

## QIAGEN Supplementary Protocol:

### Isolation of DNA from mouse tail tissue using the MagAttract® DNA Tissue M96 Kit

This protocol is designed for the isolation of total (genomic and mitochondrial) DNA from mouse tail tissue using the MagAttract® DNA Tissue M96 Kit in combination with the BioRobot® M96 workstation.

#### Introduction

The MagAttract DNA M96 System allows fully automated purification of total DNA from mouse tail tissue. MagAttract technology provides high-quality DNA, which is well suited for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot M96 performs all steps of the DNA isolation procedure.

This protocol describes first the lysis of samples with proteinase K and then follows with the simple procedure for setting up the BioRobot M96 and starting a run.

**IMPORTANT:** Please read the *MagAttract DNA M96 Handbook*, paying careful attention to the Safety Information and Important Notes sections, before beginning this procedure.

#### Starting material

Fresh or frozen mouse tail biopsies are a rich source of genomic DNA. Biopsies harvested from the tail tip often result in higher yields per mm tail (or mg tissue) than biopsies from higher up the tail. If mouse tail biopsy samples are not to be processed immediately after collection, storage at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$  is recommended. 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 M96 mouse tail procedure**

Sample type	Protocol	Starting volume	Elution volume
Mouse tail biopsies	Tissue Standard	50 $\mu\text{l}$ proteinase K-digested sample*	50–100 $\mu\text{l}$

\* See the procedure below.

#### Yield of purified DNA

DNA yields may vary depending 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. Using an elution volume of 100  $\mu\text{l}$ , yields of up to 1.4  $\mu\text{g}$  DNA are typically obtained with this procedure.

## Reagents and equipment to be supplied by the user

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

- MagAttract DNA Tissue M96 Kit, cat. no. 953436 (192) or 953438 (960)
- BioRobot M96 workstation and disposables (see *MagAttract DNA M96 Handbook*)
- Ethanol (70%)
- Distilled or deionized water (for washing of DNA)
- DNA elution solution (either RNase-free water or low-salt buffer such as 10 mM Tris-Cl, pH 7.5)
- 1.5 ml tubes with screw caps
- 96-well microplates with round-bottom wells (Greiner, cat. no. 650101)
- 96-well PCR plates (ABgene, cat. no. AB-0600) and 8-cap strips for use with them (ABgene, cat. no. AB-0602)
- 96-well microplates with 400  $\mu$ l wells (NUNC, cat. no. 267245)
- 96-well blocks with 650  $\mu$ l wells (ABgene, cat. no. AB-0765)

## Important point before starting

- Insoluble material in the digested sample may clog the BioRobot M96 tips. Such material should be removed as described in the procedure below.

## Procedure

This procedure involves digestion of mouse tail biopsy samples using QIAGEN Proteinase K, followed by automated DNA isolation using the BioRobot M96.

### Proteinase K digestion of mouse tail biopsy samples

- 1. Cut a 1 mm length section of mouse tail (approximately 6–11 mg) into a 1.5 ml sample tube with screw cap.**

At this stage, samples may be frozen (at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$ ) for later processing. When processing frozen samples, remove samples from storage and allow to thaw at room temperature before use.

- 2. Add 67  $\mu$ l Buffer G2 to the sample.**

Ensure the biopsy sample is fully submerged in Buffer G2.

- 3. Add 3  $\mu$ l QIAGEN Proteinase K, and mix by tapping the tube gently.**

- 4. Incubate at  $56^{\circ}\text{C}$  until the tissue is completely lysed.**

To ensure the sample is mixed thoroughly during incubation, place the sample in a thermomixer, in a shaking water bath, or on a rocking platform. For complete lysis of sample tissue, overnight incubation is often required.

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

**6. Homogenize the sample by pipetting up and down several times.**

Large pieces of insoluble material, such as undigested cartilage, can clog the BioRobot M96 tips and therefore should be removed. To remove insoluble material centrifuge the sample at 300 x g for 1 min, and transfer the supernatant to a new 1.5 ml sample tube. Undigested hair does not normally influence the isolation.

**7. For each sample, transfer 50 µl supernatant to one well of a 96-well block.**

## **DNA isolation**

**8. Ensure that the BioRobot M96 is switched on.**

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

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

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

Upon startup, the computer controlling the BioRobot M96 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 V1.0 for BioRobot M96.

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

**12. Select the protocol "Tissue Standard", and click the "Select" button. Select the number of samples and the elution volume in the corresponding dialog fields. Click "Next".**

QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M96 for the Tissue Standard protocol. Follow the steps detailed in the protocol message before continuing. Wear gloves when loading the required items on the worktable.

**CAUTION:** Before placing the 96-well block onto the "Samples/Deep Wells" slot, ensure that the microplate adapter there is removed. See the *BioRobot M96 User Manual* for details.

**13. 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.**

**14. Retrieve the PCR plate containing the purified DNA from the heat/cool 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, the microplate containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean microplate (see the *MagAttract DNA M96 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 M96 Handbook*. The troubleshooting described here is specific for the mouse tails application.

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### Comments and suggestions

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#### Low DNA yield

Clogging	Insoluble material, such as undigested cartilage, was not removed from the digested sample prior to starting the BioRobot M96 DNA isolation procedure.
Insufficient amount of starting material	An insufficient amount of mouse tail may have been used. Biopsies taken from further up the tail (away from the tail tip end) result in lower DNA yields per mm (or mg) tissue. Increase the size of the biopsy up to a maximum length of 1 mm (or 11 mg), and take care to remove any insoluble material prior to DNA isolation.

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