TROUBLESHOOTING GUIDE
PCR & Electrophoresis

EDVOTEK®

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR	Sample has evaporated	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)
		Make sure students close the lid of the PCR tube properly.
	Pipetting error	Make sure students pipet 20 µL primer mix and 5 µL Lambda DNA template into the PCR tube.
There's not enough sample in my QuickStrip.	The QuickStrip has dried out.	Add 40 uL water, gently pipet up and down to mix before loading.
The ladder, control DNA, and student PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
After staining, the ladder is visible but no PCR products are present.	PCR amplification was unsuccessful.	Repeat PCR with fresh PCR EdvoBeads™ and primers.
		Ensure that the thermal cycler has been properly programmed. See Module II for guidelines.
After staining, the ladder and control PCR products are visible on gel, but some student samples are not present.	Wrong volumes of DNA and primer added to PCR reaction	Practice using pipettes
DNA bands were not resolved.	Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™.