QIAGEN Supplementary Protocol:

Rapid purification of archive-quality DNA from 1–2 x 10^6, 3–5 x 10^6, or 6–9 x 10^6 cells using the Gentra® Puregene® Cell Kit

This protocol is designed for rapid purification of DNA from 1–2 x 10^6, 3–5 x 10^6, or 6–9 x 10^6 cells using the Gentra Puregene Cell Kit.

Gentra Puregene Cell 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), Gentra Puregene Cell Kit (8 x 10^8), or Gentra Puregene Cell Kit Plus (6.7 x 10^9), cat. nos. 158745, 158767, and 158788
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 1.5 ml microcentrifuge tubes (for processing up to 5 x 10^6 cells) or 2 ml microcentrifuge tubes (if processing 6–9 x 10^6 cells)
- Microcentrifuge
- Water bath heated to 65°C
- Vortexer
- Crushed ice
- 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.

Important point before starting

- In some steps of the procedure, one of three choices can be made. Choose ■ if processing 1–2 x 10^6 cells; choose ◆ if processing 3–5 x 10^6 cells; choose ● if processing 6–9 x 10^6 cells.

Things to do before starting

- Heat water bath to 65°C for use in step 19 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Add the appropriate number of cells in balanced salt solution or culture medium to a ■ 1.5 ml microcentrifuge tube, ◆ 1.5 ml microcentrifuge tube, or ● 2 ml microcentrifuge tube.**
2. **Centrifuge at 13,000–16,000 x *g* for 5 s to pellet the cells.**
3. **Remove supernatant leaving behind 10–20 µl residual liquid, and resuspend the cells by vortexing at maximum speed.**
4. **Add ■ 300 µl, ◆ 600 µl, or ● 750 µl Cell Lysis Solution to the resuspended cells, and mix by pipetting up and down.**
5. **Usually no incubation is required. If cells clumps are visible after mixing, incubate at 37°C until the solution is homogeneous.**

   Note: Samples are stable in Cell Lysis Solution for at least 2 years at room temperature (15–25°C).
6. **If you wish to include an optional RNase treatment, go to step 6a, otherwise proceed directly with step 6b.**

6a. **Add ■ 1.5 µl, ◆ 3 µl, or ● 3.75 µl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate at 37°C for 5 min. Proceed with step 7.**

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

7. **Quickly cool the sample to room temperature by placing on ice for 1 min.**
8. **Add ■ 100 µl, ◆ 200 µl, or ● 250 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**

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

   The precipitated proteins should form a tight pellet. If the protein pellet is not visible, 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 1 min.

10. **Pipet ■ 300 µl, ◆ 600 µl, or ● 750 µl isopropanol into a clean ■ 1.5 ml microcentrifuge tube, ◆ 1.5 ml microcentrifuge tube, or ● 2 ml microcentrifuge tube. Add the supernatant from the previous step by pouring carefully.**

    Make sure not to dislodge the protein pellet when transferring the supernatant.

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

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

    The DNA may 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, ◆ 600 µl, or ● 750 µl of 70% ethanol, and invert several times to wash the DNA pellet.**

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

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 ■ 50 µl, ◆ 100 µl, or ● 100 µl DNA Hydration Solution to the tube containing the pellet.**

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

20. **Incubate at room temperature overnight with gentle shaking. Ensure tube cap 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.