## RE determination of PTC Sensitivity

Set Up PCR of DNA Extract

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

-16.5$\mu$l ddH2O
-25 $\mu$l 2x ABI Taq Gold Master Mix (Master Mix contains dNTPs, Salts, and Polymerase)
-1$\mu$l Primer 1 (303-400 at 10$\mu$M)
-1$\mu$l Primer 2 (303-401 at 10$\mu$M)

This mix will be mixed gently for 5 seconds and quickly spun and then dispensed into 43.5$\mu$l aliquots in each tube. At the front bench, choose the tube with your number on it and add 6.5$\mu$l of the **supernatant** at the top from your DNA extraction that you made for the DNA fingerprinting, to this PCR tube, 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 50ul reaction will be placed in a PCR machine and run as follows:

95ºC 10minutes
55 ºC 5 minutes
40 cycles of the following:
72ºC 90 seconds
94ºC 45 seconds
55ºC 45 seconds
72ºC 10 minutes
4ºC till collected

Reactions will be put in the freezer to kill the polymerase from the PCR reaction so that it does not interfere with our restriction endonuclease reaction in the next step.

Digest Freeze Killed PCR Product

A master mix will be made as follows: for each sample to be digested, add

-6.5$\mu$l ddH2O
-3$\mu$l 10X NEB RE Buffer
-0.5$\mu$l Fnu4HI Restriction Endonuclease

This master mix will be mixed gently and quickly spun and then dispense into 10$\mu$l aliquots in each tube. Choose the tube with your number on it and add 20$\mu$l of your freeze killed PCR product. Mix gently by flicking tube with your finger (see demo) and place in 37 ºC heat block over night.

We will visualize the fragments created by this combination of PCR and RE digest by running on an agarose gel (see details next page).

Run RE Digest on gel

Students will come up to the front bench and practice loading blue juice and water into gel wells. Once everyone feels ready, we'll start. The fragments that we are trying to visualize are small and diffuse quickly. Thus, we are using a higher percentage of agarose, and trying to get the gels loaded efficiently.

1. At the front bench add 15$\mu$l of your digest to 3$\mu$l of 5X Blue Juice, mix, and load 15$\mu$l onto a 2.8% gel.
2. Make a 1 to 2 dilution of your original PCR reaction and add 8$\mu$l of the diluted sample to a 2$\mu$l aliquot of 5X Blue Juice and load the entire 10$\mu$l into the well right next to your digested sample.
3. The 2.8% agarose gel will be made, stained and run in Faster Better Buffer. The gels are prestained in SYBR Gel Green Stain, and run at 275V for 20 minutes and then visualized with a Blue Light Transilluminator.