Standard Operating Procedure for:

Purification of Filtered Samples Using the Qiagen DNeasy PowerLyzer® PowerSoil® Kit



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1. SCOPE AND APPLICATION

1.1. This method is based on the Qiagen DNeasy PowerLyzer® PowerSoil® kit user manual and describes a procedure for recovering genomic DNA (gDNA) from samples that contain PCR inhibitors. This method is used to purify samples for GFD or PMA analysis and 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 yield purified DNA.

2.2. Interferences

2.2.1. Purification removes PCR inhibitors but may result in a low yield of DNA. 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 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 designed 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 Vortex

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3.7. Centrifuge

3.8. Fisher Scientific Bead Mill 24 (S/N 24G2336)

3.9. Qiagen DNeasy PowerLyzer® PowerSoil® Kit (Catalog#12855)

3.9.1. Power Glass Bead Tubes (0.1 mm)

3.9.2. MB Spin Columns

3.9.3. 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. Qiagen DNeasy PowerLyzer® PowerSoil® Kit (Catalog#12855)

4.1.1. PowerSoil Bead Solution

4.1.2. Solution C1

4.1.3. Solution C2

4.1.4. Solution C3

4.1.5. Solution C4

4.1.6. Solution C5

4.1.7. Solution C6

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

4.3. E5 B. dorei DNA spike- Prepare per SOP PCR-3.2

4.4. E7 GFD spike – Prepare per SOP PCR-3.4

4.5. E7 BacR spike – Prepare per SOP PCR-3.5

4.6. E6 Gull2 spike – Prepare per SOP PCR-3.3

4.7. E7 DG3 spike – Prepare per SOP PCR-3.6

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4.8. E7 CowM2 spike – Prepare per SOP PCR-3.7

4.9. E7 HumM2 spike – Prepare per SOP PCR-3.8

4.10. Sterile Deionized (DI) Water

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

4.12. 80% Ethanol – Prepare per SOP IZ-09.

4.13. 100% Ethanol (for PowerVac® Manifold protocol only)

5. CALIBRATION AND STANDARDIZATION

5.1. See DEP Laboratory 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 at least 2 minutes.

6.2. Use sterile forceps to transfer a filter to a Bead Tube labeled for each sample or for controls (see section 7).

Note: Keep forceps in a small beaker of ethanol during the 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. Count the number of tubes plus one. In a 50 mL conical tube, add 750 µL of Bead Solution X total number of tubes plus one, and 2 µL of 1E4 SKETA standard X total number of tubes plus one. Invert several times to mix well. With a 1000 µL micropipette, dispense 752 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 45 seconds at a speed setting of 4. Make sure the tubes are sealed tightly and are balanced in the bead beater.

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6.7. Centrifuge at 10000 x g for 30 seconds 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.

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 the solution is centrifuged through the Spin Filter.

6.16.1. <u>Optional Vacuum processing:</u>

6.16.1.1. Instead of using the centrifuge method, a vacuum manifold can be used for the remaining steps. It can be helpful when processing many samples, however using the vacuum manifold will reduce DNA recovery. Remove the screw caps from the manifold and gently press each spin filter column into the manifold until snugly in place. Keep all unused ports of the manifold closed.

6.16.1.2. Transfer 675 µL of sample lysate (from step 6.15) to the spin filter column. Turn on the vacuum source and open the stopcock 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 675 µL. Continue until all the lysate has been loaded, then close the port.

6.16.1.3. 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.

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6.16.1.4. 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 to prevent backflow. Discard liquid from manifold into the sink.

6.16.1.5. Remove column from manifold and add to 2 mL collection tube. Spin at 10000 x g for 1 min to ensure all liquid has passed through the column and there is no ethanol residue in the spin filter.

6.16.1.6. Place column in a new 2 mL collection tube and add 100 µL of Solution C6. Centrifuge for 30 seconds at 10000 x g, being careful to space tubes and align caps so they do not snap off during spin. 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 seconds. Discard the flow through.

6.18. Centrifuge at 10000 x g for 1 min.

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 seconds and discard the Spin Filter. Store purified DNA in the freezer. Use -20°C for short term storage and -80° C for long term storage.

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 with each 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)

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7.2.1. Include an EC for each assay to be analyzed for the batch (maximum of 13 samples per batch). Place a sterile filter into a Bead Tube and spike with 5 µL of E5 *B. dorei* spike, E7 BRD3GD spike, E6 Gull2 spike, E7 CowM2 spike, or E7 HumM2 spike. Make separate ECs for each assay needed. 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/tubes 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.

9. REFERENCES

9.1. Qiagen DNeasy PowerLyzer® PowerSoil® Kit Instruction Manual (Catalog#12855)

9.2. EPA Method B (June 2010)

9.3. FDEP Laboratory Quality Assurance Manual http://publicfiles.dep.state.fl.us/dear/labs/lab_qualitymanual_19.pdf

9.4. Health and Safety Plan Health and Safety Plan

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

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

9.7. DEP SOP PCR-3.2, *Preparation of Bacteroides dorei (B. dorei) Assay Reagents and Standards for qPCR analysis*

9.8. DEP SOP PCR-3.3, *Preparation of Gull2 Assay Reagents and Standards for qPCR analysis*

9.9. DEP SOP PCR-3.4, *Preparation of GFD Assay Reagents and Standards for qPCR analysis*

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9.10. DEP SOP PCR-3.5, *Preparation of BacR Assay Reagents and Standards for qPCR analysis*

9.11. DEP SOP PCR-3.6, *Preparation of DG3 Assay Reagents and Standards for qPCR analysis*

9.12. DEP SOP PCR-3.7, *Preparation of CowM2 Assay Reagents and Standards for qPCR analysis*

9.13. DEP SOP PCR-3.8, *Preparation of HumM2 Assay Reagents and Standards for qPCR analysis*

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

9.15. DEP SOP PCR-5.1, *LIMS QC Manager Data Upload, Verification, and Sample Authorization Instructions for qPCR samples*

Appendix of Changes

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

06/05/2017 – Added extraction controls for additional markers. Replaced bead beater and added instructions specific to new bead beater.

06/05/2018 – Updated the equipment, supplies and reagents list. Update the Health and Safety plan link.

05/28/2019 – Added extraction controls for DG3, CowM2 and HumM2 markers.

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