

User-Developed Protocol:

Purification of genomic DNA from bones using the QIAamp[®] DNA Micro Kit

This protocol has been adapted by customers and is for purification of total DNA from bones or teeth using the QIAamp DNA Micro Kit. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the Safety Information and Important Notes sections in the *QIAamp DNA Micro Handbook* before beginning this procedure. 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.

Equipment and reagents to be supplied by the user

- Ethanol (96–100%)
- 1.5 ml microcentrifuge tubes
- Pipet tips (to avoid cross contamination, we recommend pipet tips with aerosol barriers)
- Disposable gloves
- Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C and 70°C
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Metal blender (e.g., Waring) or TissueLyser (cat. no. 85210 [North America], 85200 [Japan], or 85220 [rest of world]) with the Grinding Jar Set, S. Steel (cat. no. 69985)
- Liquid nitrogen

Important notes before starting

- Lysis time will vary depending on the size and density of the source material. The lysis conditions given here are intended to serve as guidelines.
- All centrifugation steps are performed at room temperature (15–25°C).

Things to do before starting

- Heat a water bath or heating block to 56°C.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that ethanol has been added to Buffers AW1 and AW2 according to the instructions in the *QIAamp DNA Micro Handbook*.

Procedure

- 1. Crush the bone into small fragments. Grind to a fine powder using a metal blender half-filled with liquid nitrogen. Alternatively, grind the bone to a fine powder using the TissueLyser and the Grinding Jar Set, S. Steel.**

When using the TissueLyser, transfer the bone sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser. Grind the bone at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of bone).

- 2. Place ≤ 100 mg of powdered bone into a 1.5 ml microcentrifuge tube.**
- 3. Add 360 μ l Buffer ATL, 20 μ l QIAGEN Proteinase K, and 1 μ l carrier RNA (1 μ g/ μ l). Incubate overnight at 56°C.**

After incubation, set the temperature to 70°C for the incubation in step 6.

- 4. Briefly centrifuge the tube to remove drops from the inside of the lid.**

- 5. Add 300 μ l Buffer AL, close the lid, and mix by pulse-vortexing for 10 s.**

To ensure efficient lysis, it is essential that the sample and Buffer AL are thoroughly mixed to yield a homogeneous solution.

A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during incubation in step 6.

- 6. Place the tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 10 min.**

If using a heating block or water bath, vortex the tube for 10 s every 3 min to improve lysis.

- 7. Centrifuge the tube at full speed (20,000 x g; 14,000 rpm) for 1 min.**

- 8. Carefully transfer the supernatant from step 7 to the QIAamp MinElute[®] column without wetting the rim.**

- 9. Close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute column is empty.

- 10. Carefully open the QIAamp MinElute column and add 600 μ l Buffer AW1 without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

- 11. Carefully open the QIAamp MinElute column and add 600 µl Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

Contact between the QIAamp MinElute column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute column. Take care when removing the QIAamp MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute Column.

- 12. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.**

This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

- 13. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 35 µl Buffer AE or water to the center of the membrane.**

Important: Ensure that Buffer AE or water is equilibrated to room temperature (15–25°C). Dispense Buffer AE or water onto the center of the membrane to ensure complete elution of bound DNA.

- 14. Close the lid 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.**

Incubating the QIAamp MinElute column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

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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