Eco-AlpsWater

Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers

Priority 3: Liveable Alpine Space. SO3.2 - Enhance the protection, the conservation and the ecological connectivity of Alpine Space

Deliverable D.T1.3.1-10

Illumina library preparation protocol: eDNA metabarcoding analyses of bacterioplankton communities

Interreg Alpine Space - Eco-AlpsWater project – WP1

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ABSTRACT

The aim of this document is to provide a synthetic description of the Illumina library preparation protocol for eDNA metabarcoding analyses of bacterial communities (Deliverable D.T1.1.2). This protocol has been used at the Sequencing and Genotyping Platform at FEM for the analysis of the samples collected in 2019 within the framework of EAW project.

Outline description of library preparation protocol for 16S bacterioplankton eDNA analyses

Using the specific bacterial primer set 341F (5'-CCTACGGGNGGCWGCAG-3', Klindworth et al. 2013) and the 850Rmod (5'-GACTACNVGGGTWTCTAATCC-3', Klindworth et al. 2013; Apprill et al. 2015) with overhang Illumina adapters, total genomic DNA was subjected to PCR amplification by targeting a ~ 460-bp fragment of the 16S rRNA variable regions V3–V4. PCR amplification was carried out using 25-µL reactions with 1 µM of each primer. Specifically, 12.5 µL of 2× KAPA HiFi HotStart ReadyMix and 5 µL of forward and reverse primers were used in combination with 2.5 µL of template DNA (5 ng µL−1). PCR amplification (GeneAmp PCR System 9700, Thermo Fisher Scientific) including a melting step (95 °C, 5 min; 1 cycle), an annealing step (95 °C, 30 s; 55 °C, 30 s; and 72 °C, 30 s; 28 cycles), and an extension step (72 °C, 5 min; 1 cycle). The PCR products were checked on 1.5% agarose gel and cleaned from free primers and primer dimer using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA). In the next step, dual indices and Illumina sequencing adapters Nextera XT Index Primer v2 (Illumina) were attached by 7 cycles of PCR (16S Metagenomic Sequencing Library Preparation, Illumina). After purification by the Agencourt AMPure XP system (Beckman), the final libraries were analyzed on a 2200 TapeStation platform (Agilent Technologies, Santa Clara, CA, USA) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) by the Synergy 2 microplate reader (Biotek). The libraries were pooled in equimolar quantities by qPCR in a final amplicon library and analyzed on a 2200 TapeStation platform (Agilent Technologies, Santa Clara, CA, USA). Barcoded library was sequenced on an Illumina® MiSeq (PE300) platform (MiSeq Control Software 2.6.2.1 and Real-Time Analysis software 1.18.54).

BASIC REFERENCES

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