QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from Gram-negative bacteria in whole blood using the Gentra® Puregene® Blood Kit

This protocol is designed for purification of DNA from 300 µl samples of whole blood using the Gentra Puregene Blood 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. Gentra Puregene Blood Kits 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.

- Gentra Puregene Blood Kit (1000 ml), cat. no. 158389
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water bath heated to 65°C
- Vortexer
- Ice and ice bucket
- 70% ethanol*
- Isopropanol
- Optional: water bath heated to 37°C if RNase A treatment is required

Things to do before starting

- Heat water bath to 65°C
- Optional: heat water bath to 37°C for step 6 of the procedure if RNase A treatment is required

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

Procedure

1. **Transfer 900 µl RBC Lysis Solution to a 1.5 ml microcentrifuge tube.**
2. **Add 300 µl whole blood, mix by inverting several times, and incubate at room temperature for 10 min.**
3. **Centrifuge at 13,000–16,000 x *g* for 20 s.**
4. **Remove the supernatant with a pipette leaving behind the visible cell pellet and approximately 10–20 µl of the residual liquid. Vortex vigorously for 20 s at high speed to resuspend the cells.**
5. **Add 300 µl Cell Lysis Solution, and mix gently by pipetting up and down until cells are resuspended.**

   Note: For some bacterial species, heating the sample to 80°C for 5 min may be required to complete cell lysis.
6. **If you wish to include an optional RNase treatment, go to step 6a, otherwise proceed with step 6b.**

6a. **Add 1.5 µl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate for 15 min to 1 h at 37°C. Proceed with step 7.**

6b. **No RNase A treatment is required. Proceed with step 7.**

7. **Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).**
8. **Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**

   Note: For bacterial species with high mucopolysaccharide content, incubating on ice for 5–15 min might 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 into a clean 1.5 ml microcentrifuge tube. Add the supernatant from the previous step by pouring carefully.**

    Be sure the protein pellet is not dislodged during pouring.
11. **Mix by inverting gently 50 times.**
12. **Centrifuge for 1 min at 13,000–16,000 x *g*.**

    The DNA might 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 300 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
15. **Centrifuge for 1 min at 13,000–16,000 x *g*.**

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 100 µl DNA Hydration Solution.**
19. **Incubate at 65°C for 1 h to dissolve the DNA.**
20. **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.