Activity #5c. Gel Electrophoresis and Bacterial Transformation Results

Learning Goals:

To test the quality and identity of the pGLO plasmid DNA we isolated last week

To learn about gel electrophoresis and to use it to separate the restriction fragments of the pGLO plasmid DNA and to calculate their sizes

To analyze the results of the pGLO plasmid bacterial transformation that we began last week, illustrating the principles of regulation of gene expression

Lab Background:

On your own, reread and review Background section from last week's lab Bacterial Transformation. Know what your research hypotheses were and be able to identify **the independent and dependent variables, and controls** for the two experiments conducted last week.

Background for gel electrophoresis:

Last week you purified plasmid DNA from bacterial cultures. How do you know whether you have DNA in the tube? How do you know whether it's the right DNA? These questions can best be answered by physical analysis. Last week, you set up restriction digestions of samples of your plasmid DNA. This week, you will separate the fragments from one another on agarose gels, and estimate their sizes by comparison to those of molecular weight markers whose size is known.

To identify specific DNA fragments, one must separate the fragments based on their size. This is accomplished by **gel electrophoresis**. Because DNA is negatively charged (due to the phosphate groups in its backbone) it is attracted to the positive electrode when subjected to an electric field. If the DNA is present within a gel, its movement toward the positive electrode will be hindered to different extents, depending on the size of the DNA molecule (would a rabbit or cow move faster through thick woods?). Thus, one can use the distance migrated by a particular DNA fragment to determine the size of the fragment. This week, we will separate the restriction fragments on agarose gels using electrophoresis..

Procedure A. Preparation of 0.8% agarose gels

Each group of 4 students should prepare one agarose gel as follows:

1. Securely attach the black rubber dams onto both ends of the gel tray. Place tray on a sheet of plastic wrap in case of leakage. Align comb in tray parallel with and 1 - 1.5 cm from the end of the tray. Make sure comb is about 1-1.5 mm up from the gel tray, to form the bottom of the sample wells. Your Professor or TA will demonstrate.

2. Add 0.32 g agarose to 40 mL water in a 125 mL erlenmeyer flask, heat mixture in microwave on high setting for 1 - 1.5 min, or until mixture begins to boil.
3. Using a folded paper towel to hold the neck of the erlenmeyer flask, swirl the gel mixture well, and return to microwave. Heat for an additional 30 - 45 sec, or until mixture begins to boil again. Bring to a boil a third time.

4. Carefully remove molten gel solution from microwave using a folded paper towel to hold the neck of the erlenmeyer flask.

5. Add 0.8 mL 50x TAE buffer (what is final concentration?________), 10 μL 2.0 mg/mL **ethidium bromide** (final conc = ____________?), swirl to mix, pour into gel tray, remove any bubbles with a pipette tip, and allow to stand at room temp for 20 - 30 min to solidify. **Note: Ethidium bromide is a carcinogen! Gloves must be worn when working with gels and gel buffers.**

B. Load samples into agarose gel, begin electrophoresis.

1. Fill gel chamber with 1x TAE buffer such that the level of liquid just covers center platform. Carefully remove the comb and dams from ends of gel, **place gel in chamber with the wells near the negative electrode (anode-black)**. Have your Professor or TA check for correct gel orientation BEFORE you begin to load your samples. Add sufficient 1x TAE to just cover the gel so that no gel bits are sticking up above the fluid. If there are air bubbles in your sample wells, use a pipette tip to dislodge them—gently! Save the plastic wrap for later.
2. Cut a small piece of parafilm and place on bench near gel, "spot" a 1-2 μL aliquot of loading dye onto parafilm for each sample to be loaded on gel. If you have 3 restriction enzyme digests, you will need 3 spots of dye. *The blue dye will help you to see your sample as you load it into the gel wells.*

3. Draw sample (15 μL) into a pipette tip, pipette up and down onto a spot of loading dye to mix (CAREFUL: try to minimize creation of air bubbles!), load sample into well of gel.
   Be careful not to poke pipette tip through bottom of well.

4. Between the samples from the two pairs of students on each gel, load the **DNA size marker** –This will give you DNA standards of known size against which you can compare your restriction digestion fragments of your plasmid DNA.
   Note: You will be using ONE of these two options today:
   Quick-Load 2-log DNA ladder, load 10 ul

5. Place cover over gel chamber, turn on power supply and set to 100 V. Confirm proper operation by checking for gas production (bubbles) at electrodes (electrolysis of water).

