

Resource Summary Report

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Diatom DNA library preparation for Illumina Miseq Sequencing using the rbcL marker gene

DOI:10.17504/protocols.io.bd94i98w

Type: Protocol

Proper Citation

Cecile Chardon, Valentin Vasselon, Marine Vautier, Sonia Lacroix, Agnès Bouchez, Frédéric Rimet, Isabelle Domaizon 2020. Diatom DNA library preparation for Illumina Miseq Sequencing using the rbcL marker gene . protocols.io
dx.doi.org/10.17504/protocols.io.bd94i98w

Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bd94i98w>

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Group: EcoALpsWater

Summary: This protocol is part of the DNA workflow applied in the Eco-ALpsWater Project, here in particular to characterize the diversity of diatom assemblage in biofilms or plankton samples. Different studies have already revealed the potential of diatom metabarcoding applications for freshwater quality assessment (Kermarrec et al. 2014; Vasselon et al. 2017ab; Visco et al. 2015). The choice of the marker gene and barcode region is key for obtaining relevant inventories of diversity and precise taxonomic assignment. For benthic diatoms, the rbcL gene has proved to be an appropriate taxonomic marker for biomonitoring (Kermarrec et al. 2013, 2014; Vasselon et al. 2017a,b) and a well-curated barcode reference library is already available to assign species names to rbcL sequences (R?Syst::diatom, Rimet et al. 2016). For the Eco-AlpsWater project, biofilms sampled in rivers and lakeshores are collected as described in the dedicated protocols ("Lake plankton sample collection ..." and "Biofilms sample collection ...") and DNA is extracted as described in the protocol "Biofilms DNA extraction" ; all these protocols are part of the Deliverable D.T1.1.2. We present here the following step in the DNA workflow (i.e. PCR amplification of selected barcodes, and wet lab methods to prepare DNA library for downstream MiSeq Sequencing). This protocol has been used in recent studies (e.g. Keck et al 2018 ; Vasselon et al 2018) where diatoms metabarcoding has been used for ecological assessment of rivers. Several primers were proposed in the literature to characterize Diatom communities through environmental DNA metabarcoding approaches, including the 18S, COI and rbcL barcodes. Following the recommendation

provided by Kermarrec et al. 2014, who compared the efficiency of those 3 barcodes to accurately characterize diatom communities from freshwater samples (lakes and rivers), the rbcL barcode will be used within the Eco-AlpsWater project as he provides a good taxonomical resolution.

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Version: 1

Publication Date: 2020

Proper Citation: Cecile Chardon, Valentin Vasselon, Marine Vautier, Sonia Lacroix, Agnès Bouchez, Frédéric Rimet, Isabelle Domaizon 2020. Diatom DNA library preparation for Illumina Miseq Sequencing using the rbcL marker gene . protocols.io
dx.doi.org/10.17504/protocols.io.bd94i98w

Record Creation Time: 20210329T220523+0000

Record Last Update: 20210329T220858+0000

Data and Source Information

Source: