVS, 1215281) with a diameter of 47 mm and a porosity of 0.45 μm
- Filtration apparatus equipped with 47 mm filter holders (clean the holder with alcohol and let the alcohol dry off before use)
- Vacuum pump
- Graduated 1L cylinder
- Cryovials (1.2 mL volume)
- Tweezers (cleaned with alcohol)
- Sterile razor blade
- Alcohol for cleaning
- Lab gloves
- Sterile Petri dish