Biotechnology Lab – Running Agarose Gels Weller

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: Set up the gel cassette and pour an agarose gel. Load samples in the wells, and run them, using size standards ('ladders') on the edges. Load 1-1.5 ug of your plant DNA in a well. For every 3ul of volume add 1ul of loading dye, mix, then put in well. When the lanes are loaded put on the lid, plug in the power and run the gel at 50V for 2-3 hours. Then stain in order to visualize the DNA. The ladders should be separate bands and your plant DNA should be a smear. You use thesizes of the bands in the ladder to estimate the size of your genomic DNA. You use the intensity of the bands of the ladder to estimate the amount of genomic DNA in your sample.

Overview:

Pour the gel.
When the gel has set, remove the comb
Place gel in tank
Add buffer to the tank until the top of the gel is barely covered.
Measure the correct volume of the samples ('aliquot')and mix with loading dye (3:1 ratio).
Load samples into gel wells.
Load size standards into wells on the edges of the gels.
Plug in the power unit, apply the 50V setting and start the sample migrating through the gel ('Electrophorese').
After 2-3 hours turn off the power and unplug the unit.
Remove the gel and place in a tray.
Add stain, mix for 10 minutes, destain if necessary
Place gel on light box – sketch approximate positions of bands and sample smears
Dr. Weller will digitally document bands on an imaging ('GelDoc') station and post them on the Web site. She will also make some small prints of the images for your lab notebooks.
Use GelDoc software to analyze the size and intensity of the gel bands.

1. Agarose gel. Since the DNA is quite large, we will use a low percent agarose (1% or 1 gm in 100ml of buffer), run with 0.5X TBE buffer.
    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
    c. Each bench (2 teams) will work together. To make the gel,
        i. you will need to weigh out some agarose, 1 gm per 100 ml of solution.
        ii. To make the solution 0.5X in TBE, you will need to dilute in some of the 10X buffer, in a 1:20 ratio, or 5ml per 100 ml total.
        iii. In order to make the agarose go into 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.

iv. You will know the agarose has dissolved if you hold the flask up to the light and it looks perfectly clear.
v. Take a 250 ml Erlenmeyer flask.
vi. Wearing gloves, take out a weigh boat and tare (zero) it.
vii. Measure out 1.0 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.
viii. Add 5 ml of 10X TBE buffer, using the a 10 ml plastic pipette and the green pump.
ix. Add 178 ml of sterile water using a graduated cylinder.
x. Swirl, then place in the microwave for 1 min on maximum power.
xi. Wearing a protective glove, swirl the solution, pointing the neck of the flask away from your face.
xii. Repeat until the solution is clear – it will be just on the point of boiling, but you don't want it to boil over.
xiii. Cover the neck of the flask with Parafilm or foil and let the mixture cool for 20 minutes or so, swirling occasionally.
xiv. Pour into the prepared apparatus (note that it is OK if the agarose solidifies, you can easily re-heat it. If you maintain it in a water bath at 60C and keep the dissolved agarose in it then it will not solidify, and you can keep it ready).

d. 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, your gel devices ('rigs') have a pouring tray with a bridge in the middle.
    i. Wear gloves, insert base in tray, place bridge in middle (make sure both sides are seated all the way down.
    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 – there are resting points to make sure the comb is correctly placed.
    iii. When the agarose is dissolved and cool enough, 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.
    iv. 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 comb.
    v. Place the mold in the electrophoresis chamber. Pour 0.5X TBE buffer into the chamber – you will probably need ~500 ml
        1. Using a 1L graduated cylinder, add 50 ml of concentrated 10X TBE buffer to 950 ml of water.
        2. Cover with Parafilm, cover that with your hand (in a glove), and invert several times to mix.
    vi. Now you are ready to load the gel, but practice with just loading dye before you get to the real samples.
        1. Place 8ul spots of loading dye on a piece of Parafilm, pull up with a pipette, place tip just below the surface of the gel in a well (but

don't stab the bottom of the gel) and slowly push the liquid out – it should settle into the bottom of the well, not float out or run over the sides. You may find it helpful to rest your elbow on the bench and use one hand to steady the wrist of your other hand.

vii. Prepare samples for loading
    1. Calculate how much of the sample (sample volume) you need to have 1-1.5micrograms total. Using a 10ul pipette, remove the volume from your sample and spot it on a piece of Parafilm.
    2. To each sample add a one-third volume of 'blue juice' gel-loading dye
    3. Mix sample and loading dye by pipetting up and down several times.
    4. Load your sample into the wells (note which well has each sample and reserve the end slots for the DNA ladders).

viii. Load the size standards at each end of the gel.

ix. 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% (three-quarters) 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).

x. Place the gel in a tray. Add the stain and let the gel sit for 10 minutes. Destain for 10 minutes. You should see violet bands in the gel.

xi. Wrap the gel in a Baggie and label with a marking pen, give to Dr. Weller.

Questions to consider:

1. What type of resolution (accuracy and precision in estimating the size and mass of your samples) are you able to achieve (the size standards will be good for this)?
2. How would you figure out the sensitivity of detection? (this is the smallest amount of a band you can see)?
3. Are the samples a single product?