Gel Electrophoresis

PCR products and many other DNA manipulations can be visualized by gel electrophoresis. During gel electrophoresis, DNA is loaded into an agarose gel where the DNA fragments are separated based on size. The agarose comes from seaweed and provides a matrix through which DNA migrates. Smaller fragments can move through the gel faster, while larger fragments will take longer to move through the gel matrix. A mold can be made out of agarose with little wells in it, into which the DNA is loaded. DNA is negatively charged and will therefore run toward a positive electrode (anode) when placed in an electric field. The gel is placed in a buffer chamber with electrodes on either end. The DNA is combined with a dye that is heavier than the buffer so that it will sink down into the wells. The electrodes are plugged in, with the one at the bottom of your gel being plugged into the positive end, causing the DNA to migrate through the gel toward that positive electrode. To actually see the DNA fragments, your gel needs to be stained with DNA Stain (EtBr, SYBR) that binds in the crevices of the double helix. When it is exposed to light of a particular wavelength, the light excites the dye and it glows, allowing the DNA to be visible. By comparing to size markers and other controls, one can determine the size or sizes of DNA in a given sample as well as estimate quantity.

1. Choose your agarose. There are many agarose products out there. All expensive so choose wisely. You want to choose the one that will best resolve the band sizes you are looking at and what you want to do with the gel after it is run. There are agaorse products that are better for 1000bp and up, or 250bp and below. There are also products that are easier to get a band out of once run on the gel.

2. Decide what percentage agarose you want to use. Percentage is also based on size

Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

3. Choose your running buffer. The most common ones used are TAE and Faster Better LB. The advantage of Faster Better is you can safely run a gel at 275V, greatly reducing running time. TAE cannot handle more than 80V but some believe it gives sharper bands. However, it is generally recognized that the type and percentage of agarose are the primary determinants of resolution.

4. Choose your DNA stain. Many still use ethidium bromide but it is a mutagen and requires very specific protocols for disposal. SYBR Safe (Life Technologies, Cat# S33102) and Gel Green (EmbiTec, Cat# EC-1995) are equally effective and far safer. SYBR safe has to be mixed 1/10,000 in the same buffer in which you made your gel and the gel can be soaked in it before or after it is run. If you are resolving small fragments, before is recommended so there is no time for diffusion after. The bath should be kept protected from the dark and is good for about 2 weeks. GelGreen can be added straight to the molten agarose before pouring, again at a 1/10,000 dilution. This can be more cost effective if you are not running many gels and is also wildly convenient over making a separate bath. In addition, GelGreen appears to be more sensitive, lighting up even the faintest bands.

5. Lastly, you need a loading dye. These often come with running buffers and they can be purchased but below is a recipe for a common 5X Blue Juice.

0.25% bromophenol blu	125mg
0.25% xylene cyanol	125mg
0.1M EDTA	25mL 0.2M EDT
30% glycerol in H_2O	15mL
10mL H_2O	
50mL TOTAL	

See Example Below.

Below is an example for a 0.8% Agarose Gel in Faster Better Buffer with SYBR Safe stain.

1. In a 250ml flask mix 0.8g of agarose in 100ml of 1X Faster Better Buffer.

2. Put in microwave and heat on high for one minute at a time, swirling in between until the solution is completely clear with no clear flakes visible. CAUTION: Flask needs to be watched or can boil over. **May also boil over when swirled** so maybe wait a minute after stopping the microwave and swirl gently once hot.

3. Best to put molten agarose covered in a 55-60^{o}C incubator to cool for an hour or overnight. Can swirl at room temp for maybe 15 minutes but risk getting a skin of agarose on top and you have to reheat. The point is, do not pour a boiling hot gel into your gel rig or you will warp it and it will never fit right again.

4. Set up gel rig with the combs you want and pour your gel to about 1/3 to half way up the combs (small rigs take 40-50mls, medium rigs about 100mls, huge rigs, 250mls). Thick gels may be necessary in order to hold a large sample but they rarely look very good.

5. Allow gel to solidify, maybe 20 minutes.

6. Meanwhile, make your SYBR Safe staining bath. You can put multiple gels in a bath, just need to make enough to cover the gels. For a single small gel, can use a small tuppy and make 200mls by adding 20µl of SYBR Safe to 200mls of 1X Faster Better Buffer.

7. Once gel is completely solidified (it will solidify around the combs last), gently pull out the combs and put the gel into the staining bath for at least an hour or can go overnight.

8. Add 3µl of your 5X BJ to 12µl or you sample and load all into a well. Repeat for all samples. Add your ladders and controls to their own wells.

9. Cover, make sure electrodes are hooked up so that your DNA will be running down the gel toward the positive electrode. Run at 275 V for 25 minutes and image with any illuminator that can excite SYBR Safe. The CMB has a Sapphire illuminator, a BioChemi Darkroom illuminator, and a Typhoon 9200. See Louie for training on any of these instruments.