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Modified DNeasy PowerWater Kit® protocol for DNA extractions from drinking water samples

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Protocol status: Working
We use this protocol and it's working

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Abstract

DNA-extractions from drinking water samples are essential for a range of subsequent microbial community quantitation and characterization methods, i.e., quantitative polymerase chain reaction (qPCR) assays targeting specific genes and the characterization of compositional and functional profiles using high throughout sequencing technologies (e.g. amplicon sequencing and shotgun metagenomic sequencing). Despite advances in the specificity and sensitivity of molecular techniques, efficient recovery of DNA from drinking water samples, particularly those with low cell counts, remains challenging. Drinking water samples, in which microbial concentrations range between $10^3$and 10^5 cells.ml^{-1}, generally requires the collection of large volume of sample and subsequent processing by filtration to concentrate microbial cells. Here we document a modified version of the DNeasy PowerWater Kit® protocol that utilizes enzymatic, chemical, and mechanical lysis strategies to enhance recovery of DNA from drinking water samples. The DNA quantities recovered using this protocol are typically at least two to three-fold higher when compared to the routine DNeasy PowerWater Kit® protocol. In our hands, this protocol consistently provides sufficient DNA of high quality from as little as 1.5 liters of filtered drinking water with cell counts in the range of 10^3-10^4 cells.ml^{-1}, while maintaining the 16S rRNA qPCR counts at least 100-1000 times higher compared to DNA extracts from negative controls (i.e., blank unused filters, filters with autoclaved deionized water filtered, and reagent blanks) processed identically as the drinking water samples.

Materials

MATERIALS

- Lysing Matrix E **MP Biomedicals** **Catalog #**116914050-CF
- Chloroform/Isoamyl Alcohol (24:1) **Acros Organics** **Catalog #**327155000
- Tris-EDTA (10X) **G-Biosciences** **Catalog #**786-033
- Lysozyme Solution (50 mg/mL) **Thermo Fisher Scientific** **Catalog #**90082
- Proteinase K Solution (20 mg/mL) **Thermo Fisher Scientific** **Catalog #**AM2546
- DNeasy PowerWater Kit **Qiagen** **Catalog #**14900-50-NF
- Sterivex-GP Pressure Filter Unit **Merck Millipore (EMD Millipore)** **Catalog #**SVGP01050
- DNA LoBind Microcentrifuge Tubes **Eppendorf** **Catalog #**022431021
- Eppendorf™ 5424 Microcentrifuge **Eppendorf** **Catalog #**05400002
- Eppendorf™ ThermoMixer C Bundle **Eppendorf** **Catalog #**2231000574
- FastPrep-24™ Classic Instrument **MP Biomedicals** **Catalog #**116004500
- Fisherbrand™ Variable Speed Mini Vortex Mixer **Fisher Scientific** **Catalog #**14955163
- Fisherbrand™ Fine Point High Precision Forceps **Fisher Scientific** **Catalog #**22327379
- Fisherbrand™ High Precision Metal Scalpels **Fisher Scientific** **Catalog #**08-920B
- Petri Dish with Clear Lid **Fisher Scientific** **Catalog #**FB0875712
- QIAcube System **Qiagen** **Catalog #**9001882

Safety warnings

Chloroform/isoamyl alcohol treatment should be performed in a laminar fume hood certified for the use of volatile organics. Please refer to the SDS of chloroform/isoamyl alcohol before using it:

Chloroform:isoamyl alcohol SDS.PDF

Before start

- Aseptically transfer the ceramic spheres, silica spheres, and glass bead contained in the 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100) to a corresponding 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021). Exclusion of these components, essential for downstream mechanical treatment (i.e. bead beating), ensures that the processed polyethersulfone (PES) membrane extracted from filter unit is fully immersed in solution during enzymatic and chemical treatment (STEPS 2 and 3, respectively).

Important points to address before start, as listed in the DNeasy PowerWater Kit Handbook:

- Solution PW1 must be warmed at 55 °C for 00:05:00 – 00:10:00 min to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
- If Solution PW3 has precipitated, heat at 55 °C for 00:05:00 – 00:10:00 to dissolve precipitate.
- Shake to mix Solution PW4 before use.

