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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® A particles should not be frozen. Extraction should be carried out the same day.

Materials

1. QIAamp Fast DNA stool Mini Kit **Qiagen Catalog #**#51604
2. qPCR DNA Extraction and Inhibition Control CY5-QXL670 **Eurogentec Catalog #**RT-SPCC-Q02
3. RNA MS2 from Bacteriophage MS2 **Roche Catalog #**10165948001
4. Glass beads, acid-washed 212-300 µM (50- 70 U.S. sieve) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G1277-500G
5. Nuclease-free water **Ambion Catalog #**AM9932
6. 1X PBS (Phosphate-buffered saline)
7. Mini BeadBeater 24 BioSpec Products Catalog #112011
8. 2ml Screw Cap Micro Tubes Thermo Scientific™ 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. SPINIX™ – 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 °C as small aliquots to avoid freeze thaw. Add 1 µL of 1/10 diluted MS2 into particle pellet.

- For Nanotrap ® samples, start the TNA extraction from step 9 in the protocol referenced below.

Protocol

NAME

Wastewater grab sample processing with Nanotrap® Microbiome A particles (40 ml)

CREATED BY

Dilip Abraham

PREVIEW

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

Protocol

NAME

Environmental sampling using Moore swabs

CREATED BY

Catherine Troman

PREVIEW

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® 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 ~ 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 700 µL Inhibitex buffer.

4 For pellets derived from other wastewater sample concentration methods: Resuspend the pellet in 3 mL of sterile PBS, and add 1ml of this suspension to 2ml Screw Cap Micro Tubes containing ~ 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 700 µL 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 2m

7 Incubate the suspensions for 00:05:00 at 95 °C 5m

8 Vortex for 00:00:15 . 15s

9 Centrifuge the samples at full speed (approximately 20,000g) for 00:01:00 to pellet the particles. 1m

10 Pipette 25 µL Proteinase K into a new 2 mL microcentrifuge tube.

11 Transfer ~ 600 µL of supernatant from step 9 into the 2 ml tube containing proteinase K.

12 Add 600 µL 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 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 flow-through 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 (~ 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 flow-through 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-through and collection tube. 3m

23 Place the QIAamp spin column in a new 2 mL collection tube and centrifuge at full speed for 00:03:00 to eliminate the chance of possible Buffer AW2 carryover. 3m

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 50 µL of eluate is stored at -80 °C and use for further analysis.

Protocol references

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