Protocol for LEO filter sample DNA extractions using PowerWater kit Version 2

MoBio PowerWater instructions amended with specific instructions

Abstract

Procedure for DNA extractions using PowerWater DNA extraction kit from filtered LEO seepage water samples and prelimnary QC by nanodrop

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Before start

A) Identify samples to extract

Determine number of samples to extract (n)

Open Google Drive spreadsheet: LEO seepage microbial communities - DNA extraction log

Itentify next n samples to be extracted - following extraction order

Make table or print from google drive with the following information

- Extraction number
- Sample number
- Sample ID
- Full sample description (sample date/time, slope, replicate)

B) Gather and prepare supplies (inside of DNA extraction cabinet)

Pipetters (P1000 and P200)

Pipette tips (filter tips)

Sharpie

Microtube rack

n x 1 Bead tubes

n x 5 Collection tubes

n x 1 Spin filter tubes

Label tubes with Sample Number on top of the lid (NOT the same as extraction number)

Label FINAL collection tube on outside (1 for each sample), label with Sample Number (41), Sample ID (Dec4-2030-W-2), your initials, and the date (you can do this with the label maker, 2 rows, 9 pt font)

Label bead tube with sample ID if not extracting right away.

Place all items in the DNA extraction cabinet, and sterilize by turning on uv lamp for 15 min (twist dial completely, door must be closed)

C) Gather weighing supplies (room 202K)

Rubber mallet

Ethanol

Kimwipes

Spatulas

Spare sample baggie

D) Prepare equipment (outside of DNA extraction cabinet)
Timer
(+4°C) Tube rack in fridge
(+55°C) Heating block

Microcentrifuge

Centrifuge

Vortex next to hood

Vortex and adapter for bead tubes

Solutions PW1-PW6 from PowerWater DNA extraction kit

n x 1 50 mL conical tube (to pack bead tube in for centrifugation, do not have to be sterile or labeled)

**Note: Three solutions are mislabeled by the manufacturer (write relevant PW# on top of lid)*

PW2 = IRS (inhibitor removal solution)

PW5 = Ethanol

PW6 = EB (elution buffer)

Notes:

Always minimize the amount of time a freezer is open by removing the box of interest, closing the freezer, adding/removing sample, and then reopening the freezer

Samples must always be balanced in centrifuge rotor!

Avoid passing your apendages over any open container by adjusting the position of your work items and your body. Store caps upside down on counters when remove - like this: U

Protocol

Weigh out samples into Bead Tube

Step 1.

* *If continuing on to step 2, start warming PW1 solution on heating block now*

Procedure to follow for one sample at a time:

Sterilize workspace with ethanol.

Prepare an ice bucket to store filters when not in use.

Label bead tube with full sample ID if not extracting sample right away.

Retrieve sample from LEO seepage sample box(es) in -80°C freezer (organized by Sample Number, not extraction order).

Crush the frozen filter into evenly sized pieces with the rubber mallet (still inside the closed baggie)

Tare the balance with empty baggie (of same size)

Weigh filter sample + baggie and record the mass of the filter.

Calculate 1/3 of the sample mass as target mass.

Weigh out target mass into bead tube:

- Double check that the Sample Number on the tube and baggie matches!
- Tare Bead Tube on balance
- Sterilize a clean spatula with ethanol and kimwipe
- Carefully transfer desired sample amount (target mass) into Bead Tube
- Record mass
- Mark baggie with date and initials to indicate extracted
- Put baggie with remaining sample back in appropriate freezer box

Clean balance and weighing areaafter use

*Note: some samples have already been subsampled as indicated by 'notes' column

Add 1 ml of Solution PW1 to the PowerWater® Bead Tube

Step 2.

Warm Solution PW1 prior to use at 55°C for 5-10 minutes. Use Solution PW1 while still warm.

What's happening:

Solution PW1 must be warmed to dissolve precipitates prior to use. Solution PW1 should be used while still warm. For samples containing organisms that are difficult to lyse (fungi, algae) an additional heating step can be include.

Solution PW1 is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic material. It is also part of the patented Inhibitor Removal Technology® (IRT). When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution PW1 should be used while it is still warm.

Vortex at maximum speed for 5 minutes using MO BIO adapter

Step 3.

Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter

Place Bead Tubes in adapter with caps towards center (4 places (13000-V1-15) or 6 places (13000-V1-5))

Turn vortexer to on, (ramping up to) maximum speed for 5 min

What's happening: The mechanical action of bead beating will break apart the surface of the filter membrane that contains trapped cells and aids in cell lysis. Use of the vortex adapter will maximize homogenization by holding the tubes equal distance and angle from the center of rotation. Avoid using tape, which can become loose and result in reduced homogenization efficiency.

Centrifuge the tubes ≤ 4000 x g for 1 minute at room temperature.
Step 4.

Place 5 mL Bead Tubes in 50 mL Conical tubes (secured with kimwipe) for centrifugation.

Thermo ST40R Centrifuge

Definition: RCF = x g

Transfer the supernatant to a clean 2 ml Collection Tube
Step 5.

Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.

Note: Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.

What's happening: The supernatant is separated and removed from the filter membrane and beads at this step.

Centrifuge at 13,000 x g for 1 minute.
Step 6.

