XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA.	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely dissolved. Then incubate sample for an additional 5 minutes.
	Ineffective DNA binding to a membrane.	After GB Buffer has been added, ensure that mixture is yellow. If it turns pink, add 10 µl of 3 M sodium acetate, pH of 5.2.
	Ethanol was not added to wash buffer.	Ensure that 96–100% ethanol was added to GW Buffer before use.
	Incomplete DNA elution from membrane.	Before applying Elution Buffer to the membrane, heat it to 70°C. Apply Elution Buffer directly to centre of membrane. Extend incubation time with Elution Buffer to 10 min. Perform second elution.
	pH of water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
Column becomes clogged during purification.	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely dissolved. Then incubate sample for an additional 5 minutes.
DNA flows out of lanes in the agarose gel.	Purified DNA contains residual ethanol.	Repeat isolation, giving particular attention to ensuring that no residual GW Buffer is left in purification minicolumn after centrifugation in step 9.
Blurred bands in gel electrophoresis image.	Running buffer contains nucleases.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
		Store 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 electrophoresis run and gel preparation.
	Purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in GW Buffer before use.
	Purified DNA contains residual ethanol.	Repeat isolation, giving particular attention to ensuring that no residual GW Buffer is left in purification minicolumn after centrifugation in step 9.

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

XIII. SAFETY INFORMATION

GB Buffer

Warning

H302, H312, H332, H412

P261, P264, P270, P271, P273, P280, P301+P312 P330, P302+P352 P312, P304+P340 P312, P363, EUH032

H302 Harmful if swallowed. **H312** Harmful in contact with skin. **H332** Harmful if inhaled. **H412** Harmful to aquatic life with long-lasting effects. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P270** Do not eat, drink or smoke when using this product. **P271** Use only outdoors or in a well-ventilated area. **P273** Avoid release to the environment. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell. Rinse mouth. **P302+P352 P312** IF ON SKIN: Wash with plenty of water. Call a POISON CENTER / doctor if you feel unwell. **P363** Wash contaminated clothing before reuse. **P304+P340 P312** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor if you feel unwell. **EUH032** Contact with acids liberates very toxic gas.