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Modified PowerMax Soil Kit Protocol for DNA Extraction from Sediment or Water Filters

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We use this protocol and it's working

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Abstract

This protocol is a modification to the Qiagen PowerMax Soil Kit for extracting DNA from sediment and water filters. It reduces bead-beating to obtain higher quality DNA and less fragmented DNA. Running the DNA out on an agarose gel with a high molecular weight DNA ladder or on a femtopulse is recommended to check DNA quality. This method was published in Wu et al. 2023 Distinct Depth-discrete Profiles of Microbial Communities and Geochemical Insights in the Subsurface Critical Zone AEM (<https://doi.org/10.1128/aem.00500-23>) and is similar to protocols found in Hug et al. 2015 ISMEJ (<https://doi.org/10.1038/ismej.2015.2>) and Kantor et al. 2013 mSphere (<https://doi.org/10.1128/mbio.00708-13>). This was originally developed using the kit by MoBio, but MoBio was bought out by Qiagen and you can purchase the kit from Qiagen now.



Materials

Reagents

- Sample (5 g of sediment or a water filter)
- Qiagen PowerSoil Max Kit
- 100% Ethanol 4°C or 100% Isopropanol
- 70% Ethanol, 3M Sodium Acetate pH 5.2 with glycogen
- Autoclaved tweezers (for water filters) or autoclaved scoopulas (for sediment)
- 3M NaAcetate pH 5.2 with glycogen (0.4 mg/ml)

Consumables

- 50mL centrifuge tubes
- 1.5mL centrifuge tubes, lobind recommended
- Pipet tips

Equipment

- Water bath
- -20C or -80C freezer
- 4C Refrigerator
- Vortex
- Centrifuge for 50mL centrifuge tubes that can spin at 10,000G
- Pipettors

Before start

NOTE1: Glycogen in the sodium acetate solution is only for aiding visual detection of the DNA pellet. If you are comfortable with ethanol precipitations, or think that you will extract >2 µg of DNA, then omitting the glycogen is fine as it can sometimes interfere with downstream applications.

NOTE2: It may be preferable to use NaCl instead of Na-Acetate to prevent the SDS from precipitating at the last step. However, we have not tested this protocol with NaCl. Make or buy a 5M NaCl solution if you would like to use NaCl instead. However, in general Na-acetate is more effective at precipitating DNA.



Tear Up Water Filters (optional)

- 1 Using autoclaved tweezers, tear up water filters in the biosafety cabinet. Usually, you can put the pieces back into the same tube, but you can transfer to another tube if it is too difficult. Be careful not to touch the inner part of the filter with the tweezers.



Modified Qiagen Powermax Protocol - Steps with Solution C1

2 Start with Qiagen kit

- 2.1 Pour garnet beads into 50 ml centrifuge tube with ~5 g sediment or torn water filter.
- 2.2 Add 15 ml of PowerBead solution to sediment tube.
- 2.3 Vortex on high for 1 minute. **Make sure that the filter pieces are getting covered by the liquid.** Shake to help get the pieces to the bottom.
- 2.4 Check Solution C1 (SDS solution). If Solution C1 is precipitated, heat the solution to 60°C until the precipitate has dissolved before use.
- 2.5 Add 1.2 ml of Solution C1 to a PowerMax Bead Solution Tube and vortex on high for 30 seconds. Make sure the filter pieces are getting covered by the liquid.

3 Freeze-thaw

- 3.1 Do a deep freeze (-20°C or -80°C) at least an hour or usually overnight.



4 Water bath

- 4.1 Thaw tubes at room temperature (takes about 45 minutes) followed by vortex for 3.5 minutes on high.
- 4.2 Incubate tubes for 30 min at 65°C water bath inverting 10x every 10 minutes.



- 4.3 Centrifuge the tubes at 2500 x g for 3 minutes at room temperature.
- 4.4 Transfer the supernatant to a clean Collection Tube (provided). The supernatant may still contain some soil particles and color. Make sure to get out all the liquid (even bubbles) otherwise DNA yield may suffer.

Continuation of the PowerMax Soil Protocol - Steps with Solutions C2-C4

- 5 NOTE: we extended the incubation steps to help remove humics from the samples. If you don't think that you have humics on your samples you can reduce the incubation steps to 10 minutes. During this time it is helpful to warm the elution solution (e.g. Solution C6 or 10mM Tris-Cl) to 55C to save time (this is optional, warmed elution solution will increase yield)
- 6 **Add 5 ml of Solution C2** to the supernatant and invert twice to mix.
 - 6.1 Incubate at 4C for at least 30 minutes.
 - 6.2 Centrifuge the tubes at 2500 x g for 4 minutes at room temperature.
 - 6.3 Avoiding the pellet, transfer the supernatant to a clean Collection Tube (provided in kit). This may require an extra spin because pellet does not stick to the tube very well.
- 7 **Add 4 ml of Solution C3** to the supernatant and invert twice to mix.
 - 7.1 Incubate at 4C for at least 30 minutes.
 - 7.2 Centrifuge the tubes at 2500 x g for 4 minutes at room temperature.
 - 7.3 Avoiding the pellet, transfer supernatant to a clean Collection Tube (provided in kit).
- 8 **Shake to mix Solution C4.** Add 30 ml of Solution C4 to the supernatant and invert twice.

Collect, Wash, and Elute DNA on Provided Spin Filters

9 Collect sample on provided spin filters. This step requires three centrifuge steps to get all of the sample through.

9.1 First, fill the Spin Filter with solution from previous step (~15mL). Centrifuge at 2500xg for 2 minutes at room temperature.

