

Analyzing PCR Results with Agarose Gel Electrophoresis

Background Information

What is Agarose Gel Electrophoresis?

Agarose gel electrophoresis is a procedure used to determine the presence and size of pieces of DNA. By analyzing the results of PCR on an agarose gel, genetic researchers are able to determine: (1) whether the PCR reaction was successful, indicated by the presence of a band on the gel; and (2) whether the PCR reaction resulted in a **PCR product** of the expected size/molecular weight. The **PCR product** is the piece of DNA you have 'amplified' or copied during the PCR.

Agarose is a powdered material made from seaweed. After mixing the agarose powder in buffer and heating it to melting in the microwave, the liquid is poured into a small mold (similar to the procedure used when making Jell-O®). A comb is placed in the liquid to make small depressions or **wells** in which to 'load' the DNA. After placing the gel in the gel box (see **Fig. 1**), DNA samples are mixed with loading dye and a single DNA sample is loaded into each well of the gel. Loading dye is often concentrated, such as 5X, and is diluted when added to the DNA sample. An electric current is then passed through the gel; since DNA is negatively charged, it travels through the gel to the positive electrode. The rate at which the DNA moves through the gel is determined by the size or molecular weight of the DNA fragment – smaller molecules travel more quickly, and larger molecules travel more slowly.

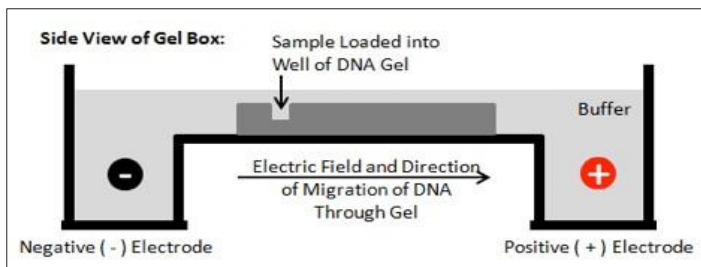


Fig. 1: Running a DNA Gel.

How Do You Know the Molecular Weight of Your DNA Samples?

We include a **molecular weight standard** on the gel to determine the size of the DNA bands or PCR products. Molecular weight standards, often abbreviated 'MW', contain pieces of DNA of known sizes. By comparing your DNA band to the band(s) of the molecular weight standard, you can estimate the size and concentration of your DNA band(s). You can also make a graph of the molecular weight standards relative to the distance each molecular weight standard band traveled through the gel. This allows for a more accurate estimation of the size of your DNA band(s).

Protocols

Pouring a DNA Gel

Note: Work in groups of 2. If your instructor has already poured your DNA gels, skip this part and proceed to 'Running a DNA Gel'.

1. Obtain the following items – to prepare a 0.8% agarose gel:
 - a. 400mg of powdered agarose
 - b. 50 mL of 1 X TAE Gel Buffer
 - c. Erlenmeyer flask or glass bottle
 - d. Labeling tape
2. Use the labeling tape to label your flask or bottle with your group's name, initials or number.
3. Pour the gel buffer into the flask or bottle. Add the agarose powder. Gently mix the agarose and the buffer by swirling.
4. Microwave the mixture on high for one minute, while watching to make sure it does not boil over. Check the liquid to see if all of the agarose has melted. If the agarose has not completely melted yet (if you can still see clearish crystals floating in the solution), microwave again for 20 seconds, and check again. Repeat microwaving for 20 seconds at a time until all of the agarose has melted.
5. Set the melted agarose aside to cool until you can pick up the flask or bottle without being burned.
6. Add GelGreen to your melted gel solution. For 50mL of gel solution, use 5uL of GelGreen. Swirl the gel solution gently to completely mix in the GelGreen. GelGreen will bind to the DNA in the gel and the bands will glow when the gel is placed on an LED light box.
7. Ask the instructor about the proper procedure for pouring a gel with the gel apparatus you are using. This will usually involve the following steps (See **Fig. 2**):
 - a. Pouring the warm agarose gel mixture into the mold.
 - b. Placing the well comb into the notches on the gel casting tray. Combs have multiple 'teeth' to create 'wells' in the gel into which you load your DNA samples.
 - c. Waiting for the gel to solidify completely.
8. You can store your gel in the gel box or in the refrigerator for up to 2 weeks, as long as the gel is wrapped in plastic wrap or submerged in DNA Gel Buffer and protected from light.

