Agarose Gels

Gels are used for quality control of biomolecules, particularly nucleic acids and proteins. Nucleic acids are negatively charged, so gels are run from black to red (color conventions of anode and cathode). Gels allow size to be estimated if the correct size standards are included. They allow integrity to be assessed - is only the right size present. They allow some level of quantification, depending on the dye used, although they are not very sensitive.
Lab Goals: run samples of the titrated oligonucleotides from last week on an agarose gel and on a polyacrylamide gel.
Overview:

Pour each type of gel.
Load gel into tank
Add buffer.
Aliquot out samples and mix with loading dye.
Load samples into gel wells.
Load size standards into wells.
Electrophoresis
Stain
Document bands on GelDoc station
Use GelDoc software to analyze the size and intensity of the gel bands.

# Agarose Gels

1. Agarose gel. We will use a 1% agarose gel in 1X TBE buffer; another good choice for oligomers is a 3% agarose gel in 1X TBE.
    a. 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/
    b. 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 weigh out 1 gm of agarose per 100 ml of buffer solution. To make the solution 1X in TBE, you will need to dilute 10X buffer with water, in a 1:10 ratio (10ml per 100 ml total).
    a. In order to make the 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. If you hold the flask up to the light it should look perfectly clear.
        i. Take a 500 ml Erlenmeyer flask.
        ii. Wearing gloves, take out a weigh boat and tare it.
        iii. Measure out 2.0 gm of agarose. If you weigh out too much do not return it to the reagent bottle, but put it in a 50 ml Falcon tube and label that. We can use it for sealing acrylamide gels, so it is not wasted.
        iv. Add 20 ml of 10X TBE buffer, using the a 10 ml plastic pipette and the green pump.

v. Add 178 ml of sterile water.
vi. Swirl, then place in the microwave for 1 min on maximum power.
vii. Wearing a protective glove, swirl the solution, pointing the neck of the flask away from your face.
viii. Repeat until the solution is clear – it will be boiling, but you don't want it to boil over.
ix. Cover the neck of the flask and let the mixture cool for 20 minutes or so, swirling occasionally.
x. Just before pouring, you can add Ethidium bromide, an intercalating agent (you can also do post-staining of the gel). Add 10ul of a 10mg/ml stock, per 100 ml of agarose gel solution, swirl to mix.
1. Note that Ethidium bromide is a mutagen, so all waste that touches it should into Hazardous Waste containers, not regular waste.
xi. Pour into the prepared apparatus – see step b (note that it is OK if this solidifies, you can easily re-heat it. If you maintain it in a water bath at 60C it will not solidify, if you anticipate having to pour a lot of gels on any given day).

b. 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 pouring out the ends of the mold. Some makers provide rubber gaskets, but tape actually works pretty well.
i. Wearing gloves, 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.
ii. 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 the each of the two combs toward the top end of each half of the mold.
iii. 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.

c. 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 come and the tape.
i. Place the mold in the electrophoresis chamber. Pour 1X TBE buffer into the chamber – you will probably need ~600 ml, so use the 1L graduated cylinder, add 100 ml of concentrated buffer, fill to the 1L line with buffer, cover with Parafilm, cover that with your hand (in a glove), and invert several times to mix.

3. Now you are ready to load the gel.
a. Calculate the amount of sample you need in order to visualize it on the gel. For an agarose gel you probably need 30-100ng per band.
i. 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.
ii. The sample volume that you can load depends on the well size. These agarose gels generally give you 10ul (mini gels), 20ul (midi gels), but this depends on the gel thickness also.

iii. 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).
iv. To each sample add an appropriate volume of blue gel-loading dye (4X LD), mix by pipetting up and down several times.
    1. You can load all of your samples, or you can just load those that the NanoDrop indicates has sufficient material (if these all seem low I will give you some samples that will show up).

b. 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!
    i. One lane on each edge will be size standards that I will give you.
    ii. Put the lid on the apparatus, plug the electrodes into the power supply (red should be at the bottom, the direction the samples are heading towards), turn on constant voltage, set at 50V. You will run this until the lower of the blue dyes is about 75% of the way down each half of the gel. This will probably take a couple of hours, so one of you will have to come back to turn off the gel and stain it (5 minutes) and take a picture (5 minutes).
    iii. Place the gel on the GelDoc transilluminator surface. Close the door, turn on the UV light. Look through the observation window. 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.