Standard Operating Procedure

Isolation of DNA from fecal and sewage samples using the QIAamp Fast DNA Stool mini kit and modified protocol

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Introduction

This protocol for DNA isolation is based on the Qiagen kit QIAamp Fast DNA stool mini kit (cat. no. 51604). The method has been tested using different sample matrices, e.g. human and animal feces and sewage. The protocol "Isolation of DNA from Stool for Pathogen Detection" supplied with the kit has been modified in order to increase the DNA yield from these complex samples, while maintaining high quality of the DNA. The main modification to the protocol supplied with the kit is the addition of a bead-beating step in the beginning of the isolation to facilitate increasing cell lysis, e.g. of Gram-positive bacteria. Additional modifications have been included as described below to increase DNA yield. For the extraction of DNA from sewage a few additional steps are included to further increase the DNA yield, and this is indicated with ▲ (triangle) in the protocol.

Sample Material

Fecal or sewage samples. The samples can either be used directly after sample collection, or stored at -80°C (-20°C) until DNA isolation. If samples are stored frozen, they must be gently thawed on ice before isolation. ▲ A pre-treatment step is required for the sewage samples. To pellet material for DNA extraction, the sewage is centrifuged at 10000 x g for 10 minutes at 4°C.

Equipment and Reagents

The collection and handling of the specimens should ideally be carried out under aseptic working conditions to limit contamination of the samples and materials. We also recommend including extraction controls, and other controls to assess background DNA levels of collection tubes, reagents, and/or other equipment involved in the collection and handling of the specimens.

Most of the reagents needed for the DNA isolation procedure are supplied with the kit, and a few reagents are to be supplied by the user due to modifications made to the standard protocol supplied with the kit (see QIAamp Fast DNA Stool Mini Handbook). For the preparation of reagents, e.g. addition of ethanol to washing buffers, please consult the kit handbook.

Equipment

- Bead beating tubes[1]
- Microcentrifuge tubes (1.5 ml, 2 ml); 1.5 ml low DNA binding tubes (e.g. Eppendorf, Axygen)
- Pipettes
- Pipet tips (Pipet filter tips)
- TissueLyserII (Qiagen)
- Microcentrifuge (for 1.5 and 2 ml tubes)
- Vortexer
- Thermomixer, heating block, water bath or other heating device

[1] We have been using Garnet Bead Tubes 0.7 mm from MoBio (cat.no. 13123-50), and our experimental results suggest other bead beating tubes provide similar results, e.g. Lysing matrix A tubes from MP Biomedicals (cat.no. 116910050).

Reagents

- Ethanol (96-100%)
- Ice
- Proteinase K[2]
- Buffer AL[2]

[2] Both, Proteinase K and Buffer AL are supplied with the kit; however the modifications described herein require larger volumes of these reagents than described in the standard protocol supplied with the kit, and which can be purchased separately (Proteinase K, cat.no. 19131; Buffer AL, cat.no. 19075).

General remarks

- All centrifugation steps in the DNA isolation protocol are carried out at 20000 x g at room temperature (15-20°C)

Literature

- QIAamp Fast DNA Stool Mini Handbook (03/2014), 1081060_HB_LS_QIAamp_FastDNAStool_0314_WW_web.pdf
- Berith E. Knudsen, Lasse Bergmark, Patrick Munk, Oksana Lukjancenko, Anders Priemé, Frank M. Aarestrup, Sünje J. Pamp (2016) Impact of Sample Type and DNA Isolation Procedure on Genomic Inference of Microbiome Composition. ***mSystems*** Oct 2016, 1 (5) e00095-16; DOI: 10.1128/mSystems.00095-16

Before getting started

- Place ethanol (96-100%) at -20°C
- Preheat heating blocks (or thermomixers) to 70°C and 95°C.
- Redissolve any precipitate in Buffer AL and InhibitEX Buffer by incubation at 37-70 °C.
- Add ethanol to Buffer AW1 and AW2 (when using a new kit)
- Mix all buffers before use
- Place TissueLyserII inserts at -20°C
- Prepare Styrofoam box with ice

Procedure

1. Weigh 0.2 g of feces or ▲0.5 g of the centrifuged sewage pellet into a bead beating tube, and place the tube on ice.
Samples are to be kept on ice until InhibitEX Buffer has been added, to reduce the risk of DNA degradation.

2. Add 1 ml InhibitEX Buffer to the tube containing the sample aliquot.

3. Vortex continuously until the sample is thoroughly homogenized.

4. Treat sample in a TissueLyser at 30 Hz for 3 x 30 s, with cooling on ice between each treatment.

5. Heat the sample at 95°C for 7 min.

6. Vortex the sample for 15 s.

7. Centrifuge the sample at full speed for 1 min to pellet sample particles.
IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.

▲ For sewage samples the following steps 8-12 can be performed by dividing each sample into two Eppendorf tubes and treating both tubes in parallel each with the indicated volume of proteinase K, Buffer AL, and ethanol. In step 13 & 14 the two lysates from the same sample will be collected on one filter spin column.

8. Pipet 30 µl proteinase K into a new 1.5 ml microcentrifuge tube (not provided).

9. Pipet 400 µl supernatant from step 7 into the 1.5 ml microcentrifuge tube containing proteinase K.

10. Add 400 µl Buffer AL and vortex for 15 s.
Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

11. Incubate at 70°C for 10 min.
Centrifuge briefly to remove drops from the inside of the tube lid.

12. Add 400 µl of ethanol (96–100%) to the lysate, and mix by vortexing.
Centrifuge briefly to remove drops from the inside of the tube lid.

13. Carefully apply 600 µl lysate from step 12 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min.
Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

14. Apply the remaining lysate from step 12 to the spin column, close the cap and centrifuge at full speed for 1 min. Place the spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.
Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

▲ For sewage samples, combine here the lysates from the two tubes (see step 7), and repeat step 13 & 14 until all lysate has been passed through the spin column.

15. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

16. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.
Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

17. Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.
This step helps to eliminate the chance of possible Buffer AW2 carryover.

18. Transfer the QIAamp spin column into a new, labeled 1.5 ml low-DNA bind microcentrifuge tube (not provided) and pipet 100 µl or ▲ 50 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 3 min. at room temperature, then centrifuge at full speed for 1 min to elute DNA.

▲ Pipet another 50 µl Buffer ATE directly onto the same QIAamp membrane. Incubate for 2 min at room temperature. Then centrifuge at full speed for 1 min. to elute DNA.
This step combines the DNA-containing eluate from 2x50 µl Buffer ATE.

19. QC the sample and quantify the isolated DNA amount. This can be done using e.g Qubit dsDNA HS Assay kit, Quant-IT DNA Assay Kit high sensitivity, or Quant-IT Picogreen dsDNA.
Nanodrop can be used to determine DNA purity. Bioanalyzer can be used to determine DNA quantity and quality.

20. The extracted DNA can be stored at -20°C or -80°C until further use.

Notes