XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Column becomes clogged during purification.	Swab sample contained food remains.	Repeat isolation, ensuring that person providing sample does not consumed any food or drink for 30 minutes prior to sample collection (refer to section IXB. Sample preparation).
Low yield of purified DNA.	Improper sample collection method. Swab contains too few peeling cells.	When collecting a sample, ensure that swab stick is scraped firmly against inside of cheek (refer to IXB. Sample preparation).
	Semen was collected from a male with oligospermia.	Use more semen material, increasing the volume of relevant solutions (SSL Buffer, Proteinase K, DTT, SSB Buffer) and of ethanol. Apply 700 µl of lysate onto a minicolumn and repeat as necessary. After each application, centrifuge minicolumn at 11 000-15 000 x g for 60 s and discard supernatant.
	Incomplete cell lysis.	Extend incubation time at 56°C. Mix by inverting at several-minute intervals.
	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual SSW2 Buffer is left in the purification minicolumn after centrifugation in step 18.
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer to membrane, heat it to 80°C. Apply Elution Buffer directly to centre of membrane. Extend incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer to 200 µl.
	pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
Isolated DNA is of poor purity.	Swab sample is of very low purity.	Use 600 µl of SSW2 Buffer during second washing step (step 16 of Isolation Protocol), and centrifuge at 11 000-15 000 x g for 60 s. Empty collection tube and re-spin dry minicolumn (step 18 of the Isolation Protocol). Ensure that person providing sample does not consume any food or drink during 30 min prior to sample collection.
	Incomplete protein digestion as a result of reduced Proteinase K activity.	Prepare a fresh Proteinase K solution. Make sure Proteinase K solution is stored at -20°C.
	One of washing steps was omitted.	Repeat isolation, performing both washing steps.
	Purified DNA contains residual alcohol.	Repeat isolation, giving particular attention to ensuring that no residual SSW2 Buffer is left in purification column after centrifugation in step 18.

Purified DNA is degraded.	Inappropriate sample storage conditions.	Storing swabs and semen samples at -80°C is recommended. Avoid subjecting sample material to repeated freeze/thaw cycles.
RNA contamination present.	Incubation with RNase A was too short.	Extend incubation time with RNase A to 30 min (step 4 of the Isolation Protocol).
	Reduced RNase A activity.	Prepare a fresh RNase A solution and repeat isolation. Ensure proper storage conditions of RNase A solution: +4°C for short-term storage and -20°C for long-term.