QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from clotted whole blood using the Gentra® Puregene® Tissue Kit or Gentra Puregene Mouse Tail Kit

This protocol is designed for purification of DNA from 50 µl or 1 ml samples of clotted whole blood 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 the 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 treatment is required: Gentra Puregene Tissue Kit (4 g) or (33 g), cat. nos. 158667 and 158689
- If no RNase treatment is required: Gentra Puregene Mouse Tail Kit, cat. no. 158267
- Glycogen Solution (500 µl), cat. no. 158930
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 1.5 ml microcentrifuge tubes (if processing 50 µl clotted blood) or 50 ml centrifuge tubes (if processing 1 ml clotted blood)
- Microcentrifuge (if processing 50 µl clotted blood) or centrifuge (if processing 1 ml clotted blood) capable of attaining 2000 x *g* with appropriate rotor for 50 ml centrifuge tubes
- Water baths heated to 55°C and 65°C
- Crushed ice

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

■ Optional: Water bath heated to 37°C if RNase A treatment is required

Important point before starting

■ In some steps of the procedure, one of two choices can be made. Choose ■ if processing 50 µl clotted blood; choose ◆ if processing 1 ml clotted blood samples.

Things to do before starting

■ Heat water baths to 55°C and 65°C for use in steps 3 and 19 of the procedure.

■ Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Add ■ 50 µl clotted blood to a sterile 1.5 ml microcentrifuge tube containing 550 µl Cell Lysis Solution or ◆ 1 ml clotted blood to a sterile 50 ml centrifuge tube containing 11 ml Cell Lysis Solution. Pipet up and down several times to mix.**
2. **Add ■ 3 µl or ◆ 60 µl Puregene Proteinase K (20 mg/ml), and mix by inverting 25 times.**
3. **Complete cell lysis by incubating at 55°C for 3 h to overnight, until all particulates are completely dissolved.**
4. **If you wish to include an optional RNase treatment, go to step 4a, otherwise proceed with step 4b.**

4a. **Add ■ 3 µl or ◆ 60 µl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate at 37°C for 15 min.**

4b. **No RNase A treatment is required. Proceed with step 5.**

5. **Quickly cool the sample to room temperature by placing on ice for 1 min.**
6. **Add ■ 200 µl or ◆ 4 ml Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.**
7. **Incubate on ice for ■ 5 min or ◆ 10 min.**
8. **Centrifuge at ■ 13,000–16,000 x *g* for 3 min or ◆ 2000 x *g* for 10 min. The precipitated proteins should form a tight, dark pellet.**
9. **Pipet ■ 600 µl isopropanol and 1 µl Glycogen Solution (20 mg/ml) or ◆ 12 ml isopropanol and 20 µl Glycogen Solution (20 mg/ml) into a clean ■ 1.5 ml microcentrifuge tube or ◆ 50 ml centrifuge tube.**
10. **Add the supernatant from step 8 by pouring carefully.**
    Make sure not to dislodge the protein pellet when transferring the supernatant.
11. **Mix by inverting gently 50 times.**

12. **Centrifuge at ■13,000–16,000 x *g* for 1 min or ◆ 2000 x *g* for 3 min.**

    The DNA should be visible as a small white pellet.

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 or ◆ 12 ml of 70% ethanol, and invert several times to wash the DNA pellet.**

15. **Centrifuge at ■13,000–16,000 x *g* for 1 min or ◆ 2000 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 or ◆ 400 µl DNA Hydration Solution.**

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.