Gel Electrophoresis of your PCR reactions

Pouring agarose gels

1. Seal both ends of the gel tray with tape or stoppers/dam.

2. Place comb in slot at the end of the gel tray.

3. Pour melted agarose into the gel tray until the gel is about 5 mm deep. Let the agarose harden, which should take 5-10 minutes. Don't touch/move your gel until it's hard. In the meantime, prepare your PCR reactions for electrophoresis.

Electrophoresis PCR reactions

1. Using a clean tip for each reaction, pipet 2 µl gel orange loading dye (OJ) into each PCR reaction tube.

You will load both your PCR reactions and standard DNA ladder into the gel. A DNA ladder has many pieces of DNA of known size so you can compare the DNA from your PCR reaction to the ladder. In this way you can determine what size your PCR product is. Two or three groups might share a gel, but only one molecular weight marker is needed per gel.

2. In your lab notebook, record where you loaded each sample, (PCR reaction(s), DNA ladder). Record the lane number and the name of the sample. Be certain to have the information of where the other groups added their samples.
3. When your gel has hardened, carefully push tray out of holder or remove the tape. Gently wiggle the comb out of the gel.
4. Place the gel tray with the gel in an electrophoresis box. Make sure that the wells are aligned with the negative end of the box.
5. Pour TBE buffer so it fills the electrophoresis box and just covers the gel.
6. Beginning with the left side of the gel load your samples in the wells. Load the DNA ladder into the middle well.

Extract the sample from the tube and making sure that the sample is visible in the very end of the tip. Place the pipet tip directly over, or just inside of, the first well. Slowly push down on the plunger to release the sample. Once the sample is expressed, remove the tip from the well and buffer before releasing the plunger.

Do not be overly concerned with getting more than the very end of the tip over, or into, the well opening as the loading dye has glycerol added which will cause the sample to sink into the wells.

7. Continue to add your samples until finished. Be sure to change tips each time and record in your lab notebook, which sample was loaded into which well.
8. Now run that gel! Plug the electrodes into your electrophoresis apparatus (**red to red, black to black**), being careful not to bump your gel too much. Make sure one more time that your wells and DNA samples are at the negative (black) end of the electrophoresis box.

9. Plug the power source into an outlet and set the voltage at 75 to 90 V.
10. Let the gel run until the dye migrates about 2 cm from far end of the gel (about 20-25 minutes).
11. Turn off the power supply, disconnect the electrodes, and remove the top of the electrophoresis apparatus.
12. Carefully remove the gel and place it on a weigh tray with your group name on it.

Staining Gel with Ethidium Bromide - Instructors will do the staining for you!

1. **Using gloves**, remove the plastic from the ethidium bromide (EtBr) sheet and place the ethidium bromide paper on the gel. Add a few drops of buffer to ensure transfer of the stain and gently rub the paper with your fingers to make sure it is contacting the gel all over.
2. Stain for about 10 minutes. Discard EtBr papers in the red EtBr trash. Throw gloves in this same trash bag if you actually handled EtBr contaminated materials.
3. Put the gel in the UV transluminator. Close the door and turn on the white light to make sure that the gel is properly aligned. Turn off the white light, turn on the UV and push "Live" to see the gel. Adjust light using + or -.
4. Take a Polaroid picture of your gel; tape Polaroid picture into lab notebook and record all relevant information with the image.

Materials Needed

1% agarose gel
Loading dye (OJ)
Ethidium Bromide sheets – Ethidium bromide is carcinogenic. Do not allow students to handle EtBr sheets or gels stained with EtBr
1 kB DNA ladder
0.5X TBE Buffer
Ethidium bromide waste bag
Staining trays

Equipment necessary

Gel boxes and casting trays
Electrophoresis power supplies
Gel doc analyzer/UV transilluminator