Dye Electrophoresis Lab

Molecular Rainbow

miniPCR bio Learning Lab™
Dye Electrophoresis Lab: Molecular Rainbow
Student's Guide

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Student's Guide Contents

Background information

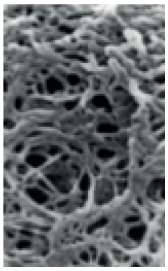
Introduction to gel electrophoresis

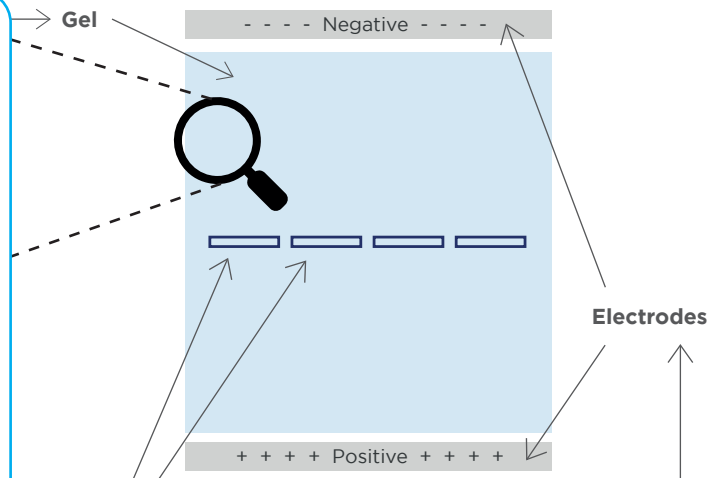
One of the most common methods for separating and observing biological molecules in the lab is called **gel electrophoresis**. The word electrophoresis means carried by electricity. During gel electrophoresis, an electric field causes molecules with an electric charge to move through a gel.

To perform gel electrophoresis you need (1) a gel, usually made of a substance called agarose, and (2) a way to conduct electricity through the gel.

1

Agarose gels feel like firm Jell-O. At the microscopic level, the inside of a gel looks like a web or a sponge. Small molecules can move through the holes without much trouble, but larger molecules get slowed down. This allows us to separate molecules of different sizes.





2

When we make a gel, we make it with small pockets called **wells**. These wells are arranged in a line across the gel. The wells allow us to add our samples into the gel.

Wells

3

Metal bars or wires are placed on either side of the gel. These act as positive and negative **electrodes**. If there is a conductive material between the electrodes, then electrical current can flow between them. During gel electrophoresis, charged molecules will move through the gel towards the oppositely charged electrode.

The gel is covered in a liquid called **electrophoresis buffer**, which helps conduct electricity between the two electrodes and through the gel. When the power is turned on, any charged molecules will move through the gel towards the electrode of the opposite charge.

Review

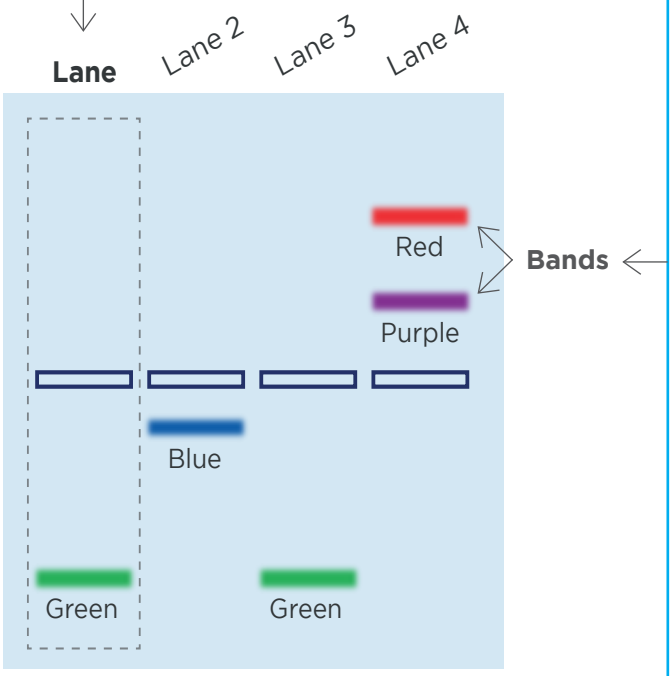
1. What causes molecules to move through the gel during gel electrophoresis?

2. What role does the gel play in gel electrophoresis?

When scientists discuss molecules separating in a gel, they talk about **bands** moving in **lanes**. Let's take a closer look at what these two terms mean.

