DNA Fingerprinting

Cheek cell extraction

1. Scrape inside of cheek with sterile loop.
2. Twirl loop in 200µl of 5% Chelex buffer.
3. Add 2ul of 10mg/ml PK to tube at front bench
4. Incubate for 15-30 minutes @ 56°C
5. Vortex for 10 seconds
6. Spin at max speed for 20 seconds
7. Boil for 8 minutes in a 110°C heat block.
8. Vortex for 10 seconds
9. Spin at max speed for 2-3 minutes.
10. Use the top, aqueous solution, called the supernatant, STAYING AWAY FROM the pellet on the bottom, for your nanodrop reading and PCR reaction.

Set Up PCR of DNA Extract

Behind the Scenes

Kit components will be thawed, mixed and spun down and a master mix will be made as follows:
for each sample to be amplified, add -5µl Reaction Mix (buffers, dNTPs,, Polymerase)
- 5µl primer mix

The primer mix contains all 10 primer pairs, with one of each pair fluorescently labelled
This mix will be vortex for 5 seconds and quickly spun and then dispense into 30ul aliquots in each tube.

In Class:

At the front bench, choose the tube with your number on it. Need 20ng of DNA template. Based on nanodrop readings, add up to 15µl of the supernatant from your DNA extraction to your reaction tube at the front bench and bring total volume up to 25µl with water. Close the tube and place in the rack. The counter and pipetman will be wiped down with 10% bleach between each template addition in an effort to limit cross contamination.
Your 25µl reaction will be placed in a PCR machine and run as follows:

96°C 1minute
30 cycles of the following:
94°C 10 seconds
59°C 1 minute
72°C 30 seconds

60°C 10minutes
4°C till collected

Loading on the 3130xl Genetic Analyzer

Behind the Scenes

A master mix will be made of Hi Di Formamide (this keeps the DNA strand denatured for cleaner results) and GS-500 Rox size standard, whereby 9.5µl of formamide and 0.5µl of size standard will be added for each sample plus 1. This will then be dispensed in 10µl aliquots into a genetic analyzer plate labeled with your numbers.

1 µl of your PCR product will be added to the appropriate well in the plate.
Samples will be heated to 95°C for 3 minutes and quick cooled in an ice bath.

These will then be placed on the genetic analyzer for you. If anyone would like another/better look at the genetic analyzers, let me know and I will show you in smaller groups.