Standard Operating Procedure for:

Purification of Filtered Samples Using the MO BIO PowerLyzer® PowerSoil® DNA Isolation Kit



DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF LABORATORIES
BIOLOGY SECTION
TALLAHASSEE FLORIDA

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

1. SCOPE AND APPLICATION

1.1. This method is based on the MOBIO PowerLyzer® PowerSoil® DNA isolation kit user manual and describes a procedure for recovering genomic DNA (gDNA) from samples that contain PCR inhibitors. This method is used to re-analyze samples when inhibition is detected after performing the crude lysate method (as described in SOP PCR-4.1).

2. SUMMARY OF METHOD

2.1. Environmental samples (water filters) are added to bead tubes and homogenized on a bead beater for rapid and thorough homogenization. Cell lysis occurs by mechanical shearing and chemical interactions. Total gDNA is bound on a silica membrane in a spin column. DNA is washed and eluted from the spin column to produce purified DNA.

2.2. Interferences

2.2.1. Purification may result in a low yield of DNA, although DNA is concentrated and PCR inhibitors are removed. Ethanol in Solution C5 can interfere with qPCR. It is important to ensure that the Spin Filter is thoroughly centrifuged to remove any traces of Solution C5 and that the Spin Filter is removed carefully to avoid splashing any Solution C5 onto the Spin Filter.

2.3. Definitions

2.3.1. qPCR: Quantitative Polymerase Chain Reaction is a procedure in which the amount of DNA amplified in each PCR cycle is measured using fluorescent dyes in order to determine the original amount of DNA in the sample.

2.3.2. Target sequence: The DNA sequence in the Gene of Interest (GOI) that is amplified by the primers/probe in each PCR cycle. The primers/probe series is developed to attach specifically to this sequence.

3. EQUIPMENT AND SUPPLIES

3.1. Gloves, nitriles

3.2. Tube racks

3.3. Forceps

3.4. Micropipettes with sterile PCR grade filter tips

3.5. CBS Optimizer PCR Workstation #2 (S/N 007632)

3.6. Fisher Mini Vortexer

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.7. Centrifuge

3.8. Mini Bead Beater-16 Model 607 (Property #145248)

3.9. MO BIO PowerLyzer® PowerSoil® DNA Isolation Kit

3.9.1. PowerLyzer® Glass Bead Tubes (0.1 mm)

3.9.2. PowerSoil® Spin Filters

3.9.3. PowerSoil® 2 mL Collection Tubes

Note: Kit reagents and components should be stored at room temperature (15-30°C).

3.10. PCR#5, Thermo Fridge/Freezer (S/N 0121174701121218), maintained at 4ºC ± 2ºC and -20ºC ± 10ºC, Room B246

3.11. PowerVac® Manifold Cat#11991 (optional)

4. REAGENTS AND STANDARDS

4.1. MO BIO PowerLyzer® PowerSoil® DNA Isolation Kit

4.1.1. PowerSoil® Bead Solution

4.1.2. PowerSoil® Solution C1

4.1.3. PowerSoil® Solution C2

4.1.4. PowerSoil® Solution C3

4.1.5. PowerSoil®Solution C4

4.1.6. PowerSoil®Solution C5

4.1.7. PowerSoil®Solution C6

4.2. 1E4 SKETA Standard (Salmon DNA) – Prepare per SOP PCR-3.1.

4.3. Sterile Deionized (DI) Water

4.4. Bleach solution, 10% (v/v)

4.5. 80% Ethanol – Prepare per SOP IZ-09.

4.6. 100% Ethanol (for PowerVac® Manifold protocol only)

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

5. CALIBRATION AND STANDARDIZATION

5.1. See FDEP Bureau of Labs Quality Manual, Section 5.5.

5.2. Clean work area before and after use. Wipe down all surfaces with 10% bleach and wait 15 minutes before rinsing with sterile water.

6. SAMPLE PURIFICATION

6.1. Place forceps in PCR Workstation #2 and turn on UV lights. Sterilize forceps for 2 minutes.

6.2. Use sterile forceps to transfer the sample filter to a Bead Tube labeled for each sample.

Note: Keep forceps in a small beaker of ethanol during purification process. Dip in the ethanol and shake off excess before grasping each filter. If forceps are accidentally dropped or placed on counter, re-sterilize in PCR Workstation #2. Make sure no ethanol residue from the forceps touches the filter.

6.3. With a 1000 µL micropipette, dispense 750 µL of Bead Solution to the Glass Bead Tube. Vortex briefly on speed 7 to mix.

6.4. If Solution C1 is precipitated, heat to 60°C until dissolved before use. It may be used while warm.

6.5. With a 100 µL micropipette, dispense 60 µL of Solution C1 into each Power Bead Tube. Vortex briefly on speed 7 or invert several times.

6.6. Place the tubes in the bead beater and shake for 45s at a max rate of 5000 rpm. Make sure the tubes are sealed tightly and are balanced in the bead beater.

6.7. Centrifuge at 10000 x g for 30 sec to pellet the debris.

6.8. With a 1000 µL micropipette, carefully transfer the supernatant to a new 2 mL collection tube for each sample. The supernatant should have a volume of 400-500 µL.

6.9. With a 1000 µL micropipette, add 250 µL of Solution C2 into each tube and vortex at speed 7 for 5 seconds. Incubate at 4°C for 5 minutes.

