Identifying a Potato Killer: Using Gel Electrophoresis **Day TWO**

Problem: Assays only tell half of a tale; you must have a method in which to determine what pathogen is infecting a particular crop. In your laboratory exercise today, you will use your PCR samples in conjunction with Gel Electrophoresis to analyze your samples infected with *P. infestans*, the pathogen responsible for the Irish Potato Famine.

Objectives: By the end of this activity, you will be able to…

- Use Gel Electrophoresis to analyze DNA Extraction and Polymerase Chain Reaction (PCR) samples.
- Read a Gel to identify areas of amplification of DNA.
- Identify the plant pathogen (*P. infestans*) that is responsible for the Irish Potato Famine.

Student Materials: (6 Lab Setups, 1 per table group)

4 Tubes from Previous Day that underwent PCR	1 premade gel	1 metric ruler
Student Lab sheet (1 per person for portfolio project)	1 Gel Electrophoresis Box	Latex Gloves
1 MicroPipette (2-20 µl)	1 bottle of 1X TBE buffer	1 tube 10x loading dye
1 tube of ready to load marker (pBR322 cut with BstN1)	1 staining tray/paper plate	1 UV light box
1 semi-log graph paper (available on my webpage!)	1 bottle of Carolina BLU™	1 camera

Safety Considerations: Be aware there are chemicals in use that are dangerous to your skin, clothing, and eyes. Please wear your **safety glasses and gloves** at all times! Know that once the box is plugged in, the liquid can **shock** you!

Special Instructions: It only takes a miniscule amount of DNA to contaminate Gel Electrophoresis. Thus use **new tips** for your pipette any time you move between your samples. This will help prevent **any cross contamination** of your samples. Remember that micropipettes have two "stops." The first stop draws up liquid that you have calibrated and when you press all the way down to the second and final "stop" you will expel the liquid you have drawn up.

Hypothesis: Gel Electrophoresis works on the basis of how far DNA fragments can move through the gel. Essentially, shorter DNA fragments move farther through the gel than larger DNA fragments and vice versa.

Create a hypothesis about how the bands would different between samples 1, 2, 3, and 4. **Be specific for each sample.**

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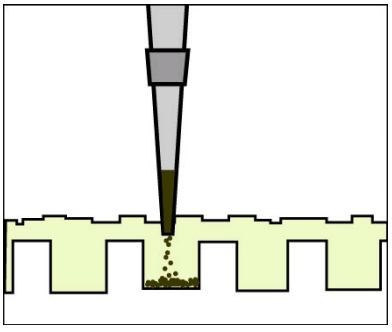
Instructions:

1. Place your gel into your gel box and add cover it (so it just covers the surface of the gel) with **1X TBE buffer**.
2. Load 20 µl of the **molecular weight marker** (pBR322/BstN1) into well 1 of your gel.
3. Add 2.5 µl 10X **loading dye to each PCR reaction tube** (1, 2, 3, and 4) and mix gently by pipeting up and down. Then load 25 µl of each reaction into separate wells of the gel as designated below. (*Calibrate accordingly!)*

Special Note: Make sure to switch tips each time!



Well 2: Negative Control (**Tube #1**)
Well 3: DNA from healthy leaf (**Tube #2**)
Well 4: DNA from infected leaf (**Tube #3**)
Well 5: DNA from positive control (**Tube #4**)

*Keep in mind, when loading a gel, make sure not go so deep to **puncture** the gel nor too light so it **does not go into the well** as you will get poor results. Steady your hand and look carefully as you are loading your sample directly into the space of each well. Good lab technique will garner good results!*

4. Put on the cover to the gel box set the voltage for 140 volts. Then plug it in to the socket. Check the side of the box for rising bubbles to ensure electricity is flowing. **DO NOT TOUCH THE ELECTRIFIED LIQUID!**

5. Wait for 20 to 30 minutes. <u>You will see the purple/blue colors move towards the **mid-line or end** of the gel to know when it is complete</u>. In the meantime, feel free to discuss with your group the analysis questions.
6. When you see the colors reach the midline or end immediately shut it off and unplug your gel box from the outlet.
7. Transfer your gel to your paper plate and hand it to your instructor. Your teacher will pour Carolina BLU™ Stain over your gel (just enough to cover it) for you to visualize later.
8. Cleanup and reset your lab station as per your teacher's instructions.
9. **Later**: Place gel on light box and view your results. Have a picture taken of your gel.

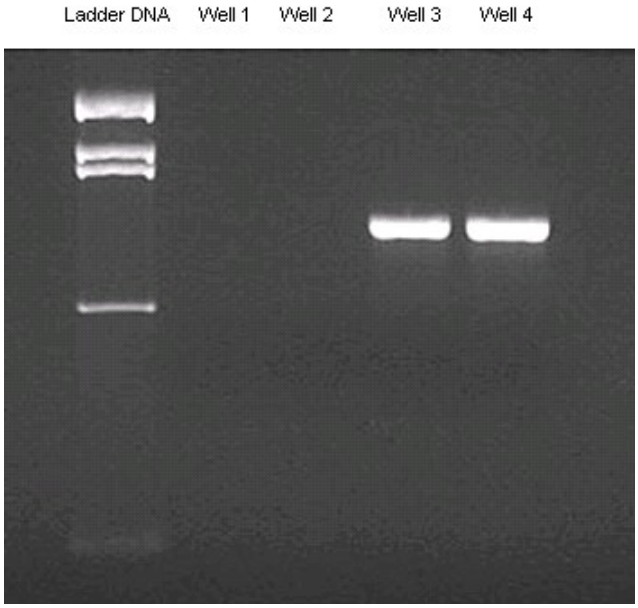
<u>Data</u>: In the space here, draw what you saw of your gel. You will put this in your formal results, but it is good to record data while it is fresh on your mind. You will use this information to answer the analysis and conclusion questions.

<u>Analysis & Conclusions:</u> Using your data, answer the following questions on a **separate sheet of paper and typed**!

1. Measure the distance that each marker DNA band migrated from the sample well, by measuring from the front of each well to the front edge of each band. The size of the DNA fragments in your marker is as follows: 1857 bp, 1058 bp, 929 bp, 383 bp, and 121 bp.

   Set up **semi-log graph paper** (*not supplied, you can find it online*) with the x-axis as the distance migrated by the DNA fragments and the y-axis (the logarithmic axis) as base pair length of the fragments. Plot the distance migrated versus base-pair length for each marker DNA fragment and connect the data points with a line. (This line will be referred to as the "standard curve.") There is a linear relationship between the distance that a DNA band travels and the log of its molecular weight. Since the molecular weight of a DNA fragment is proportional to the number of base pairs in the fragment, base pairs are frequently used in place of molecular weight when these types of calculations are done with DNA. *Also note that since you are plotting the molecular weight (in this case base pairs) on the log scale, it is **not necessary to take the log of the molecular weight prior to graphing**.*

2. Measure and record the distances migrated by the predominant DNA band in your PCR reaction. To determine the size of your DNA band, first locate the position on the x-axis that indicates the distance migrated by that band. Then, use a ruler to draw a vertical line from this point to its intersection with the "standard curve." Next, extend a horizontal line from this intersection point to the y-axis. The number on the y-axis is the calculated base-pair size of your PCR product.



3. Revisit your hypothesis based upon the graph you created. Was it supported or rejected? Why or why not? (If why not – what were some sources of error for your experiment? Make sure to include the steps of DNA Extraction and PCR.)

<u>Reference</u>: Here is a picture of a gel of expected results to help you answer question #3.