QIAGEN Supplementary Protocol:

Isolation of DNA from buccal cells using the MagAttract® DNA Mini M48 Kit

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

Introduction

The MagAttract DNA M48 System allows fully automated purification of total DNA from buccal cells. MagAttract technology provides high-quality DNA, which is ideal for genotyping and epidemiological studies as well as forensic analyses. The isolated DNA is well suited for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot M48 performs all steps of the DNA isolation procedure.

This protocol describes first how to collect buccal cell samples (using either cotton swabs or brushes); this is followed by the procedure for harvesting and lysis of cells (using proteinase K) and the simple procedure for setting up the BioRobot M48 and starting a run.

IMPORTANT: Please read the *MagAttract DNA Mini M48 Handbook*, paying careful attention to the Safety Information and Important Notes sections, before beginning this procedure.

Starting material

Collection of buccal (or epithelial) cells from the inside of the cheek is a simple, inexpensive way to collect material for DNA isolation. Buccal cell samples may be processed on the same day as collection or stored for future processing. While storage at –20°C is recommended, DNA of suitable quality for single-copy gene amplification has been documented from swabs stored (air-dried) at room temperature for over 21 months (see Table 2). The starting and elution volumes to use in this procedure are given in Table 1, below.

Table 1. Amount of starting material and elution volume for the MagAttract DNA M48 buccal cells procedure

Sample type	Protocol	Starting volume	Elution volume
Buccal cells collected on swab or brush	Buccal Cells	200 µl proteinase K-digested sample*	50–400 µl

* *See the procedure below.*

Yield of purified DNA

DNA yields vary depending on the number of nucleated cells in the sample and also on the elution volume used. Elution in smaller volumes increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application. Typical yields of DNA obtained from fresh and stored buccal cell samples are shown in Table 2, below.

Table 2. DNA yields obtained from fresh and stored buccal cell samples using the MagAttract DNA M48 System

Sample type	No. of samples tested	DNA yield (µg)*
Fresh buccal cell swabs	48	2.4 ± 1.26
21-month-old buccal cell swabs†	12	0.76 ± 0.27
21-month-old buccal cell brush samples†	12	2.05 ± 0.67

** DNA was eluted in 100 µl RNase-free water. DNA yield may be increased by using a higher elution volume.*

† Samples were stored (air-dried) at room temperature for 21 months.

Reagents and equipment to be supplied by the user

- MagAttract DNA Mini M48 Kit, cat. no. 953336
- BioRobot M48 workstation and disposables (see the *MagAttract DNA Mini M48 Handbook*)
- Sample tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692)
- Elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692) or 2 ml (Sarstedt, cat. no. 72.693)

Important notes before starting

- This protocol has been tested using the following swab types: plastic swabs with cotton or Dacron® tips. (Puritan® applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, www.hwppuritan.com, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, www.daigger.com, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.
- Check that Buffer MW1 has been prepared according to the instructions given in the Important Notes section of the *MagAttract DNA Mini M48 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.
- Before use, dilute Buffer G2 in distilled water using a ratio of 1:0.5. Prepare sufficient diluted Buffer G2 for n+1 samples (where n is the number of samples to be digested) by mixing 200 µl Buffer G2 with 100 µl distilled water for each sample. Use 290 µl of diluted Buffer G2 per sample in step 1, below. Buffer G2 may also be used undiluted although, due to the increased volume of Buffer G2 required for the buccal cells procedure, fewer isolations will be possible.

Sample collection

To collect a sample, scrape the swab or brush against the inside of each cheek 6 times. Allow the swab or brush to air-dry for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink for 30 min prior to sample collection.

Procedure

This procedure involves harvesting of cells from the samples and lysis using QIAGEN Proteinase K, and subsequent automated isolation of DNA using the BioRobot M48.

Proteinase K digestion of buccal cells

1. **Carefully cut or break off the end part of the swab or brush into a 1.5 ml sample tube (with screw cap), using scissors or by hand. Add 290 µl of diluted Buffer G2 to the sample.**

   Note: Prepare diluted Buffer G2 as described above in "Important notes before starting".

2. **Add 10 µl QIAGEN Proteinase K, and mix thoroughly by vortexing for 10 s.**

   If processing buccal cell brush samples, centrifuge the tube briefly (at 10,000 x g for 30 s) to force the brush to the bottom of the tube.

3. **Incubate at 56°C for 15 min.**

   Vortex the tube 1–2 times during the incubation, or place in a thermomixer.

4. **Centrifuge the tube briefly to remove drops from inside the lid.**

5. **Remove the swab or brush from the tube.**

   Using forceps, press the swab or brush against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 µl.

DNA isolation

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

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

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

8. **Launch the QIAsoft™ M Operating System.**

   Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup 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.

9. **Select the protocol group "MagAttract DNA Tissue" from the drop-down menu, by clicking on the dark green arrow.**

10. **Select the protocol "Buccal Cells", and click the "Select" button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.**

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

11. **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.**

12. **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, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see the *MagAttract DNA Mini M48 Handbook* appendix) in order to minimize the risk of magnetic-particle carryover.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *MagAttract DNA Mini M48 Handbook*. The troubleshooting described here is specific for the buccal cells application.

	Comments and suggestions
Low DNA yield	
Insufficient number of cells in sample	When collecting buccal cell samples, be sure to scrape the swab or brush firmly against the inside of each cheek 6 times.