QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from 1 ml whole blood with a body fluid protocol using the Gentra® Puregene® Tissue Kit

This protocol is designed for purification of DNA from 1 ml samples of whole blood using the Gentra Puregene Tissue Kit.

Note: Use this protocol if you accidentally added Cell Lysis Solution instead of RBC Lysis Solution during the first step of the whole blood protocol.

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) 158689
- Glycogen Solution (500 µl) cat. no. 158930
- 50 ml centrifuge tubes
- Microcentrifuge
- Water baths heated to 65°C and 55°C
- Vortexer
- 70% ethanol*
- Isopropanol
- Crushed ice
- 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.

Things to do before starting

- Heat water baths to 65°C for use in steps 2a and 18 and to 55°C for use in step 2b of the procedure.
- Optional: Heat water bath to 37°C for use in step 3 of the procedure if RNase A treatment is required.

Procedure

1. **Dispense 11 ml Cell Lysis Solution into a clean 50 ml centrifuge tube. Add 1 ml body fluid (whole blood), and mix by pipetting up and down.**
2. **Complete cell lysis by following step 2a or 2b below:**

2a. **Incubate at 65°C for 15 min. Proceed with step 3.**

2b. **If maximum DNA yield is required, add 60 µl of Puregene Proteinase K (20 mg/ml) and incubate lysate at 55°C for 1 h to overnight.**

3. **If you wish to include an optional RNase treatment, go to step 3a, otherwise proceed with step 3b.**

3a. **Add 60 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 4.**

3b. **No RNase A treatment is required. Proceed with step 4.**

4. **Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).**
5. **Add 4 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
6. **Incubate on ice for 5–15 min.**
7. **Centrifuge for 10 min at 2000 x *g*.**
   The precipitated proteins should form a tight pellet.
8. **Pipet 12 ml isopropanol and 20 µl Glycogen Solution into a clean 50 ml centrifuge tube. Add the supernatant from the previous step by pouring carefully.**
   Be sure the protein pellet is not dislodged during pouring.
9. **Mix by inverting gently 50 times.**
10. **Incubate at room temperature for at least 5 min.**
11. **Centrifuge for 10 min at 2,000 x *g*.**
    The DNA might be visible as a small white pellet.
12. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
13. **Add 12 ml of 70% ethanol, and invert several times to wash the DNA pellet.**
14. **Centrifuge at 2000 x *g* for 1 min.**

15. **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.
16. **Allow DNA to air dry at room temperature for 10–15 min.**
17. **Add 100 µl DNA Hydration Solution.**
18. **Incubate at 65°C for 1 h to dissolve the DNA.**
19. **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.