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Extraction of Total Nucleic Acid from Environmental Samples for the Detection of Bacterial and Viral Targets

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Abstract

This procedure describes a modified extraction procedure to isolate both DNA and RNA from wastewater samples using the QIAamp Fast DNA Stool MiniKit. The sample undergoes a lysate preparation process and includes mechanical disruption (bead beating), removal of inhibitors, purification and elution of DNA and RNA using spin columns. Extrinsic controls SPC and MS2 are added to each sample during the lysate preparation to evaluate extraction and amplification efficiency. The extracted total nucleic acid (TNA) is then stored at -80°C for testing. To rule out contamination during the extraction process, a blank is also processed through the complete protocol each day extractions are performed. Briefly, the procedure comprises the following steps:

- a. Lysis of and separation of impurities from sample pellets in InhibitEX buffer.
- b. Purification of DNA and RNA on QIAamp Mini spin columns.

Guidelines

The pellet obtained after sample concentration with Nanotrap $\mathbb R$ A particles should not be frozen. Extraction should be carried out the same day.



Materials

- 1. X QIAamp Fast DNA stool Mini Kit Qiagen Catalog ##51604
- 🛱 qPCR DNA Extraction and Inhibition Control CY5-QXL670 Eurogentec Catalog #RT-SPCC-Q02
- RNA MS2 from Bacteriophage MS2 Roche Catalog #10165948001
- \bigotimes Glass beads, acid-washed 212-300 μM (50- 70 U.S. sieve) Merck MilliporeSigma (Sigma-Aldrich) Catalog #G1277-500G
- Nuclease-free water Ambion Catalog #AM9932
- X 1X PBS (Phosphate-buffered saline)
- 7. Mini BeadBeater 24 BioSpec Products Catalog #112011
- 8. 2ml Screw Cap Micro Tubes Thermo Scientific TM Catalog #346911
- 9. 1.7 mL MaxyClear Snaplock Microcentrifuge Tube Axygen Catalog #MCT-175-C
- 10. Finnpipette F1 100 to 1000 µL Thermo Fisher Catalog #4641100N
- 11. Finnpipette F1 20 to 200 µL Thermo Fisher Catalog #4641080N
- 12. Finnpipette F1 2 to 20 µL Thermo Fisher Catalog #4641060N
- 13. Finnpipette F1 0.2 to 2 µL Thermo Fisher Catalog #4641010N
- 14. ART Barrier Specialty Pipette tips 1000 µL Thermo Fisher Catalog #2279-05PK
- 15. ART Barrier Specialty Pipette tips 200 µL Thermo Fisher Catalog #2069-05PK
- 16. ART Barrier Specialty Pipette tips 20 µL Thermo Fisher Catalog #2149P-05PK
- 17. ART Barrier Specialty Pipette tips 10 µL Thermo Fisher Catalog #2139-05PK
- 18. Ethanol Absolute Honeywell Catalog #02875
- 19. SPINIXTM Vortex Shaker Tarsons Catalog #3020
- 20. Centrifuge 5427 R Microcentrifuge Eppendorf Catalog #5429000133
- 21. Benchtop Incubator Eppendorf ThermoMixer ® C Catalog #5382000023
- 22. 2 ml Snap Cap Low Retention Microcentrifuge Tubes Thermo Fisher Catalog #3453

Protocol materials

QIAamp Fast DNA stool Mini Kit Qiagen Catalog ##51604 Materials, Step 1

QPCR DNA Extraction and Inhibition Control CY5-QXL670 Eurogentec Catalog #RT-SPCC-Q02 Materials

Nuclease-free water Ambion Catalog #AM9932 | Materials

RNA MS2 from Bacteriophage MS2 Roche Catalog #10165948001 Materials

Glass beads, acid-washed 212-300 µM (50-70 U.S. sieve) Merck MilliporeSigma (Sigma-

Aldrich) Catalog #G1277-500G

Materials



Before start

Extraction control preparation

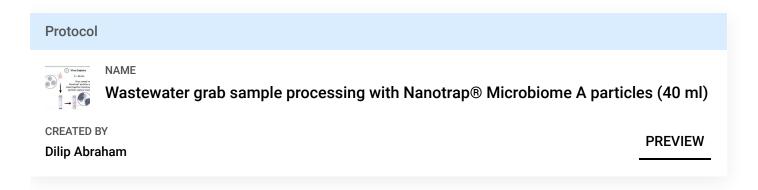
DNA control

Before first use of the sample process control (SPC), prepare a 1/10 dilution of the control DNA in nuclease free water and store them at -70 °C as small aliquots to avoid freeze thaw. Add 1 µL of 1/10 diluted SPC into particle pellet.

