QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from 5 buccal brushes using the Gentra® Puregene® Buccal Cell Kit

This protocol is designed for purification of DNA from 5 buccal brushes using the Gentra Puregene Buccal Cell Kit.

The Gentra Puregene Buccal Cell Kit enables purification of high-molecular-weight DNA from buccal cells. 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 Buccal Cell 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 Buccal Cell Kit (10), (100), or (400), cat. nos. 158822, 158845, and 158867
- Puregene Proteinase K (650 µl) or (5 ml), cat. nos. 158918 and 158920
- Lysozyme
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 55°C and 65°C
- 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.

Things to do before starting

- Heat water baths to 55°C for use in step 3b and 65°C for use in steps 3a and 18 of the procedure.
- Optional: Heat water bath to 37°C if RNase treatment is required.

Procedure

1. **Dispense 600 µl Cell Lysis Solution into a clean 1.5 ml microcentrifuge tube.**
2. **To collect buccal cells, scrape the inside of the mouth 10 times with each of 5 different sterile nylon-bristle cytology brushes. Dip brushes up and down 10 times in the Cell Lysis Solution.**

   For best results, wait at least 1 h after eating or drinking to collect buccal cells.

   DNA may be purified immediately or samples may be stored on the collection brushes for up to 1 month at room temperature (15–25°C).
3. **Complete cell lysis by following step 3a or 3b below:**

3a. **Incubate at 65°C for 15 min to 1h. Proceed with step 4.**

3b. **If maximum DNA yield is required, add 3 µl Puregene Proteinase K (20 mg/ml) and incubate lysate at 55°C for 1 h to overnight. Proceed with step 4.**

4. **If you wish to include an optional RNase treatment, go to step 4a, otherwise proceed directly to step 4b.**

4a. **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 5.**

4b. **No RNase A treatment is required. Proceed with step 5.**

5. **Quickly cool the sample to room temperature by placing on ice for 1 min.**
6. **Add 200 µl Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.**
7. **Incubate on ice for 5 min.**
8. **Centrifuge at 13,000–16,000 x *g* for 3 min.**

   The precipitated proteins should form a tight, white pellet.
9. **Pipet 600 µl isopropanol and 1 µl Glycogen Solution (20 mg/ml) into a clean 1.5 ml microcentrifuge tube, and 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 and incubate at room temperature for 5 min.**
11. **Centrifuge at 13,000–16,000 x *g* for 5 min.**

    The DNA pellet may be visible as a small white pellet, depending on yield.

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 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**
14. **Centrifuge at 13,000–16,000 x *g* for 1 min.**
15. **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.
16. **Allow DNA to air dry at room temperature for 10–15 min.**
17. **Add 50 µl DNA Hydration Solution to the tube containing the pellet.**
18. **Incubate at 65°C for 1 h to dissolve the DNA.**
19. **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.