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Extracellular DNA extraction

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Protocol Information

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Authors: Charline Giguet-Covex, Pierre Taberlet, Francesco Gentile Ficetola

Summary: Over the past decade, an increasing number of studies has used environmental DNA from lake sediments to trace past lake ecosystem and landscape changes, agricultural activities or human presence and more broadly the biodiversity. In the environment, DNA can be found as intracellular and extracellular DNA (iDNA and exDNA). The contribution of each of these pools varies according to the environments, but exDNA often represents a high proportion of the total DNA (e.g. Vuillemin et al. 2017 and reference therein). Focusing the analyses on these different pools will lead to different community composition and structure of communities (e.g. Vuillemin et al. 2017). For plants, we propose to focus on the exDNA fraction to avoid the extraction of DNA from plant macro-remains, which might lead to an overrepresentation of these taxa and limit the detection of the other, "rarer" taxa. The manipulation of ancient DNA is delicate, and the biases brought during the experiments can be multiple. Therefore, it is essential to work carefully, under strict laboratory conditions, with multiple controls and several replicates of samples or extraction or PCR (e.g. Fulton 2012). This protocol details a sampling and extraction method of exDNA from sediments. This method was firstly developed for soils by Taberlet et al. 2012. It uses a phosphate buffer to desorb the DNA fragments from particles such as clays and then, the binding, wash and elution buffers from the NucleoSpin® Soil kit (Macherey-Nagel). Here we present a modified protocol from Taberlet et al. 2012. One modification consists in the addition of a concentration step (by using the amicon ultra centrifugal filter system) after the mixing of sediments with the saturated phosphate buffer. This step allow to increase the DNA yield. The quantity of phosphate buffer is usually based on the quantity of wet sediments (e.g. for 15g of wet sediment/soil, we add 15 ml of phosphate buffer). However, because lake sediments can have very different water content (depending on the composition and compaction) and because the exDNA is adsorbed onto particles, we now propose to base the phosphate buffer quantity on the dry weight of sediments. Fulton T., 2012. Setting Up an Ancient DNA Laboratory. Book Chapter, Ancient DNA in Methods in Molecular Biology edited by

Shapiro B. and Hofreiter M., 1-11. Taberlet, P., Prud'Homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J. C., Melodelima, C., Pompanon, F. and Coissac, E, 2012. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology*, 21: 1816-1820. Vuillemin A., Horn F., Alawi M., Henny C., Wagner D., Crowe S. A., Kallmeyer J., 2017. Preservation and Significance of Extracellular DNA in Ferruginous Sediments from Lake Towuti, Indonesia. *Frontiers in Microbiology* 8, 1440.

Affiliations: [Laboratoire Environnements, DYnamiques et TERRitoires de la Montagne (EDYTEM), Université Savoie Mont Blanc, F-73376 Le Bourget du Lac, France], [Laboratoire d'Ecologie Alpine (LECA), Université Grenoble Alpes, F-38000 Grenoble, France], [Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, via Celoria 26, I- 20133 Milano, Italy; Laboratoire d'Ecologie Alpine (LECA), Université Grenoble Alpes, F-38000 Grenoble, France]

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