This step, and all remaining centrifugation steps are in microcentrifuge

What's happening: Any remaining beads, proteins, and cell debris are removed at this step. This step

is
important for removal of any remaining contaminating non-DNA organic and inorganic matter that may
reduce the DNA purity and inhibit downstream DNA applications.

Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
Step 7.

Add 200 µl of Solution PW2 and vortex briefly to mix. Incubate at 4°C for 5 minutes.
Step 8.

Incubate tubes in fridge

What's happening: Solution PW2 is another part of the patented Inhibitor Removal Technology ® (IRT) and is a second reagent to remove 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

Centrifuge the tubes at 13,000 x g for 1 minute.
Step 9.

Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
Step 10.

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

Add 650 µl of Solution PW3 and vortex briefly to mix.
Step 11.

Note: Check Solution PW3 for precipitation prior to use. Warm at 55°C for 5-10 minutes if necessary on the heating block. Solution PW3 can be used

while still warm.

What's happening: Solution PW3 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations this will adjust the DNA solution salt concentration to allow binding of the DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the spin filter.

Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x g for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
Step 12.

Note: A total of two loads for each sample processed are required.

What's happening: The DNA is selectively bound to the silica membrane in the Spin Filter basket and the
flow through containing non-DNA components is discarded.

Place the Spin Filter basket into a clean 2 ml Collection Tube
Step 13.

What's happening: Due to the high concentration of salt in solution PW3, it is important to place the Spin Filter basket into a clean 2 ml Collection Tube to aid in the subsequent wash steps and improve the DNA

purity and yield.

Shake to mix Solution PW4 before use. Add 650 µl of Solution PW4 and centrifuge at 13,000 x g for 1 minute.
Step 14.

What's happening: Solution PW4 is an alcohol 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 and other contaminants while allowing the DNA to stay bound to the silica membrane.

Discard the flow through and add 650 µl of Solution PW5 and centrifuge at 13,000 x g for 1 minute.
Step 15.

What's happening: Solution PW5 ensures complete removal of Solution PW4 which will result in higher DNA purity and yield.

Discard the flow through and centrifuge again at 13,000 x g for 2 minutes to remove residual wash.
Step 16.

What's happening: The second spin removes residual Solution PW5. It is critical to remove all traces of wash solution because the ethanol in Solution PW5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

Place the Spin Filter basket into a clean 2 ml Collection Tube
Step 17.

Note: This will be the final collection and storage tube. Make sure this final collection tube is the one you labeled with full sample details in "Before you Start" Step **B)**

Add 70 µl of Solution PW6 to the center of the white filter membrane.
Step 18.

Note: PowerWater protocol calls for 100 uL, but we will try to concentrate the DNA by only adding 70 uL (increased from 50 uL on 6/12 to have more volume for QC)

What's happening: Placing Solution PW6 (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 PW6 passes through the silica membrane, the
DNA that was bound in the presence of high salt is selectively released by Solution PW6 (10 mM Tris) which lacks salt.

Centrifuge at 13,000 x g for 1 minute.

Step 19.

Discard the Spin Filter basket. The DNA extract is now complete and is in your collection tube.

Step 20.

Double check that this tube has full sample info on label

Check DNA concentration and purity on the NanoDrop

Step 21.

Gather NanoDrop kit:

- DNA extract tubes in tube rack
- Micropipette (P10 or P3) and tips
- kimwipes
- Microtube of Water for cleaning
- Microtube of PW6 / Elution buffer solution for blanking

Nanodrop-1000 measurement procedure:

Open software
<Click Nucleic Acid>

- Lightly wipe the machine using a Kimwipe, add 2***µl*** of water to the pedestal x1, and click 'Ok'
- Add 2***µl*** PW6/EB to blank x1 'Blank'
- Add 2***µl*** of the Sample, enter the Sample ID, click 'Measure', and in your notebook record:
- 260/280
- 260/230
- ng/µL
- Run each sample 3x, calculate average values, and add to DNA extraction log table
- In between the sets of 3 sample measurements, add 2***µl*** of PW6/EB, enter 'EB' in sample name, click 'Measure', and record the ng/uL in your notebook (this provides an internal check that 'zero' is really 'zero')

Saving report; Show reports > Save Report > 'Use Date_LEO_seepage_DNA_extraction_x-y_initials' as the Name (e.g., 17061_LEO_seepage_DNA_extraction_1-2_JG)

Clean Up: Water run x1 > Wipe > Leave a Kimwipe folded in the machine

Note: previous version of the protocol used pcr water to run the internal zero check, however this was a problem when the solution used for blanking was EB. This version corrects the error and uses EB only.

Storage

Step 22.

As soon as NanoDrop readings are completed, store DNA samples in appropriate freezer box and position (see spreadsheet) in -80°C

Upload data

Step 23.

As soon as possible, upload data from your extraction into this google drive spreadsheet: LEO seepage microbial communities - DNA extraction log

Note: This will ensure that the appropriate samples are used for subsequent extractions

Add any special notes regarding the procedure that day (e.g., water bath accidentaly set to 60°C instead of 55°C) that will help us troubleshoot if needed.

Review your lab notebook to make sure it is complete with date, sample information, masses, nanodrop data, and any particular notes from the day. Inform Dr. Meredith if any supplies or low or if there was a problem that will affect subsequent extractions.

Warnings

WARNING: Solutions PW3, PW4 and PW5 contain alcohol. They are flammable.