1

Charged molecules that are put in a well will move towards the electrode that has an opposite charge. As they move through the gel, they travel in a straight line. Like runners on a track, when we talk about the molecules moving through a gel, we talk about them moving in **lanes**.



2

There are usually billions of molecules in each well of the gel. As the molecules move towards an electrode with the opposite charge, all of the molecules that have the same charge and size will move through the gel in the same direction and at the same speed. This group of molecules moving together will stay in about the same shape as the well they started in. When you look at the gel, the group of molecules will look like a small horizontal line on the gel. We call these lines **bands**.

In Lane 4, there are two bands: a red band made of billions of red molecules and a purple band made of billions of purple molecules. Both of these bands started in the well in Lane 4, but as the molecules moved through the gel the two bands separated because the red and purple molecules are different sizes and traveled through the gel at different speeds.

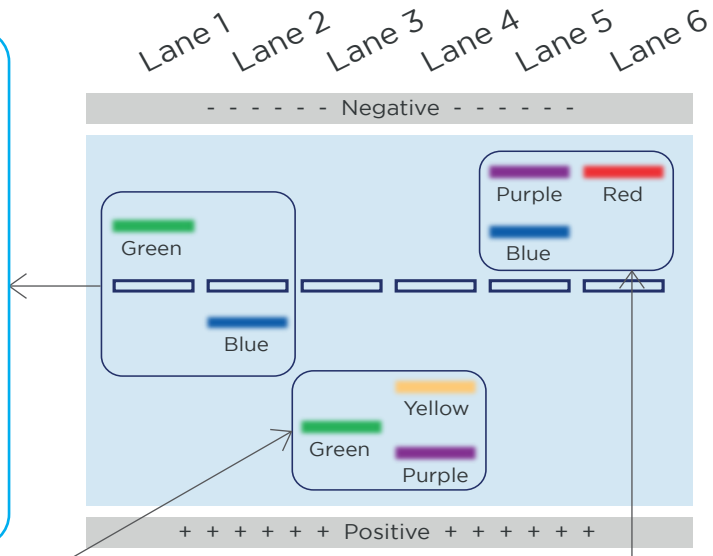
Interpreting gel results

You can get a lot of information from gel electrophoresis results! There are three pieces of information that we will focus on today: charge, composition, and size.

1

What is the charge of the molecules that were in the sample?

- Charged molecules will be attracted to the *opposite* charge.
- The attractive force will cause positive molecules to move towards the negative electrode. The green band in Lane 1 is made of positively charged molecules.
- Negative molecules will move towards the positive electrode. The blue band in Lane 2 is made of negatively charged molecules.



2

What is the composition of the molecules that were in the sample? Put another way, how many types of molecules were in the sample?

- If there is only one band in a lane it usually means that there is just one type of molecule in that sample. Lane 3 has only one green band, meaning only one type of molecule was placed in the well.
- If there is more than one band in the lane, it means there is more than one type of molecule in that sample. Lane 4 has two bands, a yellow band and a purple band, meaning a mixture of two molecules was placed in the well.

3

How big were the molecules in the sample compared to each other?

- Smaller molecules can move through the gel easily and will travel farther from the wells than larger molecules with the same charge. In Lane 5, the purple band moved farther from the well than the blue band. If we assume the purple and blue molecules have the same net charge, we can infer that the purple molecules are smaller than the blue molecules.
- Molecules that are the same size and charge will travel the same distance. The purple band in Lane 5 and the red band in Lane 6 traveled the same distance from the well. If we assume the purple and red molecules have the same net charge, we can infer that the purple and red molecules are about the same size.

Glossary

Gel electrophoresis: *A method used to separate molecules by size and charge.*
During gel electrophoresis, an electric force is used to move molecules through a gel in a direction dictated by their net electrical charge. The gel acts as a size filter; smaller molecules move more easily through the gel and travel further than larger molecules. This allows scientists to separate molecules of different sizes.

Agarose gel: *A type of gel commonly used for gel electrophoresis.*
Agarose is a sugar from seaweed. At the microscopic level, the inside of an agarose gel looks like a web or a sponge. Small molecules can move through the holes with relative ease, but larger molecules get slowed down. This allows scientists to separate molecules of different sizes.

Well: *A pocket in a gel where samples are placed at the start of a gel electrophoresis experiment.*
When making a gel, a mold called a comb is used to create pockets called wells. Samples can be placed into these wells. Then, when an electric force is applied, the molecules in the sample will move through the gel and travel towards the oppositely charged electrode.

