

XIII. TROUBLESHOOTING

Problem	Possible cause	Solution
Low yield of purified DNA	Ineffective DNA binding to membrane.	Ensure the mixture is yellow after adding CB and GB Buffers. If the color turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.
	Incomplete DNA elution from membrane.	Before applying Elution Buffer to the 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.
	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 agarose slice is completely dissolved. Then incubate sample for an additional 5 minutes.
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 the lanes in agarose gel	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual Wash Buffer is left in purification column after centrifugation in step 8 in clean-up protocol and step 9 in the gel-out protocol.
Blurred bands in gel electrophoresis image	Running buffer contains nucleases.	Always use freshly prepared buffer for both 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 isolation, paying a particular attention to whether any residual Wash Buffer is left in purification column after centrifugation in step 8 in clean-up protocol and step 9 in gel-out protocol.

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 UV light.	Minimize the DNA's exposure time to UV light during the excision from gel procedure.
	Equipment has been contaminated.	Clean scalpel or razor blade and transilluminator surface prior to gel slice excision.

XIV. SAFETY INFORMATION

GB Buffer



Warning

H302, H312, H332, H412
P261, P264, P270, P271, P273, P280, P301+P312 P330, P302+P352 P312, P363, P304+P340 P312, EUH032

CB buffer



Danger

H225, H302, H315, H319, H336
P264, P280, P301+P312 P330, P210

EUH032 Contact with acids liberates very toxic gas. **H225** Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H312** Harmful in contact with skin. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H332** Harmful if inhaled. **H336** May cause drowsiness or dizziness. **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. **P302+P352 P312** IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. **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. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.