DNA extraction method

This protocol was used for DNA extraction of Guanabara Bay (LCR_0004_2008_09_22 – eubacteria and LCR_0005_2008_09_22 - archaea) and Amazônia (LCR_0008_2008_11_26 – eubacteria and LCR_0006_2008_11_26 - archaea) samples.

⇨ DNA was extracted by the "DNeasy Tissue Kit" of Qiagen (ref. 69506) following the "Purification of Genomic DNA from Gram-positive bacteria" protocol.

⇨ Water samples were collected in Sterivex 0.22 µm filter and preserved with 1.8 mL of SET Buffer at –22°C.

⇨ 180 µL of enzymatic lysis (20 mM Tris Cl, pH 8.0; 2 mM sodium EDTA; 1.2% Triton X-100; 20 $mg.mL^{-1}$ lysozyme) buffer were added to the Sterivex filters. Samples were incubated for 30 min at 37°C.

⇨ After this time, 25 µL of proteinase K and 200 µL of buffer AL were added to the Sterivex filters. Samples were incubated at 70°C for 30 min.

⇨ Each sample was separated in 400 µL sub-samples, which were transferred to eppendorfs tubes. 200 µL of cold 100% ethanol were added to each sub-sample and mixed thoroughly by vortex.

⇨ The mixture was pipeted into DNeasy Mini spin column placed in a 2 mL collection tube. The mixture was centrifuged by ≥ 6,000 x g (8,000 rpm) for 1 min. The flow-through and the collection tube were discarded.

⇨ The DNeasy Mini spin column was placed in a new 2 mL collection tube. 500 µL of buffer AW1 were added and the mixture centrifuged by ≥ 6,000 x g (8,000 rpm) for 1 min. The flow-through and the collection tube were discarded.

⇨ The DNeasy Mini spin column was placed in a new 2 mL collection tube. 500 µL of buffer AW2 were added. The mixture was centrifuged by ≥ 20,000 x g (14,000 rpm) for 3 min to dry the DNeasy membrane. This centrifugation step ensures that no residual ethanol is carried over during the following elution. The flow-through and the collection tube were discarded.

⇨ The DNeasy Mini spin column was placed in a clear 1.5 mL microcentrifuge tube. 50 µL of DNA-RNAse free water were pipeted directly onto the DNeasy Mini spin column, incubated at room temperature for 1 min and then centrifuged for 1 min at ≥ 6,000 x g

(8,000 rpm) for 1 min to elute. The elution was repeated with more 50 µL of DNA-RNAse free water.

⇨ The eluates (100 µL) were combined, the DNA quantified and dried.