QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from clotted whole blood using Clotspin® Baskets and the Gentra® Puregene® Blood Kit

This protocol is designed for purification of DNA from 5–7 ml clotted whole blood using Clotspin Baskets and the Gentra Puregene Blood Kit.

Gentra Puregene 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. The Gentra Puregene Blood 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 Blood Kit (1000 ml), cat no. 158389
- Puregene Proteinase K (650 µl) or (5 ml), cat nos. 158918 and 158920
- Glycogen Solution (500 µl), cat. no. 158930
- Clotspin Baskets (50), cat. no. 158932
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 50 ml centrifuge tubes
- Centrifuge, capable of attaining 2000 x *g*, with appropriate rotor for 50 ml centrifuge tubes
- Water baths heated to 37°C, 55°C, and 65°C
- Vortexer
- Tube rotator

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Crushed ice

Things to do before starting

- Heat water baths to 37°C, 55°C, and 65°C for use in steps 1, 18, and 33 of the procedure.

Procedure

1. **Thaw the frozen clotted blood sample quickly at 37°C, and then place on ice.**

   Note: If clotted blood sample has not been frozen, freeze at –70°C to –80°C for a minimum of 30 min.

2. **Invert the sample tube to loosen the clot.**
3. **Pour 5–7 ml blood clot into the Clotspin Basket contained in the 50 ml tube.**
4. **Centrifuge at 2000 x *g* for 5 min to disperse the clot.**
5. **Add 15 ml RBC Lysis Solution to the Clotspin Basket to rinse as much of the residual clot material through as possible. Carefully raise the Clotspin Basket in the tube to allow the entire 15 ml of RBC Lysis Solution to flow through freely.**
6. **Remove the insert and, using a pipet tip, transfer any clot material retained in the Clotspin Basket to the filtrate in the 50 ml tube.**
7. **Vortex vigorously for 3 s at high speed to further disperse the clotted material.**
8. **Place sample on a tube rotator for 5 min at room temperature (15–25°C).**
9. **Vortex tube vigorously for 3 s at high speed.**
10. **Centrifuge at 2000 x *g* for 5 min to pellet the white blood cells and clot particulates.**

    Note: Pellet will be reddish in color.

11. **Carefully discard the supernatant, taking care that the pellet remains in the tube.**
12. **Add 5 ml RBC Lysis Solution to the pellet, and vortex vigorously for 3 s at high speed.**
13. **Incubate on a tube rotator for 5 min at room temperature.**
14. **Centrifuge at 2000 x *g* for 5 min to pellet the white blood cells and particulates.**
15. **Carefully discard the supernatant, leaving behind 200 µl of residual liquid.**
16. **Vortex the tube vigorously at high speed to resuspend the pellet in the residual liquid.** This greatly facilitates cell lysis in step 17.
17. **Add 5 ml Cell Lysis Solution and 25 µl Puregene Proteinase K (20 mg/ml). Vortex vigorously for 10 s at high speed to initiate cell lysis and protein digestion.**

    Note: For high-throughput processing of multiple samples, prepare a master mix containing Cell Lysis Solution and Puregene Proteinase K. Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

18. **Complete cell lysis by incubating at 55°C for 2 h to overnight, until all particulates are completely dissolved. To facilitate digestion, vortex vigorously for 10 s at high speed at least 3 times during the incubation.**

19. **Incubate on ice for 5 min.**
20. **Add 1.67 ml Protein Precipitation Solution to the cell lysate, and vortex vigorously for 20 s at high speed.**
21. **Centrifuge at 2000 x *g* for 10 min.**
22. **Incubate on ice for 2 min.**
23. **Pipet 5 ml isopropanol and 10 µl Glycogen Solution (20 mg/ml) into a clean 50 ml centrifuge tube.**
24. **Add the supernatant from step 21 by pouring carefully.**
    Make sure not to dislodge the protein pellet when transferring the supernatant.
25. **Mix by inverting tube gently 50 times.**
26. **Centrifuge at 2000 x *g* for 3 min.**
    The DNA should be visible as a small white pellet.
27. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
28. **Add 5 ml of 70% ethanol, and invert the tube several times to wash the DNA pellet.**
29. **Centrifuge at 2000 x *g* for 1 min.**
30. **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.
31. **Allow DNA to air dry at room temperature for 10 min.**
32. **Add 500 µl DNA Hydration Solution to the tube containing the pellet.**
33. **Incubate at 65°C for 1 h to dissolve the DNA.**
34. **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.**