QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from 25 ml Gram-negative bacteria culture medium using the Gentra® Puregene® Cell Kit

This protocol is designed for purification of DNA from 25 ml Gram-negative bacteria culture medium using the Gentra Puregene Cell 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 Cell 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.

- If no RNase treatment is required: Gentra Puregene Cell Kit (6.7 x 10^9) cat. no. 158388
- If RNase treatment is required: Gentra Puregene Cell Kit (2 x 10^8) cat. no. 158745; Gentra Puregene Cell Kit (8 x 10^8) cat. no. 158767; or Gentra Puregene Cell Kit Plus (6.7 x 10^9) cat. no. 158788
- 50 ml centrifuge tubes
- Centrifuge
- Water baths heated to 65 and 80°C
- Vortexer
- Shaker
- Ice and ice bucket
- 70% ethanol*
- Isopropanol
- Optional: water bath heated to 37°C if RNase A treatment is required

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

Things to do before starting

- Heat water baths to 80°C for use in step 4, and 65°C for use in step 19 of the procedure.
- Optional: heat water bath to 37°C for use in step 5 of the procedure if RNase A treatment is required.

Procedure

1. **Transfer 25 ml cell suspension (e.g., overnight culture containing approximately 25–75 x 10^9 cells) to a 50 ml centrifuge tube.**
2. **Centrifuge for 3 min at 1000 x *g*, and remove supernatant.**
3. **Add 15 ml Cell Lysis Solution, and mix gently by pipetting up and down until cells are resuspended.**
4. **Incubate at 80°C for 5 min.**
5. **If you wish to include an optional RNase treatment, go to step 5a, otherwise proceed with step 5b.**

5a. **Add 75 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 6.**

5b. **No RNase A treatment is required. Proceed with step 6.**

6. **Quickly cool the sample to room temperature (15–25°C) by placing on ice for 1 min.**
7. **Add 5 ml 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 5 min might be required.

8. **Centrifuge for 10 min at 2000 x *g*.**

   Note: For species with high polysaccharide content, centrifugation at 4°C might be required.

   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.

9. **Pipet 15 ml isopropanol into a clean 50 ml centrifuge tube. Add the supernatant from the previous step by pouring carefully.**

   Be sure the protein pellet is not dislodged during pouring.

10. **Mix by inverting gently 50 times.**
11. **Centrifuge for 3 min for 2000 x *g*.**

    The DNA might be visible as a small white pellet.

12. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
13. **Add 15 ml of 70% ethanol, and invert several times to wash the DNA pellet.**

14. **Centrifuge for 1 min at 2000 x *g*.**
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. **The pellet might be loose and easily dislodged.**
17. **Allow DNA to air dry at room temperature for 10–15 min.**
18. **Add 2.5 ml 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.