

User-Developed Protocol:

Isolation of genomic DNA from frozen clotted whole blood using the QIAGEN® Genomic-tip 100/G

This procedure has been adapted by customers from Genomic-tip Protocols, and is for use with the QIAGEN-tip or QIAGEN® Genomic-tip. **It has not been thoroughly tested or optimized by QIAGEN.**

The expected yield of genomic DNA from 0.5–2.5 ml of clotted whole blood should be in the range of 8–40 µg.

Please be sure to read the *QIAGEN Genomic DNA Handbook* and the detailed Sample Preparation and Lysis Protocol for Blood carefully before beginning this procedure.

Procedure

- 1. Weigh tissue. For every 50 mg add 1 ml of Lysis Buffer. Homogenize briefly to disrupt the solid mass.**

Lysis Buffer:

1 % Triton® X-100
600 mM Guanidine-HCl
10 mM EDTA
100 mM NaCl
10 mM Tris-Cl, pH 8.0

- 2. Add boiled RNase A to a final concentration of 20 µg/ml and incubate at 37°C for 1 h with gentle agitation.**
- 3. Add Proteinase K to a final concentration of 1 mg/ml and incubate at 50°C for 2–3 h with gentle agitation.**
- 4. Centrifuge sample for 10 minutes at 10–12,000 x g to pellet insoluble debris. Carefully transfer supernatant into a clean tube. Try to avoid carryover of particulate matter.**
- 5. Pre-equilibrate a QIAGEN Genomic-tip 100/G with 3 ml of Buffer QBT.**
- 6. Load the genomic sample onto the QIAGEN Genomic-tip 100/G.**
- 7. Wash the QIAGEN Genomic-tip 100/G with 3 x 10 ml of Buffer QC.**
- 8. Elute the ultrapure genomic DNA from the QIAGEN Genomic-tip 100/G with 5 ml of Buffer QF.**
- 9. Add 0.7 volumes of room-temperature isopropanol to precipitate the genomic DNA. Invert gently several times to mix. A visible, fibrous genomic precipitate should form immediately.**

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10. **Pellet DNA by centrifugation, or capture it on a Pasteur pipette which has been heated and bent into a hook at the end.**
11. **Wash genomic DNA with 3–5 ml of ice-cold 70% ethanol.**
12. **Briefly air-dry, and resuspend in an appropriate volume of TE.**
Note: Be careful not to over-dry the DNA. Ten minutes under ambient conditions should suffice.

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.asp.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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