QIAGEN Supplementary Protocol:

Purification of DNA from clotted blood using the FlexiGene® DNA Kit

This protocol is designed for purification of DNA from up to 10 ml clotted blood using the FlexiGene DNA Kit.

IMPORTANT: Please read the *FlexiGene DNA Handbook*, paying careful attention to the safety information, before beginning this procedure.

Equipment and reagents to be supplied by user

- FlexiGene DNA Kit (cat. no. 51204 or 51206)
- 100% isopropanol
- 70% ethanol*
- Pipets and sterile, DNase-free pipet tips with aerosol barrier
- 50 ml conical centrifuge tubes that tolerate 2000 x *g* and a centrifuge capable of attaining 2000 x *g*, equipped with a swing-out rotor
- Rotor–stator homogenizer (e.g., Polytron® Homogenizers from Kinematica AG, sold by Brinkmann Instruments, www.brinkmann.com/product.asp?path=36&ref=16 ; Omni Homogenizers from OMNI International, Inc., www.omni-inc.com)†
- Heating block or water bath
- Vortexer
- Disposable gloves

Important point before starting

- All centrifugation steps should be carried out at room temperature (15–25 C) in a swing-out rotor.

Things to do before starting

- Heat a heating block or water bath to 65°C for protein digestion in step 7 and dissolving the DNA in step 16.

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

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Procedure

1. **Transfer the clotted blood sample, including any residual liquid, into a 50 ml centrifuge tube.**
2. **Homogenize the sample using a rotor–stator homogenizer for at least 30 s at maximum speed until the sample is uniformly homogeneous.**
3. **Add 25 ml Buffer FG1, and mix by inverting the tube 5 times.**
4. **Centrifuge for 5 min at 2000 x *g* in a swing-out rotor.**
5. **Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 2 min, taking care that the pellet remains in the tube.**

   Note: In rare cases the pellet may be loose; so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of supernatant from the rim and sides of the tube onto the pellet.
6. **Add 5 ml Buffer FG2 and 50 µl QIAGEN® Protease. Close the tube and vortex immediately until the pellet is completely homogenized. Inspect the tube to check that homogenization is complete.**

   Note: When processing multiple samples, vortex each tube immediately after addition of Buffer FG2 and QIAGEN Protease. Do not wait until buffer has been added to all samples before vortexing.

   Usually 3–4 pulses of high-speed vortexing for 5 s each are sufficient to homogenize the pellet. However, traces of pellet with a jelly-like consistency (often barely visible) may remain. If this happens, add 1 ml Buffer FG2 and vortex again.
7. **Invert the tube 3 times, place it in a heating block or water bath, and incubate at 65°C for at least 25 min.**

   Note: The sample changes color from red to olive green, indicating protein digestion.
8. **Vortex again for 5s. Inspect the tube again to check that homogenization is complete.**
9. **Add 5 ml isopropanol (100%), and mix thoroughly by inversion until the DNA precipitate becomes visible as threads or a clump.**

   Note: Complete mixing with isopropanol is vital to precipitate the DNA and should be checked by visual inspection. For samples with very low white blood cell counts, in which the DNA may not be visible, invert the tube at least 20 times.
10. **Centrifuge for 3 min at 2000 x *g*.**

    Note: If the resulting pellets are loose, centrifugation can be prolonged or a higher *g*-force can be used.
11. **Discard the supernatant and briefly invert the tube onto a clean piece of absorbent paper, taking care that the pellet remains in the tube.**

    Note: In rare cases the pellet may be loose; so pour slowly.

    If the white blood cell count of the sample was sufficiently high, the DNA should be visible as a small white pellet.
12. **Add 5 ml 70% ethanol and vortex for 5 s.**

13. **Centrifuge for 3 min at 2000 x *g*.**

    Note: If the resulting pellets are loose, centrifugation can be prolonged or a higher *g*-force can be used.

14. **Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for at least 5 min, taking care that the pellet remains in the tube.**

    Note: In rare cases the pellet may be loose; so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of ethanol from the rim and sides of the tube onto the pellet.

15. **Air-dry the DNA pellet until all the liquid has evaporated (at least 5 min).**

    Note: Avoid over-drying the DNA pellet, since over-dried DNA is very difficult to dissolve.

16. **Add 1 ml Buffer FG3, vortex for 5 s at low speed, and dissolve the DNA by incubating for 1 h at 65°C in a heating block or water bath.**

    Note: If the DNA is not completely dissolved, incubate the solution overnight at room temperature. If a reduced volume of Buffer FG3 is used, the incubation time may need to be prolonged.