QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from 50 µl CSF using the Gentra® Puregene® Tissue Kit

This protocol is designed for purification of DNA from 50 µl samples of CSF 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), (4 g), or (33 g), cat. nos. 158622, 158667, and 158689
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 37°C, 55°C, and 65°C
- Vortexer
- Crushed ice

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

Things to do before starting

- Heat water baths to 37°C, 55°C, and 65°C for use in steps 2 and 19 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Pipet 550 µl Cell Lysis Solution into a clean 1.5 ml microcentrifuge tube, and add 50 µl CSF. Mix by pipetting up and down.**
2. **Add 3 µl Puregene Proteinase K (20 mg/ml). Mix by inverting 25 times and incubate at 55°C for 1 h to overnight.**
3. **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.**
4. **Quickly cool the sample to room temperature (15–25°C) by placing on ice for 1 min.**
5. **Add 200 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
6. **Place sample on ice for 5 min.**
7. **Centrifuge at 13,000–16,000 x *g* for 3 min.**

   The precipitated proteins should form a tight pellet.
8. **Pipet 600 µl isopropanol into a clean 1.5 ml microcentrifuge tube.**
9. **Add 1 µl Glycogen Solution (20mg/ml).**
10. **Add the supernatant from step 7 by pouring carefully.**
11. **Mix by inverting gently 50 times and incubate at room temperature for 5 min.**
12. **Centrifuge for 13,000–16,000 x *g* for 5 min.**

    The DNA may be visible as a small white pellet, depending on yield.
13. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
14. **Add 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
15. **Centrifuge at 13,000–16,000 x *g* for 1 min.**

16. **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.
17. **Allow DNA to air dry at room temperature for 10–15 min.**
18. **Add 20 µl DNA Hydration Solution to the tube containing the pellet.**
19. **Incubate at 65°C for 1 h to dissolve the DNA.**
20. **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.