TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA	Ineffective DNA binding to the membrane.	Ensure the mixture is yellow after adding CB and GB Buffers. If the colour turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.
	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 membrane. Extend the incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer.
	pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
	Ethanol was not added to Wash Buffer.	Ensure that 96-100% ethanol was added to Wash Buffer before use.
	Incomplete agarose slice dissolution.	Extend incubation at 50°C until the agarose slice is completely lysed. After lysis, incubate the sample for an additional 5 minutes.
Column becomes clogged during purification	Incomplete agarose slice dissolution.	Extend incubation at 50°C until the agarose slice is completely lysed. After lysis, incubate the sample for an additional 5 minutes.
DNA flows out of the lanes in the agarose gel	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual Wash Buffer is left in DNA Purification Micro Spin Columns after centrifugation in step 8 in the clean-up protocol and step 10 in the gel-out protocol.
Blurred bands in the gel electrophoresis image	Running buffer contains nucleases.	Always use freshly prepared buffer for both the electrophoresis run and gel preparation.
		Store the gel fragment at +4°C, under DNase-free conditions, for no more than a few days.
	Elution solution contains DNases.	Use fresh elution solution. If water is used instead of Elution Buffer, ensure that it is DNase-free.
Inhibition of downstream enzymatic reactions	Running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both the electrophoresis run and gel preparation.
	Purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in Wash Buffer before use.
	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual Wash Buffer is left in DNA Purification Micro Spin Column after centrifugation in step 8 in the clean-up protocol and step 10 in the gel-out protocol.

Incorrect DNA sequencing results.	Running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both the electrophoresis run and gel preparation.
	Extensive exposure to the UV light.	Minimize the DNA's exposure time to the UV light during the excision from the gel procedure.
	Equipment has been contaminated.	Clean the scalpel or razor blade and transilluminator surface prior to gel slice excision.