Protocol: DNA Purification from a Buccal Brush Using the Gentra Puregene Buccal Cell Kit

This protocol is for purification of genomic DNA from 1 buccal brush using the Gentra Puregene Buccal Cell Kit.

Things to do before starting

- Preheat water baths to 55°C for use in step 3b and 65°C for use in steps 3a and 17 of the procedure.
- Optional: Preheat water bath to 37°C for use in step 5 of the procedure.

Procedure

1. **To collect buccal cells, scrape the inside of the mouth 10 times with a Buccal Collection Brush.**

   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 brush for up to 1 month at room temperature (15–25°C).

2. **Dispense 300 µl Cell Lysis Solution into a 1.5 ml microcentrifuge tube. Remove the collection brush from its handle using sterile scissors or a razor blade, and place the detached head in the tube.**

   Samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

   If 300 µl Cell Lysis Solution is not sufficient to cover the head, the protocol must be scaled up to use a larger volume. Contact QIAGEN Technical Services for more information (see back cover or visit www.qiagen.com).

3. **Complete cell lysis by following step 3a or 3b below:**

3a. **Incubate at 65°C for at least 15 min (up to 60 min for maximum yield).**

3b. **If maximum yield is required, add 1.5 µl Puregene Proteinase K (cat. no. 158918), mix by inverting 25 times, and incubate at 55°C for at least 1 h (up to overnight for maximum yield).**

4. **Remove the collection brush head from the Cell Lysis Solution, scraping it on the sides of the tube to recover as much liquid as possible.**

5. **Optional: If RNA-free DNA is required, add 1.5 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Incubate for 1 min on ice to quickly cool the sample.**

   Samples can be incubated at 37°C for up to 1 h.

6. **Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**

7. **Incubate for 5 min on ice.**

8. **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, incubate on ice for 5 min and repeat the centrifugation.

9. **Pipet 300 µl isopropanol and 0.5 µl Glycogen Solution (cat. no. 158930) 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.**
11. **Centrifuge for 5 min at 13,000–16,000 x *g*.**
12. **Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
13. **Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.**
14. **Centrifuge for 1 min at 13,000–16,000 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. Allow to air dry for 5 min.**

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

16. **Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.**
17. **Incubate at 65°C for 1 h to dissolve the DNA.**
18. **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.**