XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Column becomes clogged during purification.	Inappropriate tissue homogenization.	Select the appropriate homogenization conditions (see section IXA).
	Incomplete protein degradation.	Prepare a fresh Proteinase K solution. Ensure that Proteinase K solution is stored at -20°C. Extend the vortexing time, incubate the tissue withProteinase K in TL Buffer for 16 h until the lysate is clear.
	Tissue remains were transferred onto the membrane.	Pipette the supernatant carefully, without disturbing the tissue pellet.
	DNA Purification Column is overloaded.	Do not exceed the recommended tissue amount or number of cell taken for DNA isolation.
Low yield of purified DNA.	The tissue was incorrectly stored or preserved; DNA degradation.	Store tissue at -80°C. Avoid subjecting the sample material to repeated freeze/thaw cycles. Ensure that a good quality fixative is used and that the storage conditions are adequate.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in TL Buffer. The tissue must first be fragmented into the smallest possible pieces and homogenized by an appropriate method.
	Incomplete tissue lysis.	Ensure optimal conditions for Proteinase K activity. The tissue should be as well-fragmented as possible, increase the vortexing time, incubate the tissue with Proteinase K in TL Buffer for 16 h.
	Reduced Proteinase K activity.	Inappropriate storage conditions for Proteinase K. When applicable, ensure that the sample material is thoroughly washed with PBS buffer (see section IX B, C, E and H).
	DNA Purification Column has become clogged.	See "Column becomes clogged during purification".
	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.

Isolated DNA is of low purity.	Incomplete protein degradation.	Prepare a fresh Proteinase K solution. Ensure that the solution is stored at -20°C. Extend the vortexing time, incubate the tissue with Proteinase K in TL Buffer for 16 h until the lysate is clear.
	Reduced Proteinase K activity.	Inappropriate storage conditions for Proteinase K. When applicable, ensure that the sample material is thoroughly washed with PBS buffer (see section IX B, C, E and H).
Purified DNA is degraded.	Old or damaged material was used.	Performing an isolation from fresh or properly preserved tissues is recommended.
	Inappropriate tissue storage conditions or improper preservation.	Store tissue at -80ºC. Avoid subjecting the sample material to repeated freeze/thaw cycles. Ensure that a good quality fixative is used and that the storage conditions are adequate.
	The DNA degraded as a result of over-intensive homogenization.	The recommended homogenization conditions should be applied (see section IXA).
RNA contamination present.	Tissue containing much RNA.	Perform digestion with RNase A (step 4 of the Isolation Protocol).
Inhibition of downstream enzymatic reactions.	Purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual TW2 Buffer is left in DNA Purification Column after centrifugation in step 13.