Supplementary Protocol April 2015

Purification of MAP DNA from feces using the QIAamp® *cador*® Pathogen Mini Kit

This protocol is designed for purification of *Mycobacterium avium* spp. *paratuberculosis* (MAP) DNA from up to 220 mg of feces.

In this protocol, feces are homogenized using the FastPrep®-24 Instrument or an equivalent electric homogenizer. Samples are then processed according to the standard QIAamp protocols.

IMPORTANT: Please read the *QIAamp* cador *Pathogen Mini Kit Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- QIAamp *cador* Pathogen Mini Kit (cat. no. 54104 [50] or 54106 [250])
- Buffer ASL (cat no. 19082)
- Equipment for sample disruption and homogenization
- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer or rocking platform for shaking and heating at 99°C
- Ethanol (96–100%)
- Vortexer
- *bactotype*® MAP PCR Kit (96)* (cat. no. 285905), *bactotype* MAP PCR Reagent (96)† (cat. no. 285915) or *intype*® IC-DNA (cat no. 289980)
- Optional: Feces container with conical bottom and support skirt, screw cap with spoon (e.g., Greiner cat. no. 443102)

* Not available in the USA. † Not available in Germany.

Suppliers of equipment for disruption and homogenization

MP Biomedicals

- FastPrep-24 Instrument (cat. no. 116004500)
- Lysing Matrix E (cat. no. 116914050, 116914100 or 116914500)

PEQLAB Biotechnologie GMBH

- Precellys® 24 (cat. no. 91-PCS24)
- Precellys Glas/Keramik-Kit SK38 (Cat. no. 91-PCS-SK38)

Important points before starting

- If using the QIAamp *cador* Pathogen Mini Kit for the first time, read "Important Notes" in the *QIAamp* cador *Pathogen Mini Kit Handbook*
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge

Things to do before starting

- Before beginning the procedure, read "Important Notes" (page 14) in the *QIAamp* cador *Pathogen Mini Kit Handbook*
- Add 1µl IC-DNA to 1ml Buffer ASL

  Note: Buffer ASL + IC-DNA should be prepared fresh

Procedure

1. Mix the sample thoroughly by stirring with a clean spoon or spatula (not provided).
2. Weigh 180–220 mg mixed stool in a 2 ml lysis tube (not provided) and place tube on ice.

   For a liquid sample, pipet 200 µl into the lysis 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 lysis tube on ice.

   Note: When using frozen stool samples, take care that the samples do not thaw until Buffer ASL is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade.

After addition of Buffer ASL, all following steps can be performed at room temperature (15–25°C).

3. Add 1 ml Buffer ASL + IC-DNA mix to each stool sample.
4. Homogenize the sample until the stool sample is thoroughly homogenized.

   Homogenize the sample using a conventional homogenizer until it is uniformly homogeneous.

   Disruption and homogenization using the FastPrep-24 Instrument

   1. Place the Lysing Matrix E tubes in the QuickPrep (24 x 2 ml) or HiPrep (48 x 2 ml) Adapter.
   2. Operate the FastPrep-24 Instrument for 1 min at 6.5 m/s.
5. Incubate at 99°C for 5 min at 1400 rpm.
6. Place the sample on ice for 5 min.
7. Centrifuge sample at full speed for 1 min to pellet stool particles.

At this point, use 200 µl of the supernatant from step 7 as starting material for the protocol "Purification of Pathogen Nucleic acids from Fluid Samples" in the *QIAamp* cador *Pathogen Mini Kit Handbook*.

8. Pipet 20 µl proteinase K into a 2 ml microcentrifuge tube (not provided).
9. Add 200 µl supernatant (step 7) to the proteinase K.

   Note: If processing lower sample volumes, adjust the volume to 200 µl with PBS or 0.9% NaCl.
10. Add 100 µl Buffer VXL. Close the cap and mix by pulse vortexing.

    To ensure sufficient lysis, thoroughly mix the sample and Buffer VXL to yield a homogenous solution. If using sample fluid containing Buffer ATL, for example, after enzymatic digestion of tissue, precipitates may form. Precipitates can be dissolved by brief incubation at 56°C. However, they have no influence on subsequent protocol steps.

    Note: If processing cell-free samples, ensure that 1 µg Carrier RNA is added per 100 µl of Buffer VXL before use.
11. Incubate at 20–25°C for 15 min.
12. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
13. Add 350 µl Buffer ACB to the sample, close the cap and mix thoroughly by pulse-vortexing. Ensure that isopropanol was added to the Buffer ACB concentrate before use.
14. Briefly centrifuge the 2 ml tube to remove drops from inside the lid.
15. Transfer the lysate from step 14 to the QIAamp Mini column placed in a 2 ml collection tube without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.

Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (up to 20,000 x *g*; 14,000 rpm) until the QIAamp Mini column is empty.

16. Open the QIAamp Mini column, and add 600 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
17. Open the QIAamp Mini column, and add 600 µl Buffer AW2 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
18. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 2 min to dry the membrane.
19. Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Open the QIAamp Mini column and add 150 µl Buffer AVE to the center of the membrane. Close the cap, and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

    Important: Ensure that the elution buffer is equilibrated to room temperature. To reduce noise, the centrifugation speed for elution can be set to 6000 x *g*. If this is done, the recovered eluate volume will be approximately 5 µl less than elution buffer volume applied onto the column.

Automated DNA purification on the QIAcube®

Purification of DNA from stool samples using the QIAamp *cador* Pathogen Mini Kit can be automated on the QIAcube.

The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow.

Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the QIAamp *cador* Pathogen Mini Kit for purification of high-quality DNA.

Use the QIAcube Protocol "Purification of pathogen nucleic acids from animal samples — standard" to continue processing at step 8 or use the QIAcube Protocol "Purification of pathogen nucleic acids from animal samples – Manual lysis" to continue processing at step 12.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *QIAamp* cador *Pathogen Mini Kit Handbook*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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