

## **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.T1.3.1-11**

#### **Illumina library preparation protocol: eDNA metabarcoding analyses of eukaryotic microplankton communities**

---

Interreg Alpine Space - Eco-AlpsWater project – WP1

Massimo Pindo<sup>1</sup>, Adriano Boscaini<sup>1</sup>, Nico Salmaso<sup>1</sup>, Giulia Riccioni<sup>1</sup>

<sup>1</sup> *Edmund Mach Foundation(FEM), San Michele all'Adige, Trento, Italy*

## **ABSTRACT**

The aim of this document is to provide a synthetic description of the Illumina library preparation protocol for eDNA metabarcoding analyses of eukaryotic microplankton 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 18S eukaryotic microplankton eDNA analyses

For each individual environmental sample, total genomic DNA was subjected to PCR amplification by targeting a 380-bp fragment of the 18S rRNA gene variable region V4 using the specific primer set TAREuk454FWD1 (5'-CCAGCASCYGC GGTAATTCC-3'; Stoeck et al., 2010) and TAREukREV3\_modified (5'-ACTTTCGTTCTTGATYRATGA-3'; Stoeck et al., 2010; Piredda et al., 2017) with overhang Illumina adapters. PCR amplification and library construction were performed as described in Salmaso et al. (2018). Finally, all barcoded libraries were pooled in equimolar concentrations by qPCR in a final library and checked on a Typestation 2200 platform (Agilent Technologies, Santa Clara, CA, United States). The final 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

*Piredda, R., Tomasino, M. P., D'Erchia, A. M., Manzari, C., Pesole, G., Montresor, M., et al. (2017). Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean Long Term Ecological Research site. FEMS Microbiol. Ecol. 93:fiw200. doi: 10.1093/femsec/fiw200*  
*Salmaso, N., Albanese, D., Capelli, C., Boscaini, A., Pindo, M., and Donati, C. (2018a). Diversity and cyclical seasonal transitions in the bacterial community in a large and deep Perialpine Lake. Microb. Ecol. 76, 125–143. doi: 10.1007/s00248-017-1120-x*  
*Salmaso, N., Boscaini, A., & Pindo, M. (2020). Unraveling the diversity of eukaryotic microplankton in a large and deep perialpine lake using a high throughput sequencing approach. Frontiers in Microbiology, 11, 789.*  
*Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D. M., Breiner, H. W., et al. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19, 21–31. doi: 10.1111/j.1365-294X.2009.04480.x*