PROCESSING OF THE STERIVEX-GP PRESSURE FILTER UNIT

1 On the surface of a sterile petri dish (Fisher Scientific, Cat. No: FB0875712), cut the PES filter membrane contained in the Sterivex-GP Pressure Filter Unit (EMD Millipore, Cat. No: SVGP01050) into smaller pieces using a sterile scalpel (Fisher Scientific, Cat. No: 08-920B), and subsequently transfer the cuttings into the 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100) using sterile tweezers (Fisher Scientific, Cat. No: 22327379). For details on how to extract the filter from the Sterivex-GP Pressure Filter Unit, please refer to the following video using this link: https://www.dropbox.com/s/m1ccznfsp02gy2n/IMG_5384.MOV?dl=0

Note

Before handling the Sterivex-GP Pressure Filter Unit (EMD Millipore, Cat. No: SVGP01050), aseptically transfer the ceramic spheres, silica spheres, and glass bead contained in the 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100) to a corresponding 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021). Exclusion of these components, essential for downstream mechanical treatment (i.e. bead beating), ensures that the processed polyethersulfone (PES) membrane extracted from filter unit is fully immersed in solution during enzymatic and chemical treatment (STEPS 2 and 3, respectively).

ENZYMATIC TREATMENT WITH LYSOZYME

2 Add 294 µL 10X Tris-EDTA (100 mM Tris, 10 mM EDTA, pH 8.0, G-Biosciences, Cat. No: 501035446) supplemented with 6 µl lysozyme solution (50 $mg.ml^{-1}$, Thermo Fisher Scientific, Cat. No: 90082) to the 2 ml Lysing Matrix E Tube. Vortex and then incubate for 60:00:00 min at 37 °C with light mixing at 300 rpm using the Eppendorf ThermoMixer C (Eppendorf, Cat. No: 5382000015) making sure that the filter membrane pieces are fully immersed in the solution.

Note

Final concentration of lysozyme in 300 µL solution: 1 $mg.ml^{-1}$

CHEMICAL TREATMENT AND THE ADDITION OF PROTEINASE K

3 Add 300 µL PW1 - provided in the DNeasy PowerWater kit and 30 µL Proteinase K (20 $mg.ml^{-1}$, Thermo Fisher Scientific, Cat. No: AM2546). Vortex and then

incubate for 30:00:00 min at 56 °C with light mixing at 300 rpm using the Eppendorf ThermoMixer C (Eppendorf, Cat. No: 5382000015) making sure that the filter membrane pieces are fully immersed in the solution.

Note

Final concentration of Proteinase K in 600 µL solution: 1 $mg.ml^{-1}$

CHLOROFORM/ISOAMYL ALCOHOL AND MECHANICAL TREATMENT (BEAD BEATING)

4 Aseptically transfer the ceramic spheres, silica spheres, and glass bead contained in the 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021) to corresponding 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100).

5 Add 630 µL chloroform/isoamyl alcohol 24:1 (Acros Organics, Cat. No: 327155000) and bead beat at setting 6 for 00:00:40 sec using the FastPrep -24™ Classic Instrument (MP Biomedicals, Cat. No: 116004500).

Safety information

Chloroform/isoamyl alcohol treatment should be performed in a laminar fume hood certified for the use of volatile organics. Please refer to the SDS of chloroform/isoamyl alcohol before using it: Chloroform:isoamyl alcohol SDS.PDF

6 Centrifuge for 00:10:00 min at 14000 x g at 4 °C and transfer the upper aqueous phase to a clean 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021).

Note

Expected supernatant recovery range between 600 µL and 630 µL

DNA EXTRACTION USING THE QIACUBE SYSTEM OR QIAGEN HANDBOOK

7 Use 600 µL of the recovered aqueous phase as sample for the QIACube System (QIAGEN, Cat. No: 9001882). If the sample is less than 600 µL add solution PW1 up to the final volume. Follow the instructions as indicated in the *DNeasy PowerWater Kit QiaCube Protocol Sheet*. If extractions are preformed manually, please continue with steps 11 to 23 as described in the *DNeasy PowerWater Kit Handbook.*

DNeasy PowerWater Kit QiaCube P...

DNeasy PowerWater Kit Handbook....

8 Store the DNA at -80 °C until further processing.