QIAGEN Supplementary Protocol:

Purification of archive-quality DNA from human dried blood using the Gentra® Puregene® Tissue Kit or Gentra Puregene Mouse Tail Kit

This protocol is designed for purification of DNA from 50 µl samples of dried human blood using the Gentra Puregene Tissue Kit or Gentra Puregene Mouse Tail 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.

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 Mouse Tail 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 Mouse Tail Kit (4 g), cat. no. 158267
- 100% isopropanol
- 70% ethanol*
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Water baths heated to 65°C and 55°C
- Vortexer
- Crushed ice
- Pipets and pipet tips
- Recommended: Glycogen Solution (500 µl), cat. no. 158930 (we recommend using glycogen in the purification procedure if DNA yields are expected to be <20 µg)

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

- Optional: Water bath heated to 37°C if RNase A treatment is required

Things to do before starting

- Heat water baths to 55°C and 65°C for use in steps 3 and 21 of the procedure.
- Optional: Heat water bath to 37°C if RNase A treatment is required.

Procedure

1. **Cut the fabric or filter paper containing 50 µl dried blood into small pieces, and place into a 1.5 ml microcentrifuge tube.**
2. **Add 600 µl Cell Lysis Solution and 3 µl Puregene Proteinase K (20 mg/ml).**
3. **Complete cell lysis by incubating at 55°C overnight. Invert tube periodically, if possible, or incubate with gentle shaking.**
4. **If you wish to include an optional RNase treatment, go to step 4a, otherwise proceed directly with step 4b.**

4a. **Add 3 µl RNase A Solution to the cell lysate, and mix by inverting the tube 25 times. Incubate at 37°C for 15 min.**

4b. **No RNase A treatment is required. Proceed with step 5.**

5. **Quickly cool the sample to room temperature (15–25°C) by placing on ice for 1 min.**
6. **Add 200 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
7. **Incubate on ice for 15 min.**
8. **Centrifuge at 13,000–16,000 x *g* for 3 min.**
   The precipitated proteins and pieces of fabric or filter paper should form a tight, reddish pellet.
9. **Pipet 600 µl isopropanol into a clean 1.5 ml microcentrifuge tube.**
10. **Add the supernatant from the previous step by pouring carefully.**
    Make sure not to dislodge the protein pellet when transferring the supernatant.
11. **Recommended: Add 1 µl Glycogen Solution (20 mg/ml).**
12. **Mix by inverting gently 50 times.**
13. **Incubate on ice for at least 15 min.**
14. **Centrifuge for 13,000–16,000 x *g* for 5 min.**
    The DNA may or may not be visible as a small white pellet, depending on yield.
15. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**
16. **Add 600 µl of 70% ethanol, and invert several times to wash the DNA pellet.**

17. **Centrifuge at 13,000–16,000 x *g* for 1 min.**

18. **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.

19. **Allow DNA to air dry at room temperature for 10–15 min.**

20. **Add 20 µl DNA Hydration Solution.**

    The expected yield is 0.5–2 µg DNA. Hydrating the DNA in 20 µl will give a DNA concentration of 50 ng/µl if the yield of DNA is 1 µg.

21. **Incubate at 65°C for 1 h to dissolve the DNA.**

22. **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.