EDVOTEK® Troubleshooting Guides

DNA EXTRACTION

PROBLEM:	CAUSE:	ANSWER:
My fruit flies have died.	*Drosophila* are sensitive to very hot or very cold temperatures.	Positive results can be obtained using DNA extracted from dead flies.
	Drosophila have a short life cycle.	Positive results can be obtained using DNA extracted from dead flies.
Poor DNA Extraction	Samples not mixed well enough during extraction	In addition to flicking the tube, vortex or pipet up and down to mix the sample.
	Proteinase K inactive because it was prepared too far in advance.	Prepare Proteinase K within one hour of use.
	Water bath not at proper temperature	Use a thermometer to confirm water bath set point.
	Not enough DNA	Repeat extraction. Be careful that you do not aspirate pellet. Try using two flies.
There is no pellet in the tube after the isopropyl alcohol precipitation.	Fly not adequately homogenized.	Make sure that the fly is completely mashed before proceeding.
	Sample not centrifuged at an appropriate speed.	Spin cells at maximum speed (17,000 x g) for two minutes. If your centrifuge does not reach this speed, spin at highest available speed for four minutes.
	Pellet was aspirated.	Repeat extraction. Be careful that you do not aspirate pellet.
The extracted DNA is very cloudy.	Cellular debris from pellet transferred to tube	Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.
	Cellular debris not separated from supernatant	Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.

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PCR AND ELECTROPHORESIS

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 extracted DNA into the 0.2 mL tube.
The ladder, control DNA, and *Drosophila* 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 proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer.
	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 and control PCR products are visible on the gel but some *Drosophila* samples are not present.	*Drosophila* DNA sample was not concentrated enough.	Poor DNA extraction. Repeat Module I (Isolation of *Drosophila* DNA)
	Drosophila DNA sample was degraded.	If DNA is not used right after extraction, store sample at -20°C.
	Wrong volumes of DNA and primer added to PCR reaction.	Practice using pipettes
Some *Drosophila* samples have more/less amplification than others.	Concentration of DNA varies by sample.	There is an inherent variability in the extraction process.
Low molecular weight band in PCR samples	Primer dimer	Low concentration of extracted DNA in PCR reaction.
DNA bands were not resolved.	Tracking dye should migrate at least 6 cm 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™

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