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We use this protocol and it's working

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The extraction of cross-kingdom biofilm DNA using Zymo Reasearch Quick-DNA Fecal/Soil Microbe Kits.

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ABSTRACT

The extraction of DNA from environmental samples, including soils, sediments, water, and biofilms, is a crucial stage in the analysis of environmental microbial communities and and wider environmental communities via environmental DNA (eDNA). The choice of DNA extraction methodologies significantly influences not only the yield and purity of the extracted DNA, but also the composition of the community as determined by DNA analysis techniques such as amplicon and metagenomic sequencing. Numerous studies have investigated the influence of different kit-based DNA extraction protocols on metabarcoding of various taxa, including bacteria, fungi, diatoms, microeukaryotes, and invertebrates (Dopheide et al. 2019; Giangacomo et al. 2021; Matsuoka et al. 2022; Shaffer et al. 2022; Vasselon et al. 2017; Pearman et al. 2020; Majaneva et al. 2018; Deiner et al. 2015; Djurhuus et al. 2017; Kawato et al. 2021). Here we present an amended version of the manufacturers (Zymo research) recommneded protocol for Quick-DNA Fecal/Soil Microbe Kits optimised for the extraction of cross-kingdom biofilm community DNA suitable for downstream applications such as metabarcoding.

GUIDELINES

This protocol is based upon the recommended manufacturers protocol for Zymo Quick-DNA Fecal/Soil Microbe Miniprep Kit (D6012) and Quick-DNA Fecal/Soil Microbe 96 Kit (D6011). When using the 96 Kit volumes should be adjusted as per manufacturers recomendations.

SAFETY WARNINGS

Please read material SDS sheet before starting protocol.

_d6011_quick-dna_fecalsoil_microbe_96_kit.pdf

BEFORE START INSTRUCTIONS

Add 500 µL beta-mercaptoethanol per 100ml Genomic Lysis Buffer.

Sample Preparation

15m

1 In field samples are taken by the Environment Agency, and 5 mL biofilm suspension preserved in equal amounts of preservation buffer: [M] 3.5 Molarity (M) ammonium sulphate, [M] 17 millimolar (mM) sodium citrate, [M] 13 millimolar (mM) EDTA. Samples are frozen in

Equipment

Centrifuge tube

15 ml Centrifuge tube	TYPE
Corning	BRAND
CLS430791-500EA	SKU
https://www.sigmaaldrich.com/GB/en/product/sigma/cls430791	LINK

prior to laboratory preparation.

2 Once back in the laboratory field preserved samples are first thawed and then vortexed to create a homogenous mixture.

3 Samples are pelleted at 3000 x g, 5°C for 00:15:00 minutes 15m

4 Ensure samples are fully pelleted, then remove 500 µL of buffer to separate microcentrifuge tube.

Equipment

2 ml Eppendorf Safe-Lock tube

microcentrifuge tube	TYPE
Eppendorf	BRAND
0030120094	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Laboratory-Consumables/Tubes/Eppendorf-Safe-Lock-Tubes-p-0030120094	LINK

Remaining buffer is gently aspirated to avoid disrupting pellet.

5 Pellet is resuspended in 500 µL of reserved preservation buffer, and stored in the same fully labelled microcentrifuge tube. Preserved sample is then frozen at -80 °C prior to analysis.

Sample Lysis

1h 11m

6 Thaw sample to room temperature, vortex to resuspend pellet and add 100 µL to ZR BashingBead Lysis tube. Add 900 µL DNA/RNA shield, and lyse for 00:40:00 on a horizontal vortex mixer (Vortex Genie). 40m

7 Briefly spin for 10000 x g, 18°C, 00:01:00 . Add 40 µL proteinase K, resuspend and mix through pipetting and incubate at 65 °C for 00:20:00 . 21m

8 Centrifuge ZR BashingBead tube at 10000 x g, 18°C, 00:05:00 to pellet lysate. 5m

9 Transfer up to 400 µL supernatant to a Zymo-Spin III-F Filter in a collection tube and centrifuge at 8000 x g, 18°C, 00:01:00 . 1m

10 Add 1200 µL of Genomic Lysis buffer in a fume hood to the filtrate in the collection tube from step 4. Mix well.

> **Note**
>
> Genomic Lysis buffer should have beta-mercaptoethanol added to a final dilution of 0.5% (v/v) ie 500 µL per 100 mL buffer. Beta-mercaptoethanol is a strong reducing agent added to clean tannins and other polyphenolics found in plant extracts. Please refer to material SDS and use appropraite PPE.

DNA purification

9m 30s

11 Transfer 800 µL of the mixture from step 10 to a Zymo-Spin IICR column in a collection tube and centrifuge at 10000 x g, 18°C, 00:01:00 . Discard flow through from the collection tube and repeat with remaining mixture. 1m

12 Add 200 µL DNA Pre-Wash buffer to the Zymo-Spin IICR Column in a **new** collection tube and centrifuge 10000 x g, 18°C, 00:01:00 . 1m

13 Add 500 µL g-DNA Wash Buffer to the Zymo-Spin IICR Column and centrifuge 10000 x g, 18°C, 00:01:00 . 1m

14 Transfer the Zymo-Spin IICR column to a clean 1.5 mL centrifuge tube and add 100 µL * DNA Elution buffer directly to the column matrix. Centrifuge 10000 x g, 18°C, 00:00:30 to elute the DNA. 30s

> Note
>
> * If samples exhibit a low biomass it's recommended that this volume be reduced to 50 µL in order to concentrate DNA recovered.

15 Place a Zymo-Spin III-HRC Filter in a clean collection tube and add 600 µL prep solution. Centrifuge 8000 x g, 18°C, 00:03:00 . Discard flow through and place in clean labelled 1.5 ml microcentrifuge tube. 3m

16 Transfer Eluted DNA from step 14 to a prepared Zymo-Spin III-HRC Filter and centrifuge at exactly 16000 x g, 18°C, 00:03:00 . 3m

17 The filtered DNA is now suitable for PCR and other downstream applications.