# Agarose Gel Electrophoresis

Gels are used for quality control of biomolecules, particularly nucleic acids and proteins. Molecules have a net charge (positive or negative) so they will migrate towards the opposite charge in an electric field. If you have a gel (sort of like a water-filled sponge) the molecules have to work their way through the pores in the gel also – the bigger the molecule the harder it is to fit through the pores and the slower they are.

Nucleic acids are negatively charged, so gels are run from black (negative) to red (positive) - also called the anode and cathode. Gels allow size to be estimated if the correct size standards are included.

Gel s also allow you to tell if the molecules are pure (is there only one?) and if they are intact (if there are smaller products the molecule may be degraded) – this is also called their integrity.

If you put a control molecule on, whose size and amount you know, you can use that to estimate the size and amount of your unknown molecule(s). Usually you have to use a stain of some kind in order to visualize the molecules.

Task overview:

Dissolve and melt the agarose in buffer, using a microwave oven.

Put together a gel casting tray and comb (which makes wells for holding samples).

Pour the somewhat cooled (~60C) melted agarose into the casting tray and let it solidify

Remove the comb and end pieces.

Load gel into electrophoresis tank (wells towards the black end, DNA will run down the gel toward the red end).

Add 0.5X TBE buffer until it barely covers the gel.

Measure out samples or DNA ladders (size markers) and mix with loading dye.

Load dye+samples into wells with a micropipetter- leave the two end wells blank.

Load dye+DNA ladders (size standards) into wells on either end.

Put the lid on the tank, plug in the electrodes, turn on the power, run for 2 hours.

Turn off the power and unplug, remove the tank lid.

Put the gel into a staining tray and stain for 3 minutes in 100X stain and then destain with water for 30 minutes, or stain overnight in 1X stain (cover tray with plastic wrap).

Pour the buffer back into the reagent bottles using a funnel (it is good for up to 5 runs). Place the gel on a light box/transilluminator.

With shield in place to protect your eyes, turn on the lilght box/transilluminator.

Document bands on gel with digital camera.

Analyze data with software.

