Material and Methods for Ocean Sampling Day – “from sampling to sequencing”

Sampling and Transport:

Sample collection was carried out as described in Protocol A of the OSD Handbook version 2.0 (http://www.microb3.eu/sites/default/files/osd/OSD_Handbook_v2.0.pdf). Briefly, OSD participants collected seawater using a using a Niskin bottle or 10% acid washed bucket. Seawater was filtered through 5-6 Sterivex filter units with 0.22 µm pore size Hydrophilic PVDF Durapore membrane (SVGV010RS, MERCK MILLIPORE) using either a peristaltic, vacuum or hand pump. After successful filtration sterivex filter units were stored; immediately at -20°C for short term storage and at -80°C for long term storage. Subsequently samples were shipped on dry ice to Germany to the Max Planck Institute for Marine Microbiology in Bremen and Biological Institute of the Alfred Wegner Institute in Helgoland where DNA extraction took place. Samples from Australia (OSD31-33, OSD 188-191) were processed in Australia according to OSD standardized protocols including DNA extraction and 16S amplicon sequencing. An overview of the sampling sites, sampling depth and mandatory metadata can be found in Table 1.

DNA Extraction:

Two sterivex filters per sampling site were used for DNA extraction except for the following: One sterivex for OSD113 NE Iberian Atlantic – CascaisWatch and OSD143 Skidaway Institute of Oceanography. Three sterivex for OSD96 Sao Miguel Azores I, OSD148 Wadden Sea and OSD158 Sao Miguel Azores II. Four sterivex for OSD63 Venic Aqua Alta, OSD70 Venice Lido and OSD103 Porto da Cruz (Costa Norte). DNA was extracted using Power Water DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer’s instructions. The concentration and quality of eluted DNA was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, MA, USA) and Infinite 200 NanoQuant (Tecan, Männedorf, Switzerland). Extracted DNA was shipped on dry ice to LGC Genomics (LGC Genomics GmbH, Berlin, Germany) for further processing.

16S/18S Amplicon Sequencing

Sequencing process including amplification of 16S/18S rRNA gene was carried out by LGC Genomics (LGC Genomics GmbH, Berlin, Germany). Amplification of 16S rRNA gene was performed using the primer pair 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) (Caporaso JG 2012) which was designed as part of the Earth Microbiome Project (Gilbert JA 2014). The 18S rRNA gene was amplified using the forward primer TAReuk454FWD1 (5′-CCAGCASCYGCGGTAATTCC-3′) (Stoeck T, 2010) and TAReukREV3_modified (5′-ACTTTCGTTCTTGATYRATGA-3′). The sequence of the reverse primer is based on the published primer by Stoeck et al (2010), but an additional TGA triplet was added at the 3' end. The PCRs included about 5 ng of DNA extract, 15 pmol of each forward primer 515F NNNNNNNNNNGTGCCAGCMGCCGCGGTAA and reverse primer 806R

NNNNNNNNNNGGACTACHVGGGTWTCTAAT (Eu565F NNNNNNNNNNCCAGCASCYGCGGTAATTCC and Eu981R NNNNNNNNNNACTTTCGTTCTTGATYRATGA) in 20 uL volume of MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µl of BioStabII PCR Enhancer (Sigma). For each sample, the forward and reverse primers had the same 10-nt barcode sequence. PCRs were carried out for 30 cycles using the following parameters: 2 min 96°C predenaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s. DNA concentration of amplicons of interest was determined by Gelelectrophoresis. About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying different barcodes. If needed, PCRs showing low yields were further amplified for 5 cycles (see Table 2). The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification on MinElute columns (Qiagen). About 100 ng of each purified ampliconpool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative Gelelectrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

Preparation of shot gun libraries for metagenomic samples and sequencing:

One half of the supplied DNA material per sample was sheared to about 500bp fragments using a Covaris S220 sonicator. DNA was purified and concentrated by a clean-up using MinElute columns (Qiagen). DNA concentrations were measured and 100 ng (or less, if the sample contained insufficient amounts, successful libraries could be done from as little as 5ng) were used to prepare Illumina libraries. Libraries were done with the Ovation Rapid DR multiplex 1-96 system (NuGEN). Libraries were amplified using standard Illumina primers for 8 to 15 cycles with MyTaq (Bioline) except for the following exceptions: For OSD41 and OSD151 18 cycles were necessary to generate the library. For OSD173, OSD96 and OSD168 and additional step using REPLI-g Mini Kit (Quiagen) was necessary. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

OSD sequence data and environmental data:

Sequence and contextual data are publicly available at INSDC study ERP009703 and Pangaea http://doi.pangaea.de/10.1594/PANGAEA.843778

References

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Stoeck T, B. D., Nebel M, Christen R, Jones MD, Breiner HW, Richards TA. (2010). " Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water." Molecular ecology 19(Suppl. 1): 21-31.