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Protocol 003: DNA extraction from stool using QIAamp Fast DNA Stool Mini Kit (Qiagen)

Version Number	Written by	Reviewed by	Approved by
1.0			Prof. Samuel Wanji

Principle:

The QIAamp Fast DNA Stool Mini Kit is designed for rapid purification of total DNA from up to 220 mg stool and is suitable for both fresh and frozen samples. A special protocol is provided for isolating DNA from larger amounts of stool. The fast and easy procedure comprises the following steps: Lysis of and separation of impurities from stool samples in InhibitEX Buffer

Requirements

Equipment	Consumables	Reagents
<ul style="list-style-type: none"> • Refrigerator • Freezer (-20⁰C) • Micro pipettes • Microcentrifuge • Timer • Vortex mixer • Water bath or heating block • Equipment for mechanical disruption (For tissues) 	<ul style="list-style-type: none"> • Tissue paper • Disposable gloves • 70% Ethanol • 1.5ml microcentrifuge tubes • 2 ml microcentrifuge tubes • Sterile, nuclease-free filter pipette tips • Ethanol (96-100%) • Marker pens • Ethanol resistant pens 	<p>QIAamp DNA Minikit (Qiagen)</p> <ul style="list-style-type: none"> • QIAamp Mini Spin columns • InhibitEX buffer • Collection tubes (2ml) • Buffer ATL • Buffer AL • Buffer AW1 (Concentrate) • Buffer AW2 (Concentrate) • Buffer AE • PBS (For some samples)

Storage of reagents

All QIAamp DNA Mini Kit reagents should be stored at room temperature

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Extraction procedure from stool

1. Weigh 180–220 mg stool in a 2 ml micro centrifuge tube (not provided) and place tube on ice. This protocol is optimized for use with 180–220 mg stool but can also be used with smaller amounts. There is no need to reduce the amounts of buffers when using smaller amounts of stool. If the sample is liquid, pipet 200 μ l into the micro centrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml micro centrifuge tube on ice.

Note: When using frozen stool samples, take care that the samples do not thaw until InhibitEX Buffer is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of InhibitEX Buffer, all following steps can be performed at room temperature (15–25°C).

2. Add 1 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.

Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. Heat the suspension for 5 min at 70°C. Vortex for 15 s. This heating step helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

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

5. Pipet 15 μ l proteinase K into a new 1.5 ml micro centrifuge tube (not provided).

6. Pipet 200 μ l supernatant from step 4 into the 1.5 ml micro centrifuge tube containing proteinase K.

7. Add 200 μ l 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.

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

9. Add 200 µl of ethanol (96–100%) to the lysate, and mix by vortexing. Centrifuge briefly to remove drops from the inside of the tube lid (optional).

10. Carefully apply 600 µl lysate from step 9 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate. Close each spin column 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.

11. 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.

12. 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.

13. 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.

14. Transfer the QIAamp spin column into a new, labeled 1.5 ml micro centrifuge tube (not provided) and pipet 200 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA. If yield is to be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results.

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