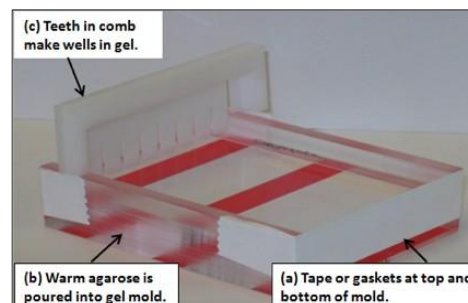


Fig 2: Procedures for Pouring an Agarose DNA Gel.

Running a DNA Gel

9. Obtain the tubes containing your PCR reactions.
10. To each of your group's PCR reaction tubes add: 5uL of 5X Loading Dye.
11. Use the table below (**Table 1**) to determine where you will load each sample on your group's DNA gel. You will also be loading molecular weight (MW) standards on your gel. Your instructor will provide a **Molecular Weight Standard Handout** illustrating the sizes of the bands contained in the MW Standard you will be using. This will help you determine whether your PCR reactions

produce DNA bands of the expected sizes (1,000 base pairs for a single copy of the HER2 gene, 2,000 base pairs for two copies, 3,000 base pairs for three copies).

Note: The well number is the slot on the gel in which you load your samples (See **Fig. 3**).

Well Number	Sample Name
1	<u>MW Marker</u>
2	<u>+2 PCR positive control</u>
3	<u>PCR negative control</u>
4	<u>Patient sample A PCR</u>
5	<u>Patient sample B PCR</u>
6	<u>Patient sample C PCR</u>

Table 1: Samples Loaded onto a DNA Gel.



Figure 3: Loading Samples into a well.

Image Source: Navaho, Wikipedia Commons.

12. Check that your gel is in your gel box and has enough DNA Gel Running Buffer in the gel box to cover the top of the gel.
Remember: Be sure that the top of your gel (the end where the wells are) is near the negative electrode on the gel box, and the bottom of the gel is near the positive electrode.
13. Have one member of the group load 10uL of your MW Standard into well #1 (the left-most well) of your gel using **Table 1** as your guide. **Figure 3** illustrates how to place your pipette tip **just above the well** before releasing the sample into the well. It is important not to push your pipette tip too deeply into the well, or you risk poking it through the bottom of the well and creating a leak.
14. Have the other member of your group load 20uL of your positive control PCR sample '+2' into well #2 of your gel, using **Table 1** as your guide. Continue to take turns loading the gel.
15. Load 20uL of the negative control PCR sample into well #3 of your gel.
16. Load 20uL of your first Patient Sample PCR into well #4 of your gel.
17. Load 20uL of your second Patient Sample PCR into well #5 of your gel.
18. Load 20uL of your third Patient Sample PCR into well #6 of your gel.
19. Put the lid on your gel box.
20. Make sure that your gel box is plugged into the power supply or directly into an electrical outlet if the gel box you are using has a built-in power supply. Set the voltage for 100V (if you are able to set the voltage) and press 'Run'.
21. Keep an eye on your gel. The gel should run 25-30 minutes to get good separation of DNA fragments. As the gel runs, the DNA loading dye will be visible in the gel. You should stop the gel, by turning off the power supply, when the bottom blue band of loading dye is about ¼ inch from the bottom of the gel.

22. Your instructor will tell you how to visualize your gel. If you are using the MiniOne gel boxes, you can turn on the built-in LED light and see the bands. If you are not using the MiniOnes, you will need to remove your gel from the gel box and place it on an LED light box in order to see your DNA bands.
23. Add each lab group's results to your **Experiment and Patient Summary Worksheet**.