9.2 Discard the flow through and add a second volume of supernatant to the same Spin Filter and centrifuge at 2500 x g for 2 minutes at room temperature.

9.3 Discard the flow through. Repeat until the entire volume has been processed

10 Wash filter

10.1 Add 10 ml of Solution C5 to the Spin Filter.

10.2 Centrifuge at 2500 x g for 3 minutes. Discard the flow through.

10.3 Centrifuge the Spin Filter at 2500 x g for 5 minutes at room temperature.

11 Transfer to Elution Tube

11.1 Carefully place the Spin Filter in a clean 50 mL tube (you will collect DNA in this tube). Avoid splashing Solution C5 onto the Spin Filter.

12 Elute DNA from filter

12.1 Add 5 ml of warm Solution C6 (55°C) to the center of the Spin Filter membrane.

12.2 Let stand for a minute. Centrifuge at 2500 x g for 3 minutes at room temperature.

- 12.3 (Optional) Repeat elution step with another 5 mL of Solution C6 to improve yield.

Concentrate DNA by Ethanol Precipitation

- 13 **Ethanol Precipitation.** The final volume of eluted DNA will be 5-10 ml from the spin filter and that generally is not a usable DNA concentration or volume.

Note1: See overall general note on the use of glycogen as a visual aid in the NaCl or Na-Acetate solutions.

Note2: Generally isopropanol precipitations are better for HMW DNA, but ethanol precipitations are better to get all the DNA in a sample. NaCl is used because it will keep the SDS soluble. Centrifuging at 10,000G instead of 15,000G will make the pellet easier to resuspend, but you will get less DNA.

- 14 **If you think you have total DNA >1 µg** proceed with this step. This means that if you measure the sample on a Qubit, the concentration should be >0.1ng/uL if you eluted in 10mL. Glycogen is omitted since this is a large enough amount of DNA to see the pellet.
- 14.1 Add either 1 mL of 3M NaAcetate pH5.2 or 0.42mL 5M NaCl (final conc 0.2M NaCl), mix by inversion
- 14.2 Add 0.6-0.7 volumes of isopropanol (7.3mL if you used the above NaCl amount). Isopropanol is generally considered better at precipitating longer pieces of DNA, but you can use 2-2.5x volume of ethanol to sample instead. Incubate 10 minutes at RT.
- 14.3 Centrifuge at 10,000G for 15-30 minutes
- 14.4 Resuspend pellet overnight in 500uL of Solution C6, sterile/DNase free water, Tris or TE.
- 14.5 Measure concentration on a Qubit fluorometer. Check DNA 260/230 and 260/280 values on a Nanodrop.
- 15 **If you have a low biomass sample (<1 µg)**
- 15.1 Add 1 ml of 3M NaAcetate pH5.2 with glycogen (.4 mg/ml) and invert 10 times to mix. Glycogen can be omitted if you don't need the visual aid. Leaving the glycogen out will



help with purity for other downstream applications, but should be okay for Illumina sequencing.

Untested: if using 5M NaCl solution, add 0.42mL (final conc 0.2M)

- 15.2 Add 27 ml of 100% 4C ethanol and invert 3-5 times to mix. Incubate overnight at 4C or -20C.
- 15.3 Let sample warm to room temperature (~30-60 mins).
- 15.4 Centrifuge at 15K x g for 30 minutes at room temperature. Use label to position the tube so you know where the pellet will end up.
- 15.5 Decant all liquid by carefully pouring off the supernatant and watching the pellet.
- 15.6 Wash the DNA pellet with 10mL 70% ethanol (room temperature). Do not let the pellet sit for a long time in the ethanol solution or it may redissolve.
- 15.7 Try to position the tube the same way as the original spin in the centrifuge. Spin at room temperature for 10 minutes.
- 15.8 Remove tubes from the centrifuge immediately after centrifuge stops. Pellet tends to get dislodged.
- 15.9 Pour off supernatant. Remove residual ethanol with the pipet rig. Air dry in biosafety cabinet for 10 minutes. DO NOT overdry the pellet or it will be difficult to resuspend and also damage the DNA.
- 15.10 Add 500ul of Solution C6, sterile/DNase free water, TE, or 10mM Tris-Cl directly to the pellet. Incubate by rotating and leaning tube so the solution is over the pellet. It is recommended to incubate overnight at room temperature. Often the amount of DNA increases two-fold overnight.
- 15.11 Quantify on a Qubit and check DNA 260/230 and 260/280 values on a Nanodrop. Some sample may contain a precipitate.
- 15.12 If samples contain precipitate, let the precipitate sink to the bottom and then pipet off the top 90% and transfer to a 1.5 mL tube.
- 16 Some samples may need to be concentrated further with another ethanol precipitation. For 500ul of sample, add 50 ul of 3M NaAcetate, mix, and then 1mL of 100% ethanol for



precipitation.

Protocol references

This method was published in Wu et al. 2023 Distinct Depth-discrete Profiles of Microbial Communities and Geochemical Insights in the Subsurface Critical Zone AEM (<https://doi.org/10.1128/aem.00500-23>) and is similar to protocols found in Hug et al. 2015 ISMEJ (<https://doi.org/10.1038/ismej.2015.2>) and Kantor et al. 2013 mSphere (<https://doi.org/10.1128/mbio.00708-13>).

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