

User-Developed Protocol:

Isolation of genomic DNA from compact bone using the QIAamp® DNA Mini Kit

This procedure has been adapted by customers from the QIAamp[®] DNA Mini Protocols, and is for use with the QIAamp DNA Mini Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

Please note that lysis time will vary depending on the size and density of the source material.

Please be sure to read the QIAGEN[®] QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook and detailed Tissue Protocol carefully before beginning this procedure.

Procedure

- Completely remove bone marrow and soft tissues using razor blades and/or sandpaper.
- 2. Crush the bone into small fragments. Grind to a fine powder using a metal blender half-filled with liquid nitrogen.
- 3. Transfer 5 g of the powder into sterile 50 ml polypropylene tubes and add 40 ml of 0.5 M EDTA, pH 7.5, to decalcify the sample. Agitate the tubes on a rotator at 4°C for 24 h.
- 4. Centrifuge the sample at 2000 x g for 15 min. Discard the supernatant. Repeat the decalcification process several times.
 - **Note:** Generally, decalcification takes 3–5 days. The decalcification process can be monitored by adding a saturated solution of ammonium oxalate, pH 3.0, to the decanted supernatant. If the solution remains clear, the decalcification process can be stopped.
- 5. Wash the pellet with 40 ml of sterile deionized water to remove ions that have accumulated during decalcification. Centrifuge the sample for 15 min at 2000 x g and discard the supernatant. Repeat this washing procedure 3 times.
- 6. To 50 mg of pellet, add 360 μl Buffer ATL. Continue with step 2 of the Tissue Protocol in the QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook.

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