QIAGEN Supplementary Protocol:

Purification of DNA from epithelial cells mixed with sperm cells using the MagAttract® DNA Mini M48 Kit

This protocol is designed for purification of total (genomic and mitochondrial) DNA from epithelial cells mixed with sperm cells using the MagAttract DNA Mini M48 Kit in combination with the BioRobot® M48 workstation.

Introduction

The BioRobot M48 system allows fully automated purification of total DNA from forensic samples. MagAttract technology provides high-quality DNA, which is ideal for forensic analyses as well as genotyping and epidemiological studies. The BioRobot M48 performs all steps of the DNA isolation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material.

This protocol first describes the lysis of samples using proteinase K and then follows with the simple procedure for setting up the BioRobot M48 and starting a run.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *MagAttract DNA Mini M48 Handbook* before beginning this procedure. Ensure that you are familiar with operating the BioRobot M48. See the *BioRobot M48 User Manual*. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Starting material

Suitable starting material is epithelial cells mixed with sperm cells. The amount of biological sample material (excluding the weight of the substrate material, e.g., clothing, paper, etc.) should not exceed 40 mg.

Equipment and reagents to be supplied by the user

- BioRobot M48 workstation, cat. no. 9000708, and disposables (see the *MagAttract DNA Mini M48 Handbook*)
- MagAttract DNA Mini M48 Kit, cat. no. 953336
- App. Package, M48, Forensics, cat. no. 9016150
- Buffer G2, cat. no. 1014636
- 1 M dithiothreitol (DTT). This solution can be made in advance, and stored at –20°C in appropriate aliquots. A thawed aliquot should be discarded after use.
- Shaking incubator at 56°C (e.g., Eppendorf Thermomixer)
- Microcentrifuge

- Sample tubes, 1.5 ml, without lids (Sarstedt, cat. no. 72.607), or with screw caps (Sarstedt, cat. no. 72.692), sample tubes, 2 ml, without lids (Sarstedt, cat. no. 72.608) or with screw caps (Sarstedt, cat. no. 72.693)
- Elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692) or 2 ml (Sarstedt, cat. no. 72.693)

Important points before starting

- Check that Buffer MW1 has been prepared according to the instructions given in the "Important Notes" section of the kit handbook.
- Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.
- As some sample types (e.g., stained fabrics) tend to be very absorbent, it may be necessary to add a greater volume of digestion buffer to the sample in step 2.

Procedure

Proteinase K digestion of epithelial and sperm cells

1. **Place the forensic sample in a 1.5 ml or 2 ml sample tube.**
2. **Add 190 µl Buffer G2 to the sample.**
3. **Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for 15 min.**

   Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. **Centrifuge the tube briefly to remove drops from inside the lid.**
6. **Remove any solid material from the tube.**

   Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.

   The sample volume should be approximately 200 µl.
7. **Centrifuge the tube at 15,000 x *g* for 5 min. Carefully transfer the supernatant to a new tube without disturbing the sperm cell pellet.**

   DNA from epithelial cells can be purified from the tube containing the supernatant, see step 14.

   Note: The cell pellet may not be visible.
8. **Wash the sperm cell pellet by resuspending the pellet in 500 µl Buffer G2. Centrifuge the tube at 15,000 x *g* for 5 min and discard the supernatant.**
9. **Repeat step 8 two or three times**.
10. **Add 180 µl Buffer G2 to the pellet and resuspend the pellet.**
11. **Add 10 µl proteinase K and 10 µl 1 M DTT, and mix thoroughly by vortexing for 10 s.**
12. **Incubate at 56°C overnight at 850 rpm in a shaking incubator or thermomixer.**

13. **Centrifuge the tube briefly to remove drops from inside the lid. DNA from sperm cells can now be purified from this tube.**

    The two tubes in which the epithelial and sperm cells have been separated are now ready for DNA purification.

DNA purification

14. **Ensure that the BioRobot M48 is switched on.**

    The power switch is on the left side of the instrument.

15. **Switch on the computer and monitor.**

16. **Launch the QIAsoft M Operating System.**

    Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software start-up window, but this setting may have been changed.

    The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft® Windows® "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

17. **Select the protocol group "Forensic" from the drop-down menu by clicking the dark green arrow, then select "gDNA".**

18. **Select the protocol "Trace Sample". Click the "Select" button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.**

    QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

19. **Place the sample tubes containing the separated epithelial and sperm cells on the worktable, plus reagent containers and plasticware according to the software instructions.**

20. **Close the workstation door and start the purification procedure. All steps are fully automated, and a software message on the screen will indicate when the procedure is finished.**

21. **Retrieve the elution tubes containing the purified DNA from the cooling block. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.**

    If the purified DNA is to be analyzed by real-time PCR or using a fluorescent capillary sequencer, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see the appendix of the *MagAttract DNA Mini M48 Handbook*) in order to minimize the risk of magnetic-particle carryover.

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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