

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.