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
- 16.5µl ddH2O
- 25 µl 2x ABI Taq Gold Master Mix
(Master Mix contains dNTPs, Salts, and Polymerase)
- 1µl Primer 1 (303-400 at 10µM)
- 1µl Primer 2 (303-401 at 10µM)

This mix will be mixed gently for 5 seconds and quickly spun and then dispensed into 43.5µl aliquots in each tube. At the front bench, choose the tube with your number on it and add 6.5µl 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:
- 95°C 10 minutes
- 55°C 5 minutes
- 40 cycles of the following:
  - 72°C 90 seconds
  - 94°C 45 seconds
  - 55°C 45 seconds
  - 72°C 10 minutes
- 4°C 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
- 6.5µl ddH2O
- 3µl 10X NEB RE Buffer
- 0.5µl Fnu4HI Restriction Endonuclease

This master mix will be mixed gently and quickly spun and then dispensed into 10µl aliquots in each tube. Choose the tube with your number on it and add 20µl of your freeze killed PCR product. Mix gently by flicking tube with your finger (see demo) and place in 37°C 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 15μl of your digest to 3μl of 5X Blue Juice, mix, and load 15μl onto a 2.8% gel.
2. Make a 1 to 2 dilution of your original PCR reaction and add 8μl of the diluted sample to a 2μl aliquot of 5X Blue Juice and load the entire 10μl 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.