**Visualizing Your PCR Products Using Gel Electrophoresis**

(Directions provided by Sarah Deel, Carleton College)

1. Making an agarose solution:

a. Each lab group should make a 40 ml solution of 2% agarose.

b. Calculate the amount of agarose you should weigh out to make your solution (you can do this before lab):

 40 ml 2% agarose:

 weigh out \_\_\_\_\_ g of agarose

 dilute in \_\_\_\_\_ ml of TBE buffer

 *Remember, use the fact that 1 g is equal to 1 ml to do this calculation. Measuring volume to the nearest milliliter is fine when you are making agarose for a gel.*

c. Check your calculations with your lab instructor or your TA before proceeding.

d. Using one of the balances in lab, carefully weigh out the appropriate amount of agarose, and mix it in the proper amount of TBE buffer (not water).

 *It will not go into solution until the next step.*

e. Microwave your agarose solution, checking the flask often to prevent boiling over. Use the “hot hands” to handle the flasks, since the glass will get very hot.

 *The agarose should be completely dissolved and the solution should be uniformly clear when you swirl it around.*

f. Take your flask back to your bench and let it cool until you can comfortably touch the glass flask.

 *You can do step 10a while you are waiting.*

2. Adding GelStar DNA stain to your agarose:

a. After your flask of agarose has cooled enough that you can touch it near the top comfortably, it is time to PUT ON GLOVES.

 *Any time you work with your agarose or gel for the remainder of the lab, you will need to be wearing gloves.*

b. Ask your TA to add 4 μl of GelStar stain to your flask. Wearing gloves, swirl the flask gently to mix, and proceed to the next step.

3. Casting a gel:

a. Wearing gloves, raise the sliding gates of your gel-casting tray and fasten them in place by *gently* tightening the plastic screws at either end. Place the 8-well plastic comb into the slots nearest the end of the gel casting tray.

 *Place the prepared tray on a level, undisturbed location on your bench so that when you pour the liquid agarose solution into the gel-casting tray it can solidify unperturbed. You will be using a comb which makes 8 wells this week, rather than the 6-well combs you have used previously.*

b. Wearing gloves, pour the agarose solution into the prepared casting tray, watching to make sure the agarose does not overflow. You may not need to use all the agarose you have prepared

 *If you pour the agarose while it is too hot, you can crack or warp the casting tray, which may cause leaking.*

c. After pouring, check to make sure that there are no large bubbles in the molten agarose. If there are bubbles, use a clean pipette tip to move the bubbles to the side of the tray.

d. Leave the gel alone to harden for several minutes while you go on to the next section.

4. Preparing your samples:

a. Label a microfuge tube with your initials and indicate the sample it contains.

b. Pipette 10 μl of your PCR product from your PCR tube into the microfuge tube.

 *If you do not have 10μl left, add as much of your PCR product as you have.*

c. Add 2 μl of loading dye to the microfuge tube. Mix thoroughly by pipetting up and down. Don’t forget to use a clean, new tip for each tube.

 *Do not throw the stock tube of loading dye away when you clean up after this lab.*

5. Preparing the marker lane:

a. Your lab group should acquire one tube of marker lane DNA with a 100 bp ladder from your lab instructor or TA.

b. Add 2 μl of loading dye to the marker lane DNA tube. Mix thoroughly by pipetting up and down.

6. Transferring your gel to the gel box:

a. Once your gel has solidified, remove the comb carefully.

b. Make sure the gates on your tray are down, and place the casting tray, still containing the gel, into the gel rig with the proper orientation: the comb end of the gel should be closest to the black (negative) electrode.

 *If there is already buffer in the rig, you can leave it there and re-use it (as long as the rig has been covered to prevent evaporation).*

c. Add enough TBE buffer to completely cover the gel.

 *Do not fill the gel rig completely (it will run too slowly); just make sure the gel is completely submerged.*

*Loading and Running the Gel*

7. Load the contents of each tube into the gel. Be careful not to let any bubbles into the pipette tip when you are drawing the sample into the tip.

 *Check your tubes before loading: microfuge briefly (around 15 seconds) if necessary. In your notebook, make a record of which sample is loaded into which well.*

8. When all of the samples have been loaded, re-check the orientation of the gel with respect to the electrical leads. Remember that DNA, having a net negative charge, will migrate toward the positive (red) electrode.

9. Placing the lid on the box connects the leads to the gel box. Connect the other ends of the leads to the power supply. Check that the power supply is set at 100-105 volts, and then turn on the "juice". The voltage will be applied to the gel for 55 minutes.

*Viewing the Gel*

10. When the gel has run for 55 minutes, disconnect the leads from the power supply and remove the lid from the gel rig.

11. Carefully, wearing gloves, remove your casting tray (still containing the gel) from the gel rig and set it on your benchtop.

12. Remove the gel from the casting tray and transfer it to the plastic sandwich container at your bench. Do not allow the gel to flip over as you make the transfer. You will view your gel on the transilluminator (UV light box). Your TA or lab instructor will help you with this. If at all possible, use a quantitative method to compare the intensities of your PCR bands in your different samples.