TROUBLESHOOTING GUIDE
DNA Extraction • Human PCR

EDVOTEK®

DNA EXTRACTION

PROBLEM:	CAUSE:	ANSWER:
There is no cell pellet after centrifuging the cheek cell suspension.	Not enough cheek cells in suspension	Mouth must be vigorously rinsed for at least 60 sec. to harvest loose cheek cells.
	Sample not centrifuged fast enough	Spin cells at maximum speed (17,000 x g) for 2 min. If your centrifuge does not reach this speed, spin at highest available speed for 4 min.
I was not able to extract DNA from hair.	Not enough hairs used for extraction	Use at least five hairs for the DNA extraction.
	No follicle was present on hair shaft	The best place to collect hairs for this experiment is the head. Pick hair follicles which have a bulbous base (sheath cells).
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 baths not at proper temperature	Use a thermometer to confirm water bath set point.
	Not enough DNA	Try cheek cell extraction. Final DNA concentrations are usually higher.
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.

PCR & ELECTROPHORESIS

PROBLEM:	CAUSE:	ANSWER:
After staining, the ladder and control PCR products are visible on gel, but some student samples are not present.	Student DNA sample was not concentrated enough.	Poor DNA extraction. Extract new DNA. Cheek cell extraction usually results in higher DNA yield.
	Student DNA sample was degraded	If DNA is not used immediately following extraction, store sample at -20°C.
	Wrong volumes of DNA and primer added to PCR reaction	Practice using pipettes
Some students have more or less amplification than others.	Concentration of DNA varies by sample.	There is an inherent variability in the extraction process. For best results, use cheek cell extraction.
Low molecular weight band in PCR samples	Primer dimer	Low concentration of extracted DNA in PCR reaction.