General remarks

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

There are several options for DNA preparations from blood. For preparations from 5-10 ml blood, the NucleoSpin Blood XL kit (Macherey and Nagel) or the QIAamp DNA Blood Maxi kit (Qiagen) are recommended. For smaller volumes (up to 2 ml), the corresponding kits for up to 2 ml (same brands) or the Invisorb Spin Blood Midi kit (0.2-2 ml; INVITEK, cat. no. 10311105) are recommended (an example is given below). Preparations are to be performed according to the instructions of the manufacturers.

Label the final sample tube with the necessary patient/sample identifier, 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 should have a unique identifier.

Material

Microcentrifuge
Eppendorf Thermomixer (70°C)

Reaction Tube 15 ml (e.g. Falcon)
Centrifuge for 15 ml Reaction Tubes, e.g. Beckman Allegra 25R
Vortex mixer
Reaction tubes (1.5 ml or 2.0 ml)
Disposable 5 ml pipettes
Variable pipettes: 20-200 µl, 100-1000 µl
Sterile H_2O
Ethanol, absolute, p.a.
Lysis Buffer A
Binding Buffer A
Elution Buffer D
Buffer EL
Label Buffer EL
Proteinase K
Wash Buffer I *(caution: contains chaotropic component)*
Wash Buffer II
2.0 ml Receiver-Tubes
1.5 ml Receiver Tubes

Spin Filter

DNA extraction from 1 ml – 2 ml blood with Invisorb Spin Blood Midi Kit from Invitek

Storage: the Invisorb® Spin Blood Midi Kit should be stored dry, at room temperature (14 – 25°C). It is stable for at least 12 months under these conditions. Proteinase K must be stored at – 20°C. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

Prepare in advance: 1) the working solution of buffer EL by mixing 30 ml EL concentrate with 970 ml H_2O and store at 4°C; 2) transfer the needed amount of Elution Buffer D into an Eppendorf Tube, place tube at 70°C.

Method (Steps 1-7: ~ 5 h/24 samples)

1. Lysis of Erythrocytes and pelleting of the nucleus containing cells

Transfer 1-2 ml of the blood sample (preserved as described in the SOP **"Collection and storage of blood for DNA preparation"**) to a 15 ml centrifuge tube and add 5 ml of cold Buffer EL (4°C); mix shortly and incubate for 5 min at room temperature.

Centrifugate at 5,000 rpm for 5 min (swing-out rotor) or at 8,000 rpm for 5 min (fixed-angle rotor), e.g. in a Beckman Allegra 25R centrifuge. Carefully discard the supernatant. Add again 5 ml of Buffer EL to the cell pellet and centrifugate as before. Carefully discard the supernatant.

***Important:* Don't remove the cell pellet !**

2. Lysis at 70°C for 15 min in Thermomixer

Add 600 µl Lysis Buffer A to the cell pellet. Pipet several times up and down and transfer the lysate completely to a 2.0 ml Receiver Tube. Add 40 µl Proteinase K, vortex shortly and incubate in a Thermomixer at 70°C for 15 min. Place a Spin Filter into a 2.0 ml Receiver Tube.

3. Preparing for optimal binding

Add 300 µl Binding Buffer A to the lysis mix and vortex shortly.

4. Load the Spin Filter and DNA binding

Transfer 600 µl of the lysate onto the Spin Filter and incubate for 1 min. Centrifugate at
12,000 rpm for 1 min. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube. Reload the Spin Filter with the residual lysate and repeat the centrifugation step. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

5. Washing I

Add 500 µl Wash Buffer I and centrifugate at 12,000 rpm for 1min. Discard the filtrate, place the filter back into the 2.0 ml Receiver Tube.

6. Washing II

Add 550 µl Wash Buffer II and centrifugate at 12,000 rpm for 1min. Discard the filtrate, place the filter again into the Receiver Tube. Repeat the washing step once again. Discard the filtrate, put the filter back into the 2.0 ml Receiver Tube and finally centrifugate for 2 min at maximum speed to remove residual ethanol completely.

7. Elution of the DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of prewarmed Elution Buffer D. Incubation for 3 min. Centrifugate for 1 min at 10,000 rpm. Add again 100 µl of the Elution Buffer D and centrifugate as before. Store the DNA containing eluate at 4°C.

Note: The DNA can also be eluted with a lower or a higher volume of Elution Buffer D (depends on the expected yield of genomic DNA). But pay attention, that the minimum volume for the elution is 50 µl. If a quite large amount of DNA is expected, the volume for elution can be increased. To maximize the final yield, the use of two elution steps with an equal volume of Elution Buffer D is recommended.

8. Determine the DNA quality and quantity

By gel electrophoretic analysis of 2 µl aliquots as described in the SOP **“DNA quality and quantity”**.

Troubleshooting

Problem/probable cause	**Comments and suggestions**
Clogged Spin Filter • Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed or time. Reduce amount of starting material.
Low amount of extracted DNA	
• Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
• Incomplete elution	Prolong the incubation time with Elution Buffer D to 5-10 min or repeat elution step once again. Take higher volume of Elution Buffer D.
• Insufficient mixing with Binding Buffer A	Mix sample with Binding Buffer A by pipetting or by vortexing prior to transfer the sample onto the Spin Filter.
Spin Filter Surface turns yellow	
• Incomplete lysis	See above.
• Insufficient washing	Wash again with Wash Buffer II.
• Too old starting material	Use protocol 2.
Low concentration of extracted DNA	
• Too much Elution Buffer D	Elute the DNA with lower volume of Elution Buffer D.
Degraded or sheared DNA	
• Incorrect storage of starting material	Ensure that the starting material is fresh or stored under appropriate conditions (for long time storage at – 20°C)!
• Old material	Avoid thawing and freezing of the material. Old material often contains degraded DNA.
RNA contaminations of extracted DNA.	RNase A digestion

Genomic DNA does not perform well in downstream-applications	
• Ethanol carryover during elution	Increase time for removing of ethanol.
• Salt carryover during elution	Ensure that Wash Buffer is at room temperature. Check up Wash Buffer for salt precipitates. If there are any precipitates, solve these precipitates by careful warming.

Version	Tracking of changes	Name	Date