QIAGEN Supplementary Protocol:

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

This protocol is designed for purification of DNA from 0.5 or 1 ml samples of Gram-positive bacteria culture medium 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
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 37°C, 65°C, and 80°C
- Vortexer
- 70% ethanol*
- Isopropanol
- Ice and ice bucket

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

Important point before starting

- In some steps of the procedure, one of 2 choices can be made. Choose ■ if processing 0.5 ml of Gram-positive bacteria culture medium or choose ◆ if processing 1 ml Gram-positive bacteria culture medium.

Things to do before starting

- Heat water baths to 37°C for use in step 5, 80°C for use in step 7, and 65°C for use in step 21 of the protocol.
- Optional: heat water bath to 37°C for use in step 8 in the protocol if RNase A treatment is required.

Procedure

1. **Transfer the appropriate amount of cell suspension (e.g., overnight culture containing approximately ■ 0.5–1.5 x 10^9 cells or ◆ 1–3 x 10^9 cells) to a 1.5 ml microcentrifuge tube on ice.**
2. **Centrifuge for 5 s at 13,000–16,000 x *g*, and remove supernatant.**

   Note: For some species centrifugation for up to 60 s might be required to obtain a tight cell pellet.
3. **Add ■ 300 µl or ◆ 600 µl Cell Suspension Solution, and mix gently by pipetting up and down until cells are resuspended.**
4. **Add ■ 1.5 µl or ◆ 3 µl Lytic Enzyme Solution, and mix by inverting 25 times.**
5. **Incubate at 37°C for 30 min. Invert tube occasionally during incubation.**
6. **Centrifuge for 1 min at 13,000–16,000 x *g*, and remove supernatant.**
7. **Add ■ 300 µl or ◆ 600 µl Cell Lysis Solution, and mix gently by pipetting up and down to lyse the cells.**

   Note: For some species heating the sample up to 80°C for 5 min might be required to complete cell lysis.
8. **If you wish to include an optional RNase treatment, go to step 8a, otherwise proceed with step 8b.**

8a. **Add ■ 1.5 µl or ◆ 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 9.**

8b. **No RNase A treatment is required. Proceed with step 9.**

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

10. **Add ■ 100 µl or ◆ 200µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**

    Note: For species with high polysaccharide content, placing the sample on ice for 15 min to 1 h might be required.

11. **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.

12. **Pipet ■ 300 µl or ◆ 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.

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

14. **Centrifuge for 1 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 ■ 300 µl or ◆ 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 ■ 100 µl or ◆ 200 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.