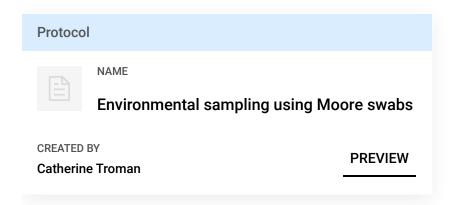
RNA control

Prepare a 1/10 dilution of the MS2 extraction control in nuclease free water and store them at -70 $^{\circ}$ C as small aliquots to avoid freeze thaw. Add 1 μ L of 1/10 diluted MS2 into particle pellet.

• For Nanotrap (R) samples, start the TNA extraction from step 9 in the protocol referenced below.



• For Moore swab samples, the pellet consists of membrane filter strips as described in the protocol below.





Total Nucleic Acid extraction (TNA) ์ 37m 30s 1 QIAamp Fast DNA stool Mini Kit Qiagen Catalog ##51604 Kit used 2 For samples processed by Nanotrap (R) A microbiome particles: Follow the steps described in the referenced protocol as described in the guidelines above. 3 For Moore swab samples: Add the cut membrane filter strips to 2ml Screw Cap Micro Tubes containing ~ 4 370 mg (one 2 ml eppendorf tube capful) of acid-washed glass beads. 3.1 Add both extraction controls to the tube, and then add $\perp \Delta 700 \mu L$ Inhibitex buffer. 4 For pellets derived from other wastewater sample concentration methods: Resuspend the pellet in 4 3 mL of sterile PBS, and add 1ml of this suspension to 2ml Screw Cap Micro Tubes containing ~ 4 370 mg (one 2 ml eppendorf tube capful) of acid-washed glass beads. 4.1 Add both extraction controls to the tube, and then add $\perp \Delta 700 \mu L$ Inhibitex buffer. 5 Include one extra tube for extraction blank (bead + InhibitEX buffer).

- 6 Shake the tubes on a bead beater at maximum speed for 00:02:00
- 7
- 8 Vortex for (5) 00:00:15 15s
- 9 Centrifuge the samples at full speed (approximately 20,000g) for (5) 00:01:00 to pellet the particles.

2m

5m

1m



- 10 Pipette 🚨 25 µL Proteinase K into a new 🚨 2 mL microcentrifuge tube.
- 11 Transfer ~ \(\Lambda \) 600 \(\mu \) of supernatant from step 9 into the 2 ml tube containing proteinase K.
- 12 Add \perp 600 μ L of Buffer AL to the mix.

Note

Do not add Proteinase K directly to the AL buffer.

13 Vortex for 00:00:15 . Mix thoroughly to form a homogeneous solution.

15s

14 Incubate at 70 °C for 00:10:00 . Centrifuge briefly to remove drops from the inside of the tube lid.

10m

- 15 Add 600 µl of ethanol (96–100%) to the lysate and mix by vortexing. Centrifuge briefly to remove drops from the inside of the tube lid.
- 16 Add A 600 µL of lysate from step 15 onto a QIAamp spin column (in a 2 ml collection tube) without moistening the rim. Close and label the cap.
- 17 Centrifuge at full speed for 00:01:00 . Retain the spin column and discard the flowthrough and collection tube.

1m

- 18 Place the QIAamp spin column in a new 🚨 2 mL | collection tube and repeat step 16 - 17 twice until all lysate ($\sim \bot$ 1800 µL) is passed through the spin column.
- 19 Place the QIAamp spin column in a new 🚨 2 mL | collection tube and add 🚨 500 µL Buffer AW1.
- 20 Centrifuge at full speed for 00:01:00. Retain the spin column and discard the flowthrough and collection tube.

1m

21 Place the QIAamp spin column in a new 🚨 2 mL collection tube and add 🚨 500 µL Buffer AW2.

22 Centrifuge at full speed for 00:03:00 . Retain the spin column and discard the flow-3m through and collection tube. 23 Place the QIAamp spin column in a new 🚨 2 mL collection tube and centrifuge at full speed 3m for 00:03:00 to eliminate the chance of possible Buffer AW2 carryover. 24 Place the QIAamp spin column in a new 🚨 1.75 mL microcentrifuge tube, add 🚨 100 µL of Buffer ATE directly onto the QIAamp membrane. 25 Incubate at | Room temperature | for 1 - 3 min. 26 Centrifuge at full speed for 00:01:00 to elute the TNA. 1m 27 Discard the spin column and save the filtrate containing TNA. 28 Aliquot 🚨 50 µL of extracted TNA into 🚨 1.75 mL screw cap tube and store at ♣ -80 °C for long term storage. 29 The remaining \perp 50 μ L of eluate is stored at \parallel -80 °C and use for further analysis.

Protocol references

1] https://www.giagen.com/us/Resources/ResourceDetail?id=2a3f2c0b-2e8a-49fd-b442-829108ae1a4a&lang=en