QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from 10–20 mg fish tissue using the Gentra® Puregene® Tissue Kit or Gentra Puregene Mouse Tail Kit

This protocol is designed for purification of DNA from 10–20 mg samples of fish tissue using the Gentra Puregene Tissue Kit or Gentra Puregene Mouse Tail 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 and Gentra Puregene Mouse Tail Kit are intended for molecular biology applications. These products are 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.

- If RNase A treatment is required: Gentra Puregene Tissue Kit (100 mg), (4 g), or (33 g), cat. nos. 158622, 158667, and 158689
- If no RNase A treatment is required: Gentra Puregene Mouse Tail Kit (100 mg) or (4 g), cat. nos. 158222 and 158267
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- Liquid nitrogen
- Mortar and pestle
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Microcentrifuge tube pestle

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

- Water baths heated to 55°C and 65°C
- Crushed ice
- 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 4b and 65°C for use in steps 4a and 20 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Dissect tissue sample quickly and freeze in liquid nitrogen. Grind tissue in liquid nitrogen with a porcelain mortar and pestle. Store ground tissue at –70°C to –80°C. Fresh tissue may also be used. Work very quickly and keep tissue on ice at all times including when tissue is weighed.**
2. **Add 10–20 mg frozen ground tissue or fresh tissue to a clean 1.5 ml microcentrifuge tube containing 600 µl Cell Lysis Solution.**
3. **Remove from ice, and homogenize thoroughly using a microcentrifuge tube pestle. Place sample back on ice until you are ready to proceed with the next step.**
4. **Complete cell lysis by following step 4a or 4b below:**

4a. **Incubate at 65°C for 15 min. Proceed with step 5.**

4b. **If maximum DNA yield is required, add 3 µl Puregene Proteinase K (20 mg/ml) and incubate lysate at 55°C for 3 h to overnight. Proceed with step 5.**

5. **If you wish to include an optional RNase treatment, go to step 5a, otherwise proceed with step 5b.**

5a. **Add 3 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 6.**

5b. **No RNase A treatment is required. Proceed with step 6.**

6. **Incubate on ice for 5 min.**
7. **Add 200 µl Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.**
8. **If processing tissues with high polysaccharide content (e.g., fin), incubate on ice for 15 min.**
9. **Centrifuge at 13,000–16,000 x *g* for 3 min.**

   Note: For samples with high polysaccharide content, centrifugation at 4°C may be required.

   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.

10. **Pipet 600 µl isopropanol into a clean 1.5 ml microcentrifuge tube.**
11. **Add the supernatant from step 9 by pouring carefully.**
    Make sure not to dislodge the protein pellet when transferring the supernatant.
12. **Mix by inverting gently 50 times.**
13. **Centrifuge at 13,000–16,000 x *g* for 1 min.**
    The DNA should be visible as a small white pellet.
14. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
15. **Add 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
16. **Centrifuge at 13,000–16,000 x *g* for 1 min.**
17. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
    The pellet might be loose and easily dislodged.
18. **Allow DNA to air dry at room temperature for 10–15 min.**
19. **Add 100 µl DNA Hydration Solution.**
20. **Incubate at 65°C for 1 h to dissolve the DNA.**
21. **Incubate at room temperature overnight with gentle shaking. Ensure tube cap 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.