XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Some red blood cells are present in the white blood cell pellet.	The red blood cells have not been completely lysed.	Discard the supernatant from over the white blood cell pellet and repeat RBC lysis step. However, if a small quantity of red blood cells remain, this will not interfere with the subsequent DNA isolation steps.
A red blood cell clot was formed and cannot be lysed by RBC Lysis Buffer.	Old material or improper material storage; also the source of the blood (for example, rat's blood).	Follow the procedure given in Section IX. Sample preparation (Red blood cell clot formation).
	Insufficient quantity of anticoagulant in the blood sample.	Collect the sample material again, doubling the amount of anticoagulant or follow the procedure given in Section IX. Sample preparation (red blood cell clot formation).
The column becomes clogged during purification.	Improper storage of the sample material.	Storing blood samples at -80°C is highly recommended. Avoid subjecting the samples to repeated freeze/thaw cycles. Store blood samples at +4°C no longer than 24 h.
	Incomplete white blood cell lysis.	Resuspend the white blood cell pellet thoroughly and/or the extend incubation time at 55°C, vortexing the lysate at several-minute intervals.
Low yield of purified DNA.	Material contains few white blood cells.	Increase sample volume and/or decrease Elution Buffer volume to 20 µl.
	Incomplete white blood cell lysis.	Resuspend the white blood cell pellet thoroughly and/or the extend incubation time at 55°C, vortexing the lysate at several-minute intervals.
	The purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual BW2 Buffer is left in DNA Purification Column after the final centrifugation step.
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer to the membrane, heat it to 80°C. Apply Elution Buffer directly to the centre of the membrane. Extend the incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer to 200 µl.
	The pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.

Purified DNA is degraded.	Inappropriate sample storage conditions.	Storing blood samples at -80°C is recommended. Avoid subjecting the sample material to repeated freeze/thaw cycles. Store blood samples at +4°C no longer than 24h.
Isolated DNA is of poor purity.	Incomplete protein digestion due the reduced Proteinase K activity.	Prepare a fresh Proteinase K solution. Ensure Proteinase K solution is stored at -20°C.
	One of the washing steps was omitted.	Repeat the isolation, performing both washing steps.
	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual BW2 Buffer is left in DNA Purification Column after centrifugation in step 16.
RNA contamination present.	Both DNA and RNA were bound to membrane.	If RNA could interfere in downstream applications, the additional RNase A step should be included (see Section VIII. Recommendations and important notes).