GEL & PCR CLEAN-UP KIT
TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Low yield of purified DNA	Ineffective DNA binding to the membrane.	Ensure the mixture is yellow after adding the ACL and AEX buffers. If the colour turns pink, add 10µl of 3 M sodium acetate, pH 5.2.
	Incomplete DNA elution from the membrane.	Before applying the AEB buffer to the membrane, heat it to 80°C. Apply the Elution Buffer directly to the centre of the membrane. Extend the incubation time with the AEB buffer to 10 min. Perform second elution.
		Increase volume of the AEB buffer to 200µl.
	The pH of the water used for elution is lower than 7.0.	Use AEB buffer for DNA elution.
	Ethanol was not added to the wash buffer.	Ensure that 96-100% ethanol was added to the Wash buffer before use.
	Incomplete agarose slice dissolution.	Extend the 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 the 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	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual Wash buffer is left in the purification column after centrifugation in step 13 in the PCR clean-up protocol and step 12 in the gel clean-up protocol.
Blurred bands in the gel electrophoresis image	The 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.
	The elution solution contains DNases.	Use fresh elution solution. If water is used instead of the AEB buffer, ensure that it is DNase-free.

Problem	Cause	Solution
Inhibition of downstream enzymatic reactions	The running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both the electrophoresis run and gel preparation.
	The purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in the Wash buffer before use.
	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual Wash buffer is left in the purification column after centrifugation in step 13 in the PCR clean-up protocol and step 12 in the gel clean-up 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.