Electrode: *A conductor that is used to establish a current through an object or material.*
During a gel electrophoresis experiment, an electrical current is used to move charged molecules through the gel. Metal wires or bars are placed on either end of the gel and connected to a power source. These wires serve as negative and positive electrodes that drive the flow of electricity through the gel.

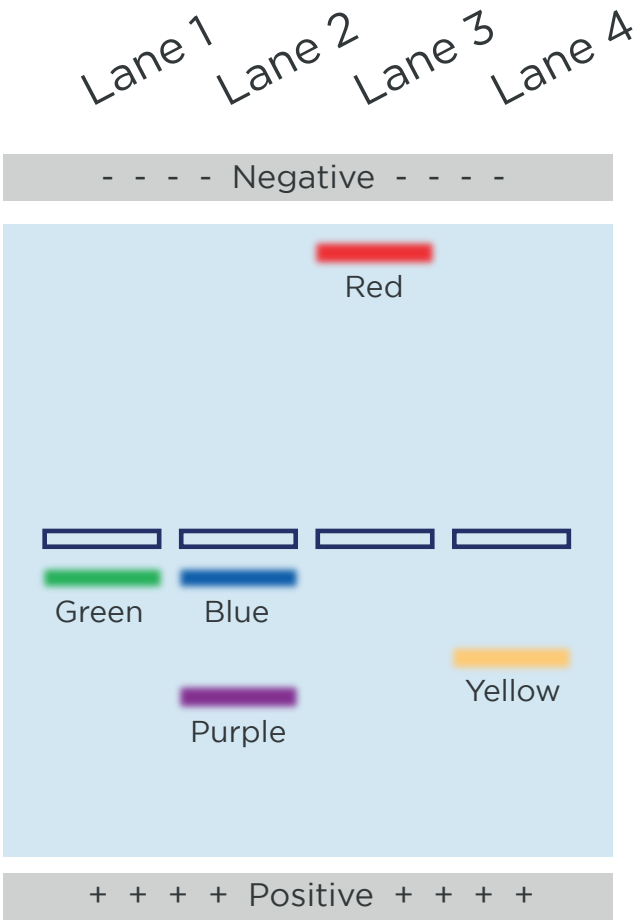
Electrophoresis buffer: *A solution used to conduct electricity between the electrodes during gel electrophoresis.*
During a gel electrophoresis experiment, charged molecules that are put in a well will move towards the electrode that has an opposite charge. The gel is covered in a salty liquid that conducts electricity between the electrodes and through the gel.

Lane: *The straight line through which molecules travel through a gel.*
During a gel electrophoresis experiment, charged molecules that are put in a well will move towards the electrode that has an opposite charge. As they move through the gel, molecules travel in a straight line that is the same width as the well. This area is referred to as a lane.

Band: *A visible group of molecules that traveled together through a gel.*
During a gel electrophoresis experiment, the molecules in a sample move through the gel towards an electrode with the opposite charge. All of the molecules that have the same charge and size will move through the gel in the same direction and at the same speed. The molecules will stay in about the same shape as the well they started in. When you look at the gel, the group of molecules will look like a small horizontal line, or band, on the gel.

Review

Refer to the gel on the right to answer the following questions:



Charge:

1. Which band(s) are made up of negatively charged molecules? Select as many answers as are correct.
   a. Green band
   b. Blue band
   c. Purple band
   d. Red band
   e. Yellow band

   Explain how you can tell:

2. Which band(s) are made up of positively charged molecules? Select as many answers as are correct.
   a. Green band
   b. Blue band
   c. Purple band
   d. Red band
   e. Yellow band

   Explain how you can tell:

Composition of samples:

3. Which lane(s) contained a single type of dye? Select as many answers as are correct.
    a. Lane 1
    b. Lane 2
    c. Lane 3
    d. Lane 4

    Explain how you can tell:

4. Which lane(s) were mixtures and contained more than one type of dye? Select as many answers as are correct.
    a. Lane 1
    b. Lane 2
    c. Lane 3
    d. Lane 4

    Explain how you can tell:

Size:

5. Let's focus on the green, blue, purple and yellow bands on the bottom half of the gel. If you assume all the molecules have the same net charge, which band(s) in the gel are made up of the smallest molecules? Select as many answers as are correct.
    a. Green band
    b. Blue band
    c. Purple band
    d. Yellow band

    Explain how you can tell:

6. If you assume all the molecules have the same net charge, which band(s) in the gel are made up of the largest molecules? Select as many answers as are correct.
    a. Green band
    b. Blue band
    c. Purple band
    d. Yellow band

Explain how you can tell:

Today's lab

Today, you will use gel electrophoresis to analyze dyes.

