QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from umbilical cord blood using the Gentra® Puregene® Tissue Kit or Gentra Puregene Blood Kit

This protocol is designed for purification of DNA from 2 ml samples of umbilical cord blood using the Gentra Puregene Tissue Kit or 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 Tissue Kit and the Gentra Puregene Blood Kit are intended for molecular biology applications. These products are 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.

- If RNase treatment is required: Gentra Puregene Tissue Kit (4 g) or (33 g), cat. nos. 158667 and 158689
- If no RNase treatment is required: Gentra Puregene Blood Kit (1000 ml), cat. no. 158389; we also recommend using Puregene Proteinase K (650 µl) or (5 ml), cat. nos. 158918 and 158920
- Glycogen Solution (500 µl), cat. no. 158930
- 100% isopropanol
- 70% ethanol*
- Pipets and pipet tips
- 15 ml centrifuge tubes
- Centrifuge, capable of attaining 2000 x g, with appropriate rotor for 15 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, 9a, and 23 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. If sample is frozen, thaw quickly in a 37°C water bath. Immediately place thawed sample on ice until ready to proceed with the DNA purification procedure.

2. Pipet 6 ml RBC Lysis Solution into a clean 15 ml centrifuge tube. Add 2 ml umbilical cord blood to the tube containing RBC Lysis Solution.

3. Mix by inverting, and incubate for 15 min at room temperature (15–25°C) on a tube rotator.

4. Centrifuge at 2000 x *g* for 5 min.

5. Carefully discard the supernatant leaving behind the white blood cell pellet and approximately 100–200 µl of the residual liquid.

6. Vortex vigorously to resuspend the cells in the residual liquid. This greatly facilitates cell lysis in step 7, below.

7. Add 2 ml Cell Lysis Solution to the resuspended cells and vortex vigorously for 10 s to lyse the cells.

8. Incubate samples overnight at room temperature to ensure that the samples are homogeneous.

Note: Samples are stable in Cell Lysis Solution for at least 2 years at room temperature. To store samples in Cell Lysis Solution, process samples in a laminar flow hood with new reagents or reagents only opened in the hood. Lyse cells using aseptic technique during cell lysis (steps 1–7).

9. If cell or tissue clumps are visible in the lysate, go to step 9a, otherwise proceed directly with step 9b.

9a. Recommended: Add 10 µl Puregene Proteinase K (20 mg/ml), and mix by inverting 25 times. Incubate at 55°C overnight or until tissue has dissolved. If possible, invert tube periodically during the incubation.

9b. No cell or tissue clumps are visible. Proceed with step 10.

10. If you wish to include an optional RNase treatment, go to step 10a, otherwise proceed with step 10b.

10a. Add 10 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.

10b. No RNase A treatment is required. Proceed with step 11.

11. **Quickly cool the sample to room temperature by placing on ice for 1 min.**
12. **Add 667 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
13. **Centrifuge at 2000 x *g* for 5 min.**

    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.
14. **Pipet 2 ml isopropanol and 20 µl Glycogen Solution (20 mg/ml) into a clean 15 ml centrifuge tube. Add the supernatant from step 13 by pouring carefully.**

    Make sure not to dislodge the protein pellet when transferring the supernatant.
15. **Mix by inverting gently 50 times.**
16. **Centrifuge for 2000 x *g* for 3 min.**

    The DNA will be visible as a small white pellet.
17. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
18. **Add 2 ml of 70% ethanol, and invert several times to wash the DNA pellet.**
19. **Centrifuge at 2000 x *g* for 1 min.**
20. **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.
21. **Allow DNA to air dry at room temperature for 5–10 min.**
22. **Add 150 µl DNA Hydration Solution to the tube containing the pellet.**
23. **Incubate at 65°C for 1 h to dissolve the DNA.**
24. **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.