QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from marine invertebrate tissue using the Gentra® Puregene® Tissue Kit

This protocol is designed for purification of DNA from 5–20 mg samples of marine invertebrate tissue 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
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Microfuge tube pestle
- Water baths heated to 55°C and 65°C
- Crushed ice
- 70% ethanol*
- Isopropanol
- Liquid nitrogen
- Optional: Water bath heated to 37°C if RNase A treatment is required

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

Important points before starting

- In some steps of the procedure, one of 2 choices can be made. Choose ■ if processing 5–10 mg marine invertebrate tissue; choose ◆ if processing 10–20 mg marine invertebrate tissue samples.

Things to do before starting

- Heat water baths to 65°C for use in steps 4a and 20 and 55°C for use in step 4b of the procedure.
- Optional: Heat water bath to 37°C for use in step 5 of the procedure if RNase A treatment is required.

Procedure

1. Dissect tissue sample quickly and freeze in liquid nitrogen.

Store at –70° 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. Transfer ■ 5–10 mg frozen ground tissue to a sterile 1.5 ml microcentrifuge tube containing 300 µl Cell Lysis Solution or ◆ 10–20 mg frozen ground tissue to a sterile 1.5 ml microcentrifuge tube containing 600 µl Cell Lysis Solution.

3. Remove from ice, and homogenize thoroughly using a microfuge tube pestle. Place sample on ice until ready to perform the next step.

4. Complete cell lysis by following step 4a or 4b below:

4a. Incubate lysate at 65°C for 15 min to 1 h. Proceed with step 5.

4b. If maximum DNA yield is required, add ■ 1.5 µl or ◆ 3 µl Puregene Proteinase K (20 mg/ml) to the lysate, and mix by inverting 25 times. Incubate at 55°C for 3 h to overnight. Invert tube periodically during the incubation, if possible. 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 ■ 1.5 µl or ◆ 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 1 min to quickly cool the sample to room temperature (15–25°C).

7. Add ■ 100 µl or ◆ 200 µl Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.

Note: For samples with high polysaccharide content, it may be necessary to place the sample on ice for 15 min to 1 h.

8. Incubate on ice for 5–15 min.

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

9. **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.

10. **Pipet ■ 300 µl isopropanol or ◆ 600 µl isopropanol into a clean 1.5 ml microcentrifuge tube. Add the supernatant from the previous step by pouring carefully.**
11. **Be sure the protein pellet is not dislodged during pouring.**
12. **Mix by inverting gently 50 times.**
13. **Centrifuge for 1 min at 13,000–16,000 x *g*.**

    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 ■ 300 µl or ◆ 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
16. **Centrifuge for 1 min at 13,000–16,000 x *g*.**
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 ■ 50 µl or ◆ 100 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.