The way each dye will move through the gel will depend on the characteristics of the molecules that make that dye.

- The direction that dye molecules will move through the gel depends on the charge of the molecules. Negatively charged molecules will move towards the positive electrode, while positively charged molecules will move towards the negative electrode.
- How fast molecules will move through the gel will depend on the size of the molecules—smaller molecules will typically move faster.

The dyes:

Your task is to use gel electrophoresis to study the dye samples. You will determine:

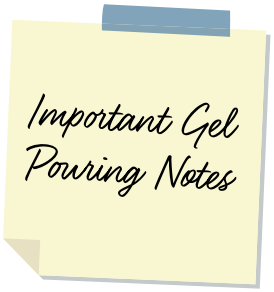
- **Charge:** Which dye molecules are positively charged and which ones are negatively charged?
- **Composition:** Which dye samples contain only one type of dye and which ones are mixtures of more than one dye?
- **Size:** Which dye molecules appear to be large and which ones seem small compared to each other?

Time to analyze the molecular rainbow!

Laboratory guide

Protective gloves and eyewear should be worn for the entirety of this experiment.

Pouring gels (before or during class period)



- Gels can be prepared up to five days ahead of time and stored at room temperature if placed in an airtight container.
- These instructions are designed for use with the pre-weighed Agarose Tabs™ provided in the lab kit.
- One Agarose Tab™ will yield one gel for use in either a Bandit™ or blueGel™ electrophoresis system by miniPCR bio™.
- If using a different electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions. You can use gels of any percentage between 1-2%.

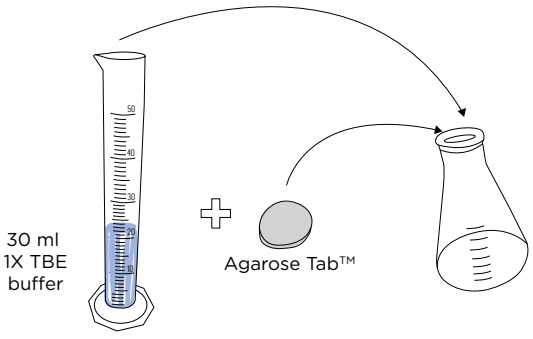
If using a Bandit™ STEM Electrophoresis Kit, detailed instructions for pouring and running gels can be found on page 29

A. Prepare 1X TBE buffer (to be completed by teacher in advance)

- Prepare at least 60 ml of buffer for every Bandit™ or blueGel™ electrophoresis system you plan to use.
- 30 ml of the buffer will be used to make your gel and 30 ml will be used as running buffer.
- Refer to page 6 for detailed instructions on preparing 1X TBE buffer.

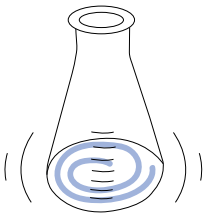
B. Prepare an agarose solution

- Obtain a heat-resistant container such as a glass Erlenmeyer flask or beaker that is at least three times the volume you wish to add.
- Combine 30 ml room temperature 1X TBE buffer and one Agarose Tab™ for each gel you plan to pour.
- Allow the tabs to soak until they fully disintegrate (this could take a few minutes).
- Swirl the flask or beaker to ensure the tabs have fully disintegrated before heating.



C. Heat solution

- Expect to heat for about 60 seconds per 30 ml of liquid in a standard microwave.
- Heat until the solution boils and continue until agarose is fully dissolved. No agarose particles should remain.



Caution: The solution may boil over the top of some containers. The solution will be very hot.

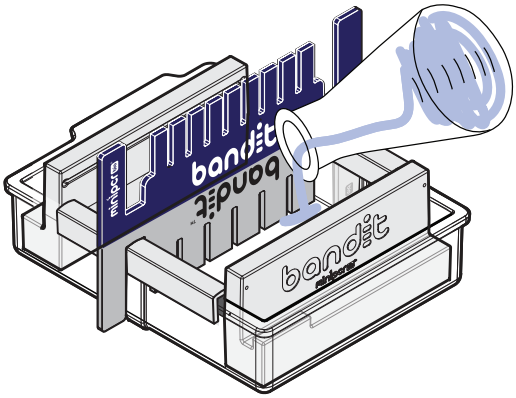
D. Set up your gel casting system with the comb in the center position

- You will need six lanes per gel.
- If using a Bandit™ STEM electrophoresis kit, make sure Electrodams™ are firmly in place before pouring the gel. Refer to page 29 for detailed assembly instructions.
- If using a different electrophoresis system, refer to the manufacturer's instructions for how to set up your particular gel casting system.

