DNA Purification from ISWAB-DNA Using the Gentra Puregene Blood Kit

This protocol is for purification of genomic DNA from ISWAB-DNA samples with 300µl or 1mL using the Gentra Puregene Blood Kit.

Things to do before starting

1. Preheat water bath to 65°C for use step 19 of the procedure.
2. Optional: Preheat water bath to 37°C for use in step 8 of the procedure.

Protocol
Skip steps 1-6 from the Gentra Puragene whole Blood gDNA extraction protocol

Start at Step 7 below:

7. If you use 300µL input volume from iSWAB sample then add ■300µl Lysis Solution. If you use the full volume in the collected iSWAB tube then add ▲1ml, Lysis Solution, and pipet up and down to lyse the cells or vortex vigorously for 10 seconds.

   - Usually no incubation is required; however, if cell clumps are visible, incubate at 37°C until the solution is homogeneous. Samples are stable in the Cell Lysis Solution for at least 2 years at room temperature.

8. Optional: If RNA-free DNA is required, add ■ 1.5µl or ▲15µl, RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Then incubate for ■1 min or ▲3 min on ice to quickly cool the sample.

9. Add ■100µl, or▲ 1ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.

10. Centrifuge for ■1 min at 13,000–16,000 x g, or ▲5 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. Pipette ■300µl isopropanol into a clean 1.5ml tube, or ▲3ml isopropanol into a clean 15 ml tube, and 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 until the DNA is visible as threads or a clump.

13. Centrifuge for ■1 min at 13,000–16,000 x g, or ▲3 min at 2,000 x g.

    - The DNA may be visible as a small white pellet.

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 ■300µl, or ▲3ml of 70% ethanol and invert several times to wash the DNA pellet.

16. Centrifuge for ■1 min at 13,000–16,000 x g, or ▲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. Air dry the pellet for ■5 min, or ▲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 ■100µl, or ▲300µl DNA Hydration Solution and vortex for 5 second at medium speed to mix.

19. Incubate at 65°C for ■5 min, or ▲1 hour 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.