PCR for ISSRs

1. Check the thermocycler to make sure that the correct program is loaded (different primers have different annealing temps).

2. Fill a tub with ice and place the DNA samples in it.

3. Set out solutions for ddH_2O, 10x buffer, $MgCl_2$, and 1.25 mM dNTP's; be careful to watch to make sure that they thaw but do not warm up completely (place on ice before this happens).

4. Label 0.5 ml tubes with the run number and sample number.

5. Make cocktail first in one tube for each set of samples, making sure to add in enough for another sample (to cover pipetting error). Always add the greatest volume first and check off the list as you add to the cocktail. Always use filtered tips.

   ddH_20 > dNTP's > 10 x buffer > $MgCl_2$ > Primer (from freezer) > Taq (from freezer)

   NOTE: Always add Taq last. Keep Taq in the freezer until you are ready to add it to the cocktail. When pipetting out Taq, don't put the tip all the way down in the tube. Make sure all of the Taq solution is out of the tip!!!!

   NOTE: Primer is ordered as a lyophilized solution and you must bring to 200µM stock solution (50 µl per tube) with UVed TE (#BM x 5 = volume of TE to add) – then dilute to 20µM for use:

   5 µl stock primer
   45 µl TE

6. Flick cocktail solutions for 10-15 seconds until foamy and well-mixed (so Taq goes into solution). Spin for a few seconds in small centrifuge to ensure complete mixing. Place cocktail solutions on ice.

7. Set pipetter for **24.5 µl** with filtered tips and add cocktail solution into each tube (doesn't need to be on ice, but still a good idea). A little of the cocktail solution should be left over.

8. Add **0.5 µl** of DNA to each tube containing the cocktail solution. Use very small tips with filter halfway down and a separate tip for each sample to prevent contamination. After adding DNA to each tube, pipette up and down to mix thoroughly. Leave tubes open.

9. Make sure to have a negative control (cocktail solution only).

10. Spin tubes quickly in the small centrifuge (to get bubbles out).

11. Place tubes in PCR machine and begin cycle. When complete, samples can be run immediately on a gel or frozen in the freezer to be run another day.