6. After 2-3 minutes, observe your gel. Make sure the blue dye is migrating in the correct direction—towards the larger portion of the gel, towards the positive electrode. If it is migrating backwards, you have switched the orientation of the gel and you must reverse it immediately to get your DNA to run in the correct direction.

C. Examine electrophoresis gel.

1. When the fastest migrating dye has migrated over half the length of the gel (after 20-30 minutes), turn off power supply.

2. Wear gloves to remove gel tray from the chamber. Slide gel off tray onto the plastic wrap.

3. Place gel on UV transilluminator, view and photograph gel. ****Caution – do not look directly at transilluminator surface, except through UV-blocking cove**r.

D. Bacterial Transformation data analysis

Observe transformation plates in normal light, and when exposed to long-wave ultraviolet light.

Record your observations on this page below—**create a chart or table to hold your data**. Count the numbers of colonies on each plate (if possible), estimate the percentage that glow green on each plate. Record all this information below.

When you are done observing your transformation plates, discard plates in the autoclave bag on the side bench.

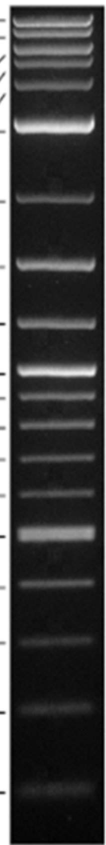
E. Data analysis
Gel Electrophoresis

1. Measure the distance (in mm) migrated by each DNA fragment on your gel by **measuring from the front (leading edge) of the well to the front of each band** and enter the data for your marker DNA in the appropriate table below. The migration of linear DNA through a gel shows an inverse, logarithmic relationship with its size (mass, length). On the graph paper provided, plot three graphs, **each with migration on the X-axis**:

    a. Length vs. migration on standard graph paper, for your marker DNA only
    b. Length vs. migration on semi-log paper, for your marker DNA only
    c. Log of length vs. migration on standard graph paper, for your marker DNA only

Draw a straight line through the data points on graphs b and c (points at the extremes probably will not fall on the line due to a limited range of effective separation by agarose and measurement errors). These are your standard curves.

Migration of DNA bands for Quick-Load 2-log DNA ladder.
Measure the migration of the bands indicated with asterisks (*).

