QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from bone fragments using the Gentra® Puregene® Tissue Kit

This protocol is designed for purification of DNA from 0.1–0.3 mg samples of bone fragments using the Gentra Puregene Tissue Kit.

Gentra Puregene Kits enable purification of high-molecular-weight DNA from a variety of sample sources. The convenient purification procedure removes contaminants and enzyme inhibitors, and purified DNA is ready for immediate use in sensitive downstream applications or for archiving. Purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9 and is up to 200 kb in size.

IMPORTANT: Please read the *Gentra Puregene Handbook*, paying careful attention to the safety information, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, consult the appropriate material safety data sheets (MSDSs), available from the product supplier. The Gentra Puregene Tissue Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by 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.

- Gentra Puregene Tissue Kit (100 mg) cat. no. 158622, Gentra Puregene Tissue Kit (4 g) cat. no. 158667, or Gentra Puregene Tissue Kit (33 g) cat. no. 158689
- Glycogen Solution (500 µl) cat. no. 158930
- 2 ml spin tube
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 65°C and 55°C
- Vortex
- 70% ethanol*
- Isopropanol
- Crushed ice
- Liquid nitrogen

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Hammer

- Plastic pouch
- Aluminium foil
- Optional: Water bath heated to 37°C if RNase A treatment is required

Things to do before starting

- Heat water baths to 55°C for use in step 4 and 65°C for step 20 of the procedure.
- Optional: Heat water bath to 37°C for step 8 of the procedure if RNase A treatment is required.

Procedure

1. **Place bone in a sealed plastic pouch, wrap it in aluminum foil, and freeze it in liquid nitrogen. Pulverize the bone with a hammer.**
2. **Transfer 0.1 to 0.3 mg of small bone fragments into the insert of a 2 ml spin tube.**
3. **Add 300 µl Cell Lysis Solution and 1.5 µl Proteinase K Solution (20 mg/ml).**
4. **Complete cell lysis by incubating at 55°C overnight. Invert tube periodically if possible.**
5. **Centrifuge for 1 min at 13,000–16,000 x *g*.**
6. **Remove the insert from the spin tube and discard.**
7. **If you wish to include an optional RNase treatment, go to step 7a, otherwise proceed with step 7b.**

7a. **Add 1.5 µl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate at 37°C for 15 min to 1 h. Proceed with step 8.**

7b. **No RNase A treatment is required. Proceed with step 8.**

8. **Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).**
9. **Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
10. **Incubate on ice for 10 min.**
11. **Centrifuge for 3 min at 13,000–16,000 x *g*.**

    The precipitated proteins should form a tight pellet. If the protein pellet is not tight, vortex vigorously for 20 s at high speed, and then incubate on ice for 5 min. Centrifuge at 13,000–16,000 x *g* for 3 min.

12. **Pipet 300 µl isopropanol and 0.5 µl Glycogen Solution (20 mg/ml) into a clean 1.5 ml microcentrifuge tube. Add the supernatant from the previous step by pouring carefully.**

    Be sure the protein pellet is not dislodged during pouring.

13. **Incubate on ice for 10 min to 1 h.**

14. **Centrifuge for 10 min at 13,000–16,000 x *g*.**

    A white DNA pellet might be visible.

15. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
16. **Add 300 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
17. **Centrifuge for 1 min at 13,000–16,000 x *g*.**
18. **Allow DNA to air dry at room temperature for 10–15 min.**
19. **Add 20 µl DNA Hydration Solution.**
20. **Incubate at 65°C for 1 h to dissolve the DNA.**
21. **Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure tube lid is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.**

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.