# Agarose Gels

1. Introduction to agarose gels. We will prepare a 1% agarose gel in 1X TBE buffer
   1. Setting up, pouring and running a gel is kind of shown here, although a narrative would have been much more useful than the music. <http://www.molecularstation.com/science-videos/video/18/agarose-gel-electrophoresis-method/>
   2. Here is another one – this requires Quicktime: <http://www.queensu.ca/cloe/projects/public/AGE/content/description_of_technique.php>
2. To make the gel, you will need to weigh out some agarose, for a 1% gel you would weigh out 1 gm of agarose and add 100 ml of buffer solution. We are going to make 100ml of a 1.5% solution, so you will need to weigh out 1.5gm of agarose.
   1. In order to make the agarose dissolve and make a solution you have to heat it – for this we use the microwave, and because the agarose is granular, you do have to worry about superheating – when you remove the flask to swirl the solution be sure to wear a protective glove, glasses and to point the neck of the flask away from your face. When the solution is complete, if you hold the flask up to the light it should look perfectly clear. If you see any floating bits, even if they seem clear, you must heat the solution a little longer
      1. Take a 250 ml Erlenmeyer flask.
      2. Wearing gloves, take out a weigh boat and tare it.
      3. Measure out 1.5 gm of agarose from your 15 ml stock tube.
         1. If you weigh out too much you can return it to your tube.
      4. Add 100 ml of 1X TBE buffer, using a graduated cylinder to measure, then pour into the flask
      5. Swirl the powder into the buffer, then place the flask in the microwave for 1 min on maximum power.
      6. Wearing a protective glove and protective glasses, swirl the solution, pointing the neck of the flask away from your face.
      7. Repeat until the solution is clear – it will be just barely boiling, but you don’t want it to boil over.
      8. Cover the neck of the flask with foil or Parafilm and let the mixture cool for 20 minutes or so on the bench, swirling occasionally.
         1. This can be put into a 60C water bath, which will keep it molten indefinitely – you can directly pour agarose at this temperature.
      9. Just before pouring, you can add Ethidium Bromide, an intercalating agent (you can also do post-staining of the gel with FastBlue – check with the instructor which we will be doing).
         1. Add 20ul of a 10mg/ml stock (in a 1.5ml microfuge tube on your bench), swirl to mix
         2. Note that Ethidium bromide is a mutagen, so all waste that touches it should into Hazardous Waste containers, not regular waste.
      10. Pour the mixture into the prepared casting tray (see next step for directions), with end gaskets and a comb inserted.
   2. To prepare the apparatus: We are pouring a horizontal, or flat-bed gel, so we are making a thin layer of agarose across the surface. We have to keep it from leaking out the ends of the casting tray - some makers provide rubber gaskets, but tape actually works pretty well if you can’t find them.
      1. Wearing gloves, fit the gaskets across each open end, or tear off a piece of tape long enough to more than reach across the ends of the mold. Attach so that the sides and bottom appear to be well sealed, repeat for the other end.
      2. You need a place to put the samples – this is made by creating ‘wells’ in the gel material, with a device called a comb. Insert a comb toward the top end of each half of the mold.
         1. Some casting trays allow multiple combs to be used – in that case use a slot towards one end and a slot in the middle if you are going to need more spaces for samples.
      3. When the agarose is ready, pour it into the prepared mold – you need a thin continuous layer, but you don’t want it to be very thick – it should not take all of the agarose you prepared.
   3. Let the agarose harden – this should take about 20 minutes, depending on how hot it was. You will be able to tell that it is ready when it becomes translucent rather than clear. Remove the gaskets or tape and the comb.
      1. Place the gel on the tray in the electrophoresis chamber. Pour 0.5X TBE buffer into the chamber – you will probably need ~600 ml
      2. If you do not have a reagent bottle already on your bench you can make 0.5X buffer: use the 1L graduated cylinder, add 50 ml of 10X TBE buffer, fill to the 1L line with Nanopure water (in the big carboy by the sink), cover with Parafilm, cover that with your hand to make a seal (wear a glove), and invert several times to mix.
3. Now you are ready to load the gel.
   1. Calculate the amount of sample you need in order to visualize it on the gel. For an agarose gel you probably need 50-100ng per band (we will do this for our PCR products), or 1ug if you are looking at a broad smear (this is what you should do for genomic DNA). You will need a reading from the spectrophotometer before you can do this calculation.
      1. You may need to dilute a sample if you would have to measure out less than 1ul, since this is not very accurate. Samples that are too dilute have to be re-concentrated – precipitate them with ethanol and resuspend in a smaller volume.
      2. The sample volume that you can load depends on the well size. These agarose gels generally give you volumes of 10ul (mini gels), 20ul (midi gels), in a well, but this depends on the gel thickness also.
         1. You can test this by just putting in some loading dye (this is also a good way to practice the technique – you can just flush out the well with buffer after you have practiced).
      3. Ideally you want at least 3ul of sample to load, less than that is difficult to deliver quantitatively. If your sample is less than 3ul, you can add buffer to make up the difference – use the new total to determine how much loading dye to add (next step).
      4. To each sample add an appropriate volume of blue gel-loading dye (4X LD), mix by pipetting up and down several times.
         1. If you have 3ul of sample you will add 1ul of loading dye, if you have 4ul of sample use 1.5ul of loading dye (the math is not perfect but it is hard to measure less than 0.5ul accurately).
   2. Loading requires a steady hand- you need to layer the sample in the bottom of each well, *without* stabbing the pipette tip through the agarose that forms the container, nor by floating the sample over the top of the gel. Practice is the only answer! You can steady the loading hand on your other arm.
      1. One lane on each edge will be size standards
         1. Load 5ul of the DNA ladder +2ul of loading dye = 7ul in each well.
      2. Put the lid on the apparatus, plug the electrodes into the power supply, turn on constant voltage, set at 50V. You will run this until the lower (fastest) of the blue dyes is about 75% of the way down each half of the gel. This will probably take a couple of hours.
      3. If your gel contains ethidium bromide, immediately put it on the transilluminator surface.
         1. Cover the transilluminator with the protective shield, turn on the light. You should see glowing bands in the gel – if you do start the imaging software and take a picture. If you do not, make a note in your notebook that the gel failed. If you see bands, save the image, email it to yourself and your partner so that you can print it out and put it in your notebook.
      4. Staining – if you did not use ethidium bromide, put the gel in a staining tray, add 100ml of staining solution - the directions vary depending on what stain and destain you use, look for specific instructions from the teacher.
         1. Once the gel is destained, remove the gel from the solution and place on the transilluminator – cover with the protective shield, turn it on, take a picture to document bands.