Fragment Length (bp)	**Log of length**	**Distance Migrated (mm)**	Mass (ng) Kilobases 40 10.0 40 8.0 48 6.0 40 5.0 32 4.0 120 3.0 40 2.0 57 1.5 45 1.2 122 1.0 34 0.9 31 0.8 27 0.7 23 0.6 124 0.5 49 0.4 37 0.3 32 0.2 61 0.1 
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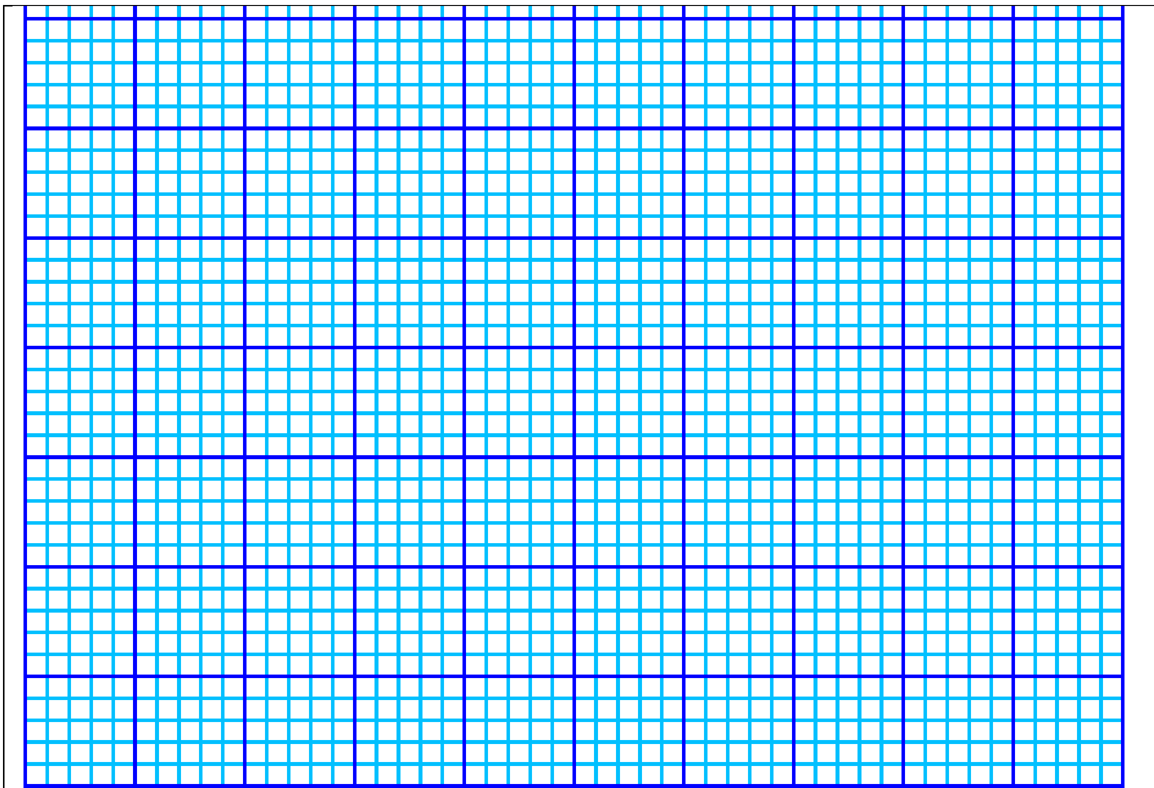
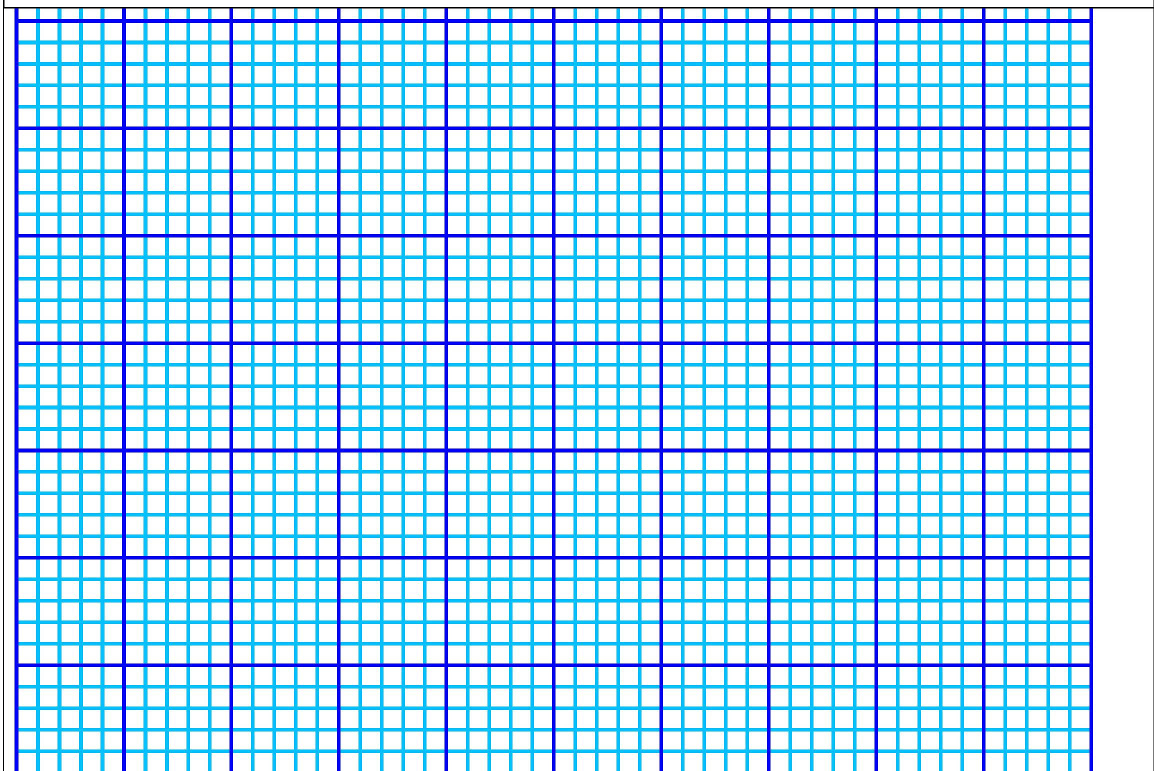
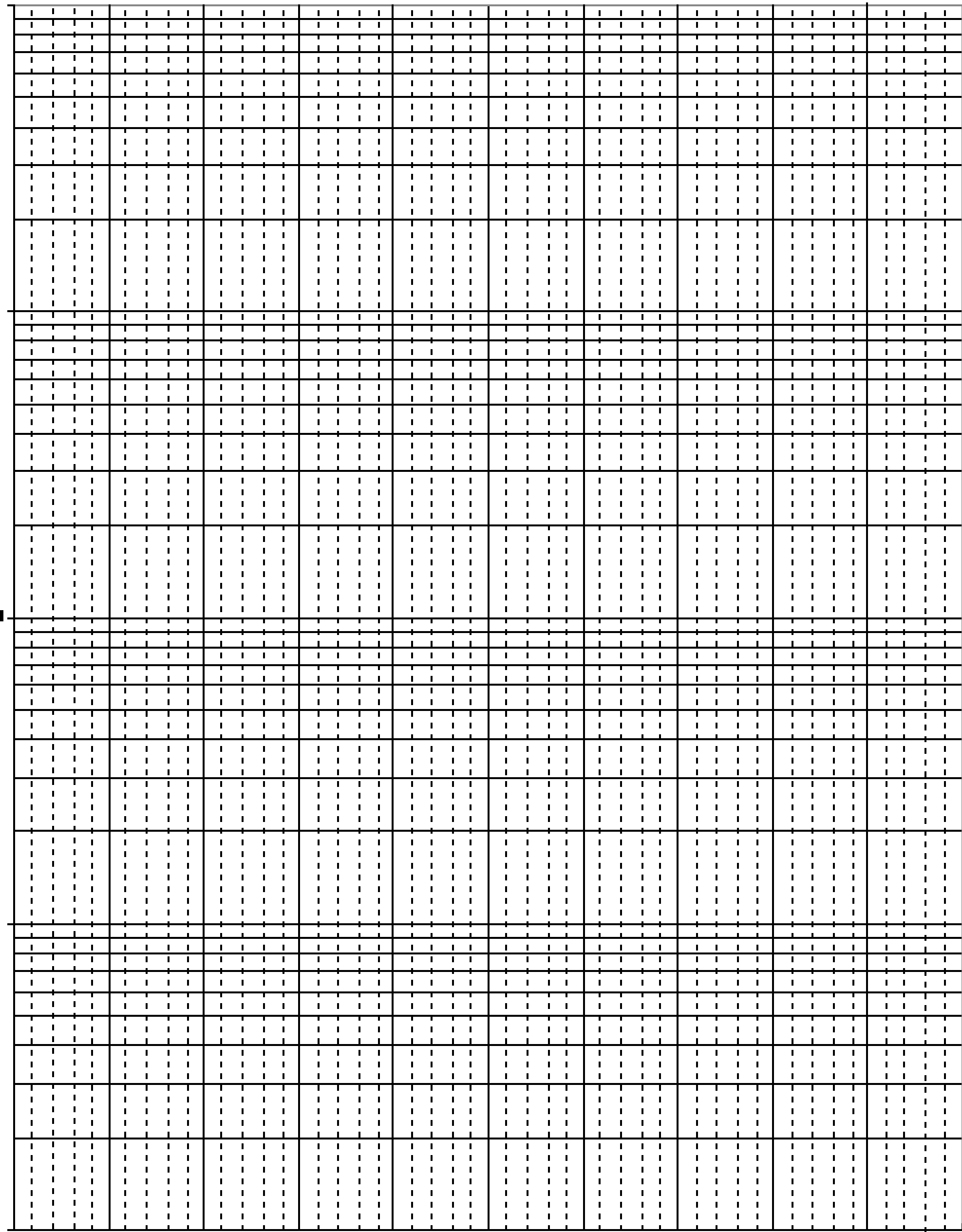
Migration of unknown bands from YOUR plasmid restriction digestion, in mm:

Xho I—1 band:

BamHI—3 different bands:







Summary Questions:

1. **Use your 3 standard curves to estimate the size of your pGLO DNA restriction fragments here:**

   Sizes of our pGLO DNA cut with BamHI as extrapolated from your own gel:

   Sizes of our pGLO DNA cut with XhoI as extrapolated from your own gel:

2. The green fluorescent protein is 229 amino acids long. How large a piece of DNA would be required to encode this protein? Are any of the fragments you obtained near that length?

3. Are the fragments obtained consistent with the hypotheses/predictions that were made during the sequence analysis experiment (Exercise 6a)?

4. What is the relationship between DNA migration in the gel and the size of the DNA molecules? Why are the lines in your graphs different from one another?

5. It is easy to understand why multiple bands would be present in the lanes containing DNA that had been cut with a restriction enzyme, but why do you think there are several bands present in the lane containing undigested plasmid DNA?

Transformation

1. Which plates should be compared to determine whether genetic transformation has occurred? Why?

2. What is meant by control plate? What purpose does a control serve?

3. How much bacterial growth do you see on each plate? If you observe any difference, what might be the cause of this?

4. What color are the bacteria under white light? Under long wave UV light? Explain any differences between the plates.