Troubleshooting Guide for the Electrophoresis of DNA Markers

Problem	Probable Causes	Recommended Solutions
Faint or no DNA bands	• **Quantity of DNA**	Increase the amount of DNA. A low concentration may be due to volume added per well width. Detection of DNA in polyacrylamide is less sensitive than in agarose.
	• **DNA was degrade**	Avoid nuclease contamination of the DNA markers.
	• **DNA was electrophoresed off the gel**	Electrophorese the gel for less time, at a lower voltage, or in a higher percentage gel.
	• **DNA was denatured**	Do not heat DNA markers (except Lambda-derived markers) prior to electrophoresis. Dilute markers in TE or in a buffer containing 20mM NaCl.
Missing DNA bands	• **Small DNA bands were electrophoresed off the gel**	Electrophorese the gel for less time, at lower voltage, or in a higher percentage gel.
	• **DNA bands of similar molecular size were not resolved**	Increase the electrophoresis time and check the proper percentage gel for resolution.
Smeared DNA bands	• **DNA was degraded**	Avoid nuclease contamination of DNA markers.
	• **DNA was denatured**	Do not heat DNA markers (except Lambda-derived markers) prior to electrophoresis. Dilute markers in TE or in a buffer containing 20mM NaCl.
	• **To much DNA was loaded on the gel**	Decrease the amount of DNA in the gel.
	• **Inproper electrophoresis conditions were used**	Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30°C during electrophoresis. Check that the electrophoresis buffer used has sufficient buffering capacity.
	• **DNA contained too much salt**	Remove excess salt before electrophoresis by ethanol precipitation.
	• **DNA was contaminated by protein**	Remove proteins before electrophoresis by phenol extraction.
Anomalous DNA band migration	• **DNA was denatured**	Do not heat DNA markers (except Lambda-derived markers) prior to electrophoresis. Dilute markers in TE or in a buffer containing 20mM NaCl.
	• **Inproper electrophoresis conditions were used**	Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30°C during electrophoresis. Check that the electrophoresis buffer used has sufficient buffering capacity.
	• **Lambda DNA fragments, the *cos* site reannealed**	Heat DNA at 65°C for 5 min before electrophoresis.