**General remarks**

Take the appropriate safety measures for working in laboratories, especially for working with biological material.

The folowing method is a modification of Malferrari et al.: “High-quality genomic DNA from human whole blood and mononuclear cells”, Biotechniques 33(6): 1228-1230, 2002.

It is suited for the semi-automated isolation of many samples in 96-well format, e.g., using a Multiprobe II Plus pipetting roboter from Perkin-Elmer.

Label the final sample tube or plate with the necessary patient/sample identifier(s), preferably in machine (e.g., bar-code)- and human-readable form as suggested in “Sample_ID_memo.doc” by GEM-Bonn. Since there is a one-to-many relationship between a blood and a DNA sample, each DNA sample tube or plate should have a unique identifier.

**Material**

| Lysis buffer | 200 ml (sterilize by filtration, store at 4°C) |
|---|---|
| 3M Guanidine thiocyanate | 71 g |
| 20mM EDTA | 8 ml 0.5M EDTA, pH 8 |
| 10mM Tris-Cl, pH 6.8 | 4 ml 0.5M Tris-Cl, pH 6.8 |
| 40mg/ml Triton X-100 (1,06 g/ml) | 7.5 ml 100% or 37.5 ml 20% |
| 10 mg/ml DTT | 2 g DTT |
| Aqua dest., autoclaved | ad 200 ml |

Binding solution: 50 mg/ml Silica (2-10µm particles)

Suspend 2.5 g Silicon dioxide (Sigma, cat. no. S5631) in ~40 ml autoclaved auqa dest. and sedimend at 8 g for 12 min. Discard the turbid supernatant (to remove too small particles) and resuspend the pellet in 50 ml lysis buffer. Store at 4°C.

| Washing solution | 200 ml (store at room temperature) |
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| 25% ethanol, p.a. | 50 ml |
| 25% isopropanol, p.a. | 50 ml |
| 100mM NaCl | 5 ml 4 M NaCl |
| 10mM Tris-l, pH 8 | 2 ml 1M Tris-Cl, pH 8 |
| Aqua dest., autoclaved | ad 200 ml |

| Elution buffer (TE) | 1 l |
|---|---|
| 10mM Tris-Cl, pH 8 | 10 ml 1M Tris-Cl, pH 8 |
| 1mM EDTA | 2 ml 0.5M EDTA, pH 8 |
| Aqua dest., autoclaved | ad 1 l |

96-well plates (1.2 ml/well; ABgene, cat. no. 564)

Autoclaved sealing mats (ABgene, cat. no. AB0674); reusable.

Variable pipette: 200-1000 µl or pipetting roboter

Centrifuge such as Beckman Allegra 25R

**Method (Steps 1-9: ~ 5 h/48 samples, each sample divided into two 500 µl aliquots)**

1. Per well of a 96-well plate, add 250 µl lysis buffer to 500 µl blood (preserved as described in the SOP **"Collection and storage of blood for DNA preparation"**), close the plate with a sealing mat and mix thoroughly.

2. Add 250 µl binding solution, close the plate with a new sealing mat and mix thoroughly.

3. Centrifuge 2 min. at 114 g and remove the supernatant by pipetting. Be careful not to disturb the pellet.

4. Add 250 µl lysis buffer, close the plate with a new sealing mat and mix thoroughly.
Centrifuge 2 min. at 114 g and remove the supernatant by pipetting. Do not disturb the pellet.

5. Repeat step 4.

6. Add 250 µl washing solution, close the plate with a new sealing mat and mix thoroughly.
Centrifuge 2 min. at 114 g and remove the supernatant by pipetting. Do not disturb the pellet.
Optional: repeat the washing step.

7. Add 250 µl ethanol, abs., p.a., close the plate with a new sealing mat and mix thoroughly.
Centrifuge 2 min. at 114 g and remove the supernatant by pipetting. Do not disturb the pellet.
Air-dry the pellet.

8. Add 800 µl elution buffer, resuspend the pellet and incubate at 65°C for 3 min. Centrifuge at $v_{max}$ (e.g., at 4,100 rpm in the Allegra 25R).

9. Transfer the DNA-containing supernatants to a new 96-well plate. Close this plate with a new sealing mat or adhesive tape and store at 4°C. Use a unique identifier, e.g., bar code, for each 96-well plate. Document the sample distribution in the plate in computer-readable form, e.g., as an Excel file.

10. Determine the DNA quality and quantity by gel electrophoretic analysis of 10 µl aliquots as described in the SOP **"DNA quality and quantity"**.

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