



DNA extraction using MoBio PowerSoil DNA Isolation Kit for filters

Forked from 16S Amplicon Library Generation for Reef Corals

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ABSTRACT

This protocol describes how to extract DNA from 47-mm 0.2/0.1-µm filters.

EXTERNAL LINK

<https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf>

GUIDELINES

This protocol describes how we use the PowerSoil DNA Isolation Kit.

All steps should be performed in the pre-PCR lab using designated pipettes with filter tips.

Why we use soil kit for water samples? Soil kits have the technology to remove humic substances (that are in many water samples that we work with) that are very inhibitory for downstream processes such as PCR.

MATERIALS

NAME	CATALOG #	VENDOR
PowerSoil DNA Isolation Kit	12888-100	Mobio
Pall membrane filters	Z269212	Sigma Aldrich

BEFORE STARTING

Clean all surfaces before beginning to ensure removal of contaminants. To do this wipe sequentially with a combination of (1) MQ, (2) 70% ethanol and (3) DNase Away.

Cleaning of pipettes is also highly recommended.

You can also apply 10 minutes UV exposure in the UV-box for pipettes, tips, racks, etc.

- For each sample label (1) BeadBeating tube, (4) 2 mL collection tubes and (1) SpinFilter.

Get your samples from the freezer, keeping them on ice until extraction begins is recommended.

Extract up to 24 samples at once, randomize the samples extracted across the experiment design. Finally, extract sample blanks for each extraction batch to test for contamination.

- 2 **Add one wrapped filter** to each PowerBead Tubes with open end facing the bottom **and invert several times or vortex gently** the tubes to make sure that the buffer in the tube soaks the filters.

Alternatively, you can cut the filter into smaller pieces but usefulness of this technique has never been proved.

Cutting can be also used to save part of the filter. In this case work as fast as you can with clean scissors and tweezers above a sterile Petri dish. Keep in mind that once thawed the DNA is exposed to DNAses. Avoid freezing and thawing too many times.

What's happening: The PowerBead Tube contains a buffer that will begin to dissolve humic acids and protect nucleic acids from degradation.

- 3 Make sure C1 solution is dissolved (heat solution if not dissolved on heatblock or waterbath at 60°C)
Add 60 µl of Solution C1.

What's happening: Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

 60 µl

- 4 Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24).

Vortex at maximum speed for 10 minutes.

Note: If you are using the 24 place Vortex Adapter for more than 12 preps, MoBio recommends increase the vortex time by 5-10 minutes but take into count that this increases the shearing of your DNA, which can be a problem for some downstream procedures requiring long DNA fragments. For amplicon sequencing or metagenomics it's OK.

What's happening: Cells are lysed by a combination of chemical agents and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

 00:10:00

- 5 **Centrifuge tubes at room temperature at 10,000 x g for 30 seconds .**

Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Be sure not to exceed 10,000 x g or tubes may break.

 00:00:30

- 6 Transfer supernatant to the provided clean pre-labeled 2 mL collection tube (expect between 400 µL and 500 µL of supernatant).

The supernatant may be dark in appearance and still contain some particles. The presence of a dark color in the mixture is expected in many soil types at this step. Subsequent steps in the protocol will remove both carry over soil and coloration of the mixture.

 500 µl

- 7 **Add 250 µL of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.**

What's happening: Solution C2 is an inhibitor removal solution. It contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

 250 µl

 00:05:00

8 **Centrifuge tubes at room temperature for 1 minute at 10,000 g.**

 00:01:00

9 Avoiding the pellet, **transfer up to 600 µL of supernatant** to a clean, pre-labeled 2 ml collection tube (provided).

What's happening: The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

 600 µl

10 **Add 200 µL of solution C3 and vortex briefly. Incubate at 4°C for 5 minutes**

What's happening: Solution C3 is inhibitor removal solution and is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

 200 µl

 00:05:00

11 **Centrifuge the tubes at room temperature for 1 minute at 10,000 g.**

12 **Transfer up to 750 µL of supernatant** to a clean, pre-labeled 2 ml collection tube (provided).

What's happening: The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

 750 µl

13 Before use shake to mix the Solution C4.

Add 1.2 mL of solution C4 to supernatant and vortex for 5 seconds.

What's happening: Solution C4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

14 Load approximately 675 µL of the supernatant and solution onto a spin filter and centrifuge at 10,000 g for 1 minute at room temperature.

Discard flow through and add an additional 675 µL of supernatant to the spin filter, centrifuge at 10,000 g for 1 minute.

Load remaining supernatant on to the spin filter and centrifuge at 10,000 g for 1 minute, discard the flow through.

What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

 675 µl

 00:01:00

- 15 **Add 500 µL of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 g.** Discard the flow through into waste container.

What's happening: Solution C5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

 500 µl

 00:00:30

- 16 **Centrifuge again the filter and 2 ml collection tube for 1 minute at 10,000 g.**

What's happening: This second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

 00:01:00

- 17 **Carefully place spin filter into a new pre-labeled clean 2 ml collection tube.**

Do not splash Solution C5 onto the filter!!! It is important to avoid any traces of the ethanol based wash solution.

- 18 **Add 100 µl of Solution C6 to the center of the filter and centrifuge at room temperature for 30 seconds at 10,000 g.**

Placing the Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution C6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris) which lacks salt.

Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution. Solution C6 contains no EDTA. If DNA degradation is a concern, Sterile TE may also be used instead of Solution C6 for elution of DNA from the Spin Filter.

 100 µl

 00:00:30

- 19 **Discard the spin filter and store DNA at -20°C until further processing or at -80°C for long-term storage.**