Crude Lysate Extraction Method for qPCR Analysis

1. SCOPE AND APPLICATION

    1.1. This method describes a procedure for genomic DNA (gDNA) extraction from environmental water samples that have been prepared by membrane filtration.

2. SUMMARY OF METHOD

    2.1. After membrane filtration, environmental samples are added to bead tubes and placed in a bead beater for rapid and thorough homogenization. Cell lysis occurs by mechanical shearing in Salmon DNA extraction buffer. The extract is clarified by centrifugation and can be analyzed with qPCR amplification and detection in a thermocycler system using a primer/probe assay.

    2.2. Interferences

        2.2.1. Some substances, including humic and tannic acids, can act as inhibitors of PCR amplification and reduce efficiency by either binding to the DNA polymerase or interfering with the annealing and extension cycles. Sample purification or dilution can help alleviate the effects of inhibitors.

3. EQUIPMENT AND SUPPLIES

    3.1. Gloves, latex or nitriles

    3.2. Tube racks

    3.3. Centrifuge tubes, 1.5 mL

    3.4. Forceps

    3.5. Micropipettes with sterile PCR grade filter tips (use per SOP PCR-2.4.)

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

    3.7. Fisher Mini Vortexer

    3.8. Centrifuge

    3.9. Bead Beater

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

        3.9.2. Fisher Scientific™ Bead Mill 24 Homogenizer (Property #152281)

4. REAGENTS AND STANDARDS

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4.1. Salmon DNA extraction buffer - Prepare per SOP PCR-3.1.

4.2. *B.dorei*, Gull2, BacR, DG3, CowM2, and HumM2 Extraction Controls - Prepare per SOP PCR-3.2, PCR-3.3, PCR-3.5, PCR-3.6, PCR-3.7, and PCR-3.8.

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

4.4. 80% Ethanol – Prepare per SOP IZ-09.

5. CALIBRATION AND STANDARDIZATION

5.1. See DEP Laboratory Quality Manual, Section 5.5

5.2. Clean work area counters after use. Spray surface of work area with 10% bleach solution and wipe down after 15 minutes. Spray and wipe down with sterile DI water.

6. SAMPLE PREPARATION

6.1. Prepare samples per SOP PCR-4.0.

6.2. For each sample and control bead tube, set up two sets of 1.5 mL centrifuge tubes. Label the top of the tubes with the last 3 numbers of the sample ID. Label the Extraction Control tubes as EC along with the assay name. Label the Extraction Blank tubes as EB. Label the Method Blank tubes as MB. Place small sample labels on the second set of tubes. See SOP PCR-5.0 for instructions on printing labels.

6.3. Set up Extraction Blank and Extraction Control bead tubes per Section 9.

6.4. Add 590 µL of Salmon DNA extraction buffer into each sample and MB bead tubes. Add 595 µL of Salmon DNA extraction buffer to EC and 600 µL to EB.

6.5. Place the tubes in the bead beater (make sure tubes are tightly sealed) and homogenize for 60s at max speed in the mini bead beater or 45s at speed 4 in the Bead Mill 24 (Program 01).

6.6. Centrifuge at 14000 X g for 5 min to pellet the debris.

6.7. With a 1000 µL micropipette, carefully transfer approximately 400 µL (minimum of 200 µL) of supernatant to a new 1.5 mL centrifuge tube and avoid pellet debris and beads. If some pellet debris or beads carry over, it will not affect the sample at this stage. It may be necessary to push down into the filter to remove enough supernatant.

6.8. Tap the tube or invert several times to mix the supernatant. Centrifuge crude supernatant for 10 min at 14000 X g.

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6.9. Carefully, without disturbing the pellet, use a 1000 µL pipette to transfer the clarified supernatant (minimum of 100 µL) to a new labeled centrifuge tube. If any of the pellet is aspirated or pulled up into the supernatant, repeat Step 6.6.

6.10. Refrigerate extracts at 4°C if analysis will begin within 24 hours. Otherwise, freeze extracts at -20°C until analysis.

7. SAMPLE ANALYSIS

7.1. Refer to SOP PCR-1.0 for HF183 qPCR sample analysis.

7.2. Refer to SOP PCR-1.2 for GULL2 qPCR sample analysis.

7.3. Refer to SOP PCR-1.4 for BacR qPCR sample analysis.

7.4. Refer to SOP PCR-1.5 for DG3 qPCR sample analysis.

7.5. Refer to SOP PCR-1.6 for CowM2 qPCR sample analysis.

7.6. Refer to SOP PCR-1.7 for HumM2 qPCR sample analysis.

8. DATA ARCHIVAL

8.1. Refer to SOP PCR-5.0 for prep worksheet instructions.

9. QUALITY CONTROL

9.1. Analyze an Extraction Blank (EB) as a negative control for each set of samples extracted (maximum of 13 per batch). Place a sterile filter into a bead tube and extract and analyze along with the same batch of samples.

9.2. Analyze an Extraction Control (EC) for each applicable assay as a positive control 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 *B. dorei*, Gull2, BacR, DG3, CowM2, or HumM2 Extraction Control as needed. Extract and analyze along with the samples.

9.3. Refer to the method SOPs for further details on required quality controls, acceptance limits, and corrective actions.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. For sample extraction and analysis, wear safety glasses, gloves, and a clean disposable lab coat. If any substance falls on the gloves during processing, change into a new pair immediately to prevent any possible contamination of samples or controls.

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

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11. REFERENCES

11.1. FDEP Laboratory Quality Manual

11.2. Health and Safety Plan

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

11.4. DEP SOP PCR-1.0, *Human-Associated Bacteroidales HF183 Marker in Water by Quantitative Polymerase Chain Reaction (qPCR) Assay*

11.5. DEP SOP PCR-1.2, *Gull-Associated GULL2 Marker by Quantitative Polymerase Chain Reaction (qPCR) Assay*

11.6. DEP SOP PCR-1.4, *Ruminant-associated Bacteroidetes BacR Marker by Quantitative Polymerase Chain Reaction (qPCR) Assay*

11.7. DEP SOP PCR-1.5, *Canine-associated Bacteroidales DG3 Marker by Quantitative Polymerase Chain Reaction (qPCR) Assay*

11.8. DEP SOP PCR-1.6, *Cow-associated CowM2 Marker by Quantitative Polymerase Chain Reaction (qPCR) Assay*

11.9. DEP SOP PCR-1.7, *Human-associated HumM2 Marker by Quantitative Polymerase Chain Reaction (qPCR) Assay*

11.10. DEP SOP PCR-2.4, *Calibration and Use of Mechanical Pipettes using the PCS Pipette Calibration System*

11.11. DEP SOP PCR-3.1, *Preparation of SKETA Assay Reagents and Standards for qPCR Analysis*

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

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

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

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

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

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

11.18. DEP SOP PCR-4.0, *Preparation of Samples for qPCR Analysis*

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

Appendix of Changes

11/27/2017	Added new bead beater and BacR analysis. Updated hyperlinks.
11/21/2018	Added DG3 analysis. Updated hyperlink
11/13/2019	Added sample analysis reference for CowM2 and HumM2

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