E. Pour the agarose solution into the prepared casting platform with a gel tray and comb

- The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).
- Note: Because this lab uses colored dyes as experimental samples, there is no need to add DNA stain.

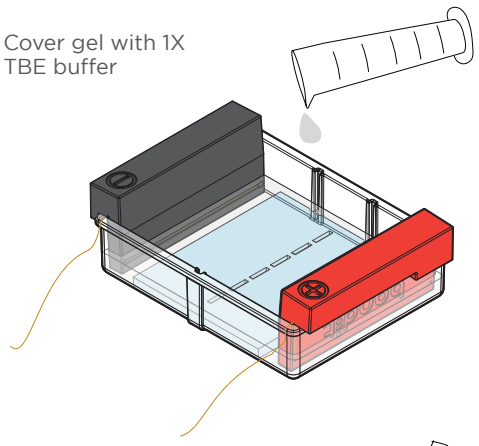
Prepare an agarose gel with comb in center position



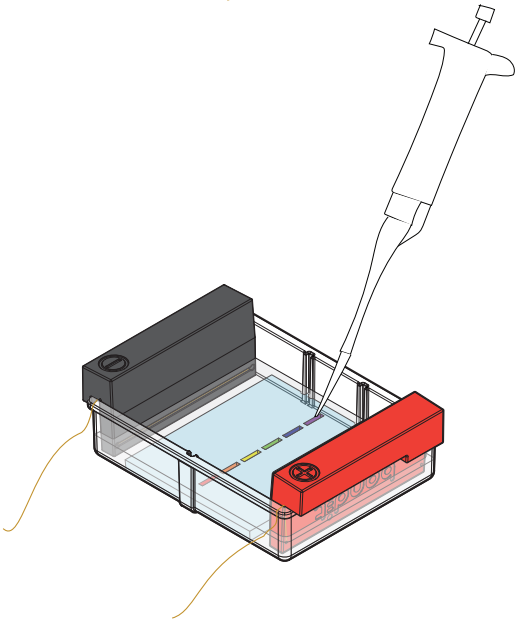
F. Allow gel to solidify completely

- Gel is ready when cool and firm to the touch.
- Gels will typically be ready in about 10 minutes.
- Gels can be stored in an airtight container at room temperature for up to five days before use.
- You can remove the comb and disassemble the gel casting apparatus before storing the gel.

Running the gel



1. **Submerge your gel in enough 1X TBE buffer to just cover the gel and fill the wells**
   - If using a Bandit™ STEM electrophoresis kit, your gel is already in the buffer chamber. Ensure that the Electrodams™ are in the correct orientation and have the electrode wires threaded (refer to page 33 for detailed instructions).
   - If using a Bandit™ or blueGel™ electrophoresis system you will need approximately 30 ml of 1X TBE buffer.



2. **Use a micropipette to load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl **Red Dye**
   - **Lane 2:** 10 µl **Orange Dye**
   - **Lane 3:** 10 µl **Yellow Dye**
   - **Lane 4:** 10 µl **Green Dye**
   - **Lane 5:** 10 µl **Blue Dye**
   - **Lane 6:** 10 µl **Purple Dye**

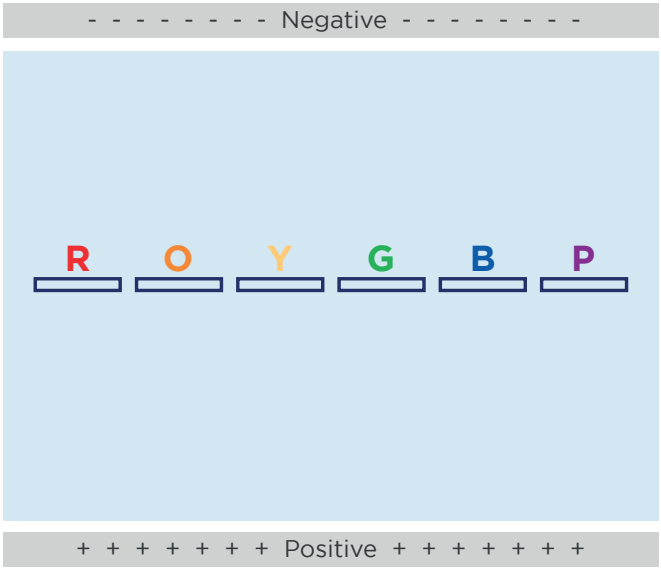
3. **Connect the