QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from Gram-positive bacteria in whole blood using the Gentra® Puregene® Yeast/Bact. Kit

This protocol is designed for purification of DNA from 300 µl samples of whole blood using the Gentra Puregene Yeast/Bact. 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 Yeast/Bact. 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 Yeast/Bact. Kit (5 ml), cat. no. 158522 or Gentra Puregene Yeast/Bact. Kit (200 ml), cat. no. 158567
- RBC Lysis Solution (450 ml), cat. no. 158902 or RBC Lysis Solution (1000 ml), cat. no. 158904
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 37°C, 65°C, and 80°C
- Vortexer
- Ice and ice bucket
- 70% ethanol*
- Isopropanol

* 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 for use in step 8, 80°C for use in step 10, and 65°C for use in step 24 of the protocol.
- Optional: heat water bath to 37°C for use in step 11 of the protocol if RNase A treatment is required.

Procedure

1. **Transfer 900 µl RBC Lysis Solution into a 1.5 ml microcentrifuge tube.**
2. **Add 300 µl whole blood, and mix by inverting several times.**
3. **Incubate at room temperature for 10 min, and mix by inverting at least once during the incubation.**
4. **Centrifuge for 20 s at 13,000–16,000 x *g*.**
5. **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.**
6. **Add 300 µl Cell Suspension Solution, and mix gently by pipetting up and down.**
7. **Add 1.5 µl Lytic Enzyme Solution, and mix by inverting 25 times.**
8. **Incubate at 37°C for 30 min. Invert tube occasionally during incubation.**
9. **Centrifuge for 1 min at 13,000–16,000 x *g*, and remove supernatant.**
10. **Add 300 µl Cell Lysis Solution, and mix gently by pipetting up and down to lyse the cells.**

    Note: For some bacterial species heating the sample to 80°C for 5 min might be required to complete cell lysis.

11. **If you wish to include an optional RNase treatment, go to step 11a, otherwise proceed with step 11b.**

11a. **Add 1.5 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 12.**

11b. **No RNase A treatment is required. Proceed with step 12.**

12. **Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).**
13. **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 necessary.

14. **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, incubate on ice for 5 min and repeat the centrifugation.

15. **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.

16. **Mix by inverting gently 50 times.**

17. **Centrifuge for 1 min for 13,000–16,000 x *g*.**

    The DNA might be visible as a small white pellet.

18. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**

19. **Add 300 µl of 70% ethanol, and invert several times to wash the DNA pellet.**

20. **Centrifuge for 1 min at 13,000–16,000 x *g*.**

21. **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.

22. **Allow DNA to air dry at room temperature for 10–15 min.**

23. **Add 100 µl DNA Hydration Solution.**

24. **Incubate at 65°C for 1 h to dissolve the DNA.**

25. **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.