QIAGEN Supplementary Protocol:

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

This protocol is designed for purification of DNA from 1 g samples of soil 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
- Recommended: Glycogen Solution (500 µl) cat. no. 158930 (if DNA yields are expected to be low)
- Tween 20
- 100 mM sodium phosphate buffer (pH 8)
- 15 ml tubes, 1.5 ml microcentrifuge tubes
- Centrifuge, microcentrifuge
- Water baths heated to 37, 65, and 80°C
- Vortexer
- Shaker
- 70% ethanol*
- Isopropanol
- Ice and ice bucket

* 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 steps 6 and 7, 80°C for use in step 6, and 65°C for use in step 21 of the protocol.

Procedure

1. **Transfer 1 g soil to a 15 ml centrifuge tube containing 2 ml 100 mM sodium phosphate buffer.**
2. **Add 1 µl of Tween 20 and shake at 250 rpm for 30 min.**
3. **Centrifuge for 3 min at 2000 x *g*.**
4. **Dispense 500 µl Cell Lysis Solution into a clean 1.5 ml microcentrifuge tube. Add 100 µl supernantant from the previous step, and mix thoroughly by pipetting up and down several times.**
5. **Add 3 µl Lytic Enzyme Solution, and mix by inverting 25 times.**
6. **Incubate at 37°C for 30 min. Invert tube occasionally during incubation.**

   Note: For some bacterial species heating the sample to 80°C for 5 min might be required to complete cell lysis.
7. **If you wish to include an optional RNase treatment, go to step 7a, otherwise proceed with step 7b.**

7a. **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. Proceed with step 8.**

7b. **No RNase A treatment is required. Proceed with step 8.**

8. **Incubate on ice for 1 min to quickly cool the sample to room temperature (15–25°C).**
9. **Add 200 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
10. **Centrifuge for 3 min at 13,000–16,000 x *g*.**

    The precipitated proteins should form a tight pellet.
11. **Pipet 600 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.
12. **Recommended: Add 1 µl Glycogen Solution.**
13. **Mix by inverting gently 50 times.**
14. **Centrifuge for 5 min at 13,000–16,000 x *g*.**

    The DNA might be visible as a small white pellet.
15. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**

16. **Add 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
17. **Centrifuge for 1 min at 13,000–16,000 x *g*.**
18. **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.
19. **Allow DNA to air dry at room temperature for 10–15 min.**
20. **Add 20 µl DNA Hydration Solution.**
21. **Incubate at 65°C for 1 h to dissolve the DNA.**
22. **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.