Protocol: DNA Purification from Compromised Blood Samples Using the Gentra Puregene Blood Kit

This protocol is for purification of genomic DNA from 10 ml compromised whole blood using the Gentra Puregene Blood Kit. Blood samples stored at –20°C, or at room temperature (15–25°C) for more than 24 hours, or at 2–8°C for more than 5 days are considered compromised.

Things to do before starting

- Preheat water baths to 37°C and 65°C for use in steps 7 and 19 of the procedure.
- Frozen blood samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

Procedure

1. **Dispense 30 ml RBC Lysis Solution into a 50 ml centrifuge tube. Add 10 ml whole blood or bone marrow, and mix by inverting.**
2. **Incubate for 5 min at room temperature (15–25°C). Invert gently at least once during the incubation.**
3. **Centrifuge for 5 min at 2000 x *g*.**
4. **Carefully discard the supernatant by pipetting and leave approximately 3.5 ml of the supernatant and the brownish pellet in the tube.**
5. **Vortex the tube vigorously to resuspend the pellet in the residual liquid.**

   Vortexing greatly facilitates cell lysis in the next step.

   The pellet should be completely dispersed after vortexing.
6. **Add 10 ml Cell Lysis Solution, and vortex vigorously for 10 s to lyse the cells.**
7. **Incubate at 37°C for at least 2 h.**

   Samples can additionally be incubated at room temperature overnight to ensure that the solution is homogenous.

   Note: Do not incubate overnight at 37°C.
8. **Optional: If RNA-free DNA is required, add 50 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Then incubate for 5 min on ice to quickly cool the sample.**
9. **Add 4.5 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
10. **Centrifuge for 10 min at 2000 x *g*.**

    The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

11. **Pipet 13.5 ml isopropanol into a clean 50 ml centrifuge tube. Add 135 µl Glycogen Solution (cat. no. 158930). Add the supernatant from the previous step by pouring carefully.**

    Be sure the protein pellet is not dislodged during pouring.

12. **Mix by inverting gently 50 times.**
13. **Centrifuge for 3 min at 2000 x *g*.**

    The DNA will be visible as a small white pellet.

    The pellet might be loose and easily dislodged.

14. **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.**
15. **Add 10 ml of 70% ethanol and invert several times to wash the DNA pellet.**
16. **Centrifuge for 1 min at 2000 x *g*.**
17. **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 5–10 min.**

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

18. **Add 500 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.**
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.**