**Tips for One Step RT PCR**

1. Getting ready for *Chamaecrista* one step RT PCR:
   1. Suggestion: Team should divide tasks and then report back to group. One can do UV readings and calculations, one can work on the primers, one can work on mastermix.
   2. Students will take UV spec readings of 1:50 dilutions of *Chamaecrista* RNA and calculate the concentration of RNA in each sample. They have directions on their handout from last week and they can also download the Excel spreadsheet from Moodle to plug their numbers in. The 260 readings are used to calculate the RNA concentration. The 260/280 ratio tells them how pure their RNA is (1.6 to 1.7 is good).
   3. Students will calculate what dilution and volume of each *Chamaecrista* RNA sample they will need for their one step RT PCR reaction. It is important that they realize they will have to work from a dilution, not their original RNA stock. The volume they can add to their RT PCR reaction cannot exceed 5 ul.
   4. Students will calculate how to dilute their primers and run this by one of us before actually making their dilutions. In order to make comparisons between treatments, equal amounts of RNA should be used in each reaction. Students sometimes have difficulty realizing this and conversations with the TA or instructor help.
      1. Example: If they receive 0.05 umol of primer they must calculate how much RNase free water to add to make 100uM primer solution. 0.05uM/X = 100uM/l = 100uM/106ul. Solving the equation gives you X=500ul RNase free water. They should label the tube 100 uM and date it. Then they need to do a 1:10 dilution to get 10uM. So, 1 ul in 10 ul of RNase free water. They will be using 3 ul of the 10 uM primer solution in the RT PCR reaction. It is important that they use both the forward and reverse primer.
   5. Students will calculate how much mastermix they need for their RT PCR reaction following the directions on the handout. They have to show one of us their calculations before they can make their mastermix.
   6. Students will combine their mastermix, RNA, and RNase free water in a PCR tube for each reaction.
   7. The PCR machine in the lab will be used for RT PCR. Most of their primers have a Tm of about 60 so we’ll go with an annealing temperature of 55. The program should already be in the PCR machine, we just need to double check the annealing temperature.
   8. Student must label the PCR sheet (letter/number) for each of their samples and be sure to get it in the right spot.
   9. TAs, please come back later and put the PCR projects in our class box in exactly the same order you find them in the PCR machine. We’ll run their PCR products on a gel next week.

Some students chose to test for SNP variants by using restriction enzymes that cut one variant, but not the other. If this is something you and your students are interested in pursuing, here is a protocol. The enzymes would need to be identified and ordered when the primers are ordered. My students found that Student Biology Workbench (<http://bighorn.animal.uiuc.edu/cgi-bin/sib.py>) provided them with the necessary design tools. Use the “Identify restriction enzyme sites in nucleic sequence(s)” tool on the nucleic acids page.

*Restriction Enzyme Digest*

1. Locate your PCR tube from last week, and set it out at your bench to thaw. Your lab group will need to thaw one tube of 10X restriction buffer (“10X RB”).

2. Get a clean microfuge tube and label it with your name or initials, and some indication that you are digesting the DNA in the tube.

3. Pipette 8 μl of your PCR product into your microfuge tube. *Be very careful not to pipette more than 8* μ*l; you will need most of the remainder of your PCR product to run uncut on your gel.*

4. Add 1 μl of 10X Restriction Buffer to your tube. Mix well by pipetting up and down. *You may use a P20 pipettor even though it is generally used only to 2* μ*l.*

5. Add 1 μl of your restriction enzyme to each of the experimental microfuge tubes from your group. You will want a control for each sample where you do not add restriction enzyme.

6. Find a floater for your microfuge tubes, and put all four tubes from your group (one for each member of your group plus one tube with the TA’s sample) in one floater. Incubate your tubes in the 37°C water bath for 90 minutes.