6.10. Centrifuge at 10000 x g for 1 min to pellet the debris.

6.11. Carefully, without disturbing the pellet, transfer up to 600 µL of the supernatant to a new 2 mL collection tube for each sample.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.12. With a 200 µL micropipette, add 200 µL of Solution C3 into each tube and vortex at speed 7 for 5 seconds. Incubate at 4°C for 5 minutes.

6.13. Centrifuge at 10000 x g for 1 min to pellet the debris.

6.14. Carefully, without disturbing the pellet, transfer up to 750 µL of the supernatant to a new 2 mL collection tube for each sample.

6.15. Shake Solution C4 to mix before use. With a 1-10mL micropipette, add 1200 µL of Solution C4 into each tube. Ensure that the solution does not surpass the rim of the tube as the tube will be nearly full. Vortex for 5 seconds.

6.16. With a 1000 µL micropipette, transfer 675 µL of the solution to a Spin Filter for each sample and centrifuge at 10000 x g for 1 min. Discard the flow through. Repeat two times until all of the solution is centrifuged through the Spin Filter.

6.16.1. Optional: Instead of using the centrifuge method, a vacuum manifold can be used for the remaining steps. It can be helpful when processing many samples. Attach one PowerVac™ Mini Spin Filter Adapter into the manifold for each sample to be processed. Gently press a spin filter column into the adapter until snugly in place. Close all unused ports of the manifold.

6.16.2. Transfer 650 µL of sample lysate (from step 6.15) to the spin filter column. Turn on the vacuum source and open the stockcock of the port. Hold the tube in place when opening the stopcock to keep the spin filter steady. After the lysate has passed through completely, load again with the next 650 µL. Continue until all of the lysate has been loaded, then close the ports.

6.16.3. Load 800 µL of 100% ethanol into the spin filter. Open the stopcock while holding the column steady. Allow to pass through then close the stopcock.

6.16.4. Add 500 µL of Solution C5 to each filter. Apply vacuum until solution has passed through, then continue to pull vacuum for an additional minute to dry the membrane. Close each port. Turn off the vacuum and vent the manifold by opening an unused port. Make sure all vacuum pressure is released before continuing.

6.16.5. Remove column to a new 2 mL collection tube and add 100 µL Solution C6. Centrifuge for 30 seconds at 10,000 x g. Discard the column and store DNA at -20º to -80ºC. Skip Steps 6.17-6.21.

6.17. With a 1000 µL micropipette, add 500 µL of Solution C5 into each tube and centrifuge at 10000 x g for 30 sec. Discard the flow through.

6.18. Centrifuge at 10000 x g for 1 min.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.19. Carefully transfer the Spin Filter to a new 2 mL collection tube. Avoid splashing any of Solution C5 onto the Spin Filter.

Note: Solution C5 is an ethanol based solution. Any trace of this solution can interfere with qPCR.

6.20. With a 100 µL micropipette, add 100 µL of Solution C6 onto the center of each white filter membrane.

6.21. Centrifuge at 10000 x g for 30 sec and discard the Spin Filter. Store purified DNA in the freezer. Use -20°for short term storage. Use -80° C for long term storage of greater than 5 days.

7. QUALITY CONTROL

7.1. Negative Controls

7.1.1. Analyze a Method Blank (MB) control for each set of samples filtered (maximum of 13 per batch). Filter 50 mL of sterile PBS after each sample batch and analyze with samples.

7.1.2. Analyze an Extraction Blank (EB) control for each set of samples extracted (maximum of 13 per batch). Place a sterile filter into a Bead Tube and extract along with samples in the batch.

7.2. Extraction Control (EC)

7.2.1. Analyze an EC for each set of samples extracted (maximum of 13 per batch). Place a sterile filter into a Bead Tube and spike with 5 µl of 10^5 *B. dorei* standard DNA. Extract along with samples in the batch.

7.3. Sample Processing Control (SPC)

7.3.1. Add 2 µL of 1E4 SKETA standard to each Bead Tube after adding the sample/control filter in Step 6.2.

7.4. Refer to SOP PCR-5.1 for further details on uploading quality control data into LIMS, including calculations and control limits.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Wear gloves and avoid all skin contact with kit reagents.

8.2. All media/bacteria/plates must be autoclaved for 45 minutes before disposal.

8.3. Refer to the Health and Safety Plan. Read MSDS information for reagent handling and disposal.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9. REFERENCES

9.1. MO BIO PowerLyzer® PowerSoil® DNA Isolation Kit Instruction Manual

9.2. EPA Method B (June 2010)

9.3. FDEP Bureau of Labs Quality Manual
http://www.dep.state.fl.us/labs/library/progplan.htm

9.4. Health and Safety Plan http://depnet/burlabs/safety.htm

9.5. DEP SOP IZ-09, *Preparation of 80% Ethanol*

9.6. DEP SOP PCR-1.0, *Human-Associated Bacteroidales HF183 Marker in Water by qPCR Assay*

9.7. DEP SOP PCR-2.4, *Calibration and Use of Mechanical Pipettes using PCS Pipette Calibration System and Artel Pipette Tracker*

9.8. DEP SOP PCR-3.1, *Preparation of SKETA assay standards and reagents for qPCR analysis*

9.9. DEP SOP PCR-4.1, *Crude Lysate Extraction Method for qPCR Analysis*

9.10. DEP SOP PCR-5.0, *Sample Custody, Preparation Labels, and Worksheet Instructions for Molecular Biology*

Appendix of Changes

05/11/2016 – Added optional method of purification that includes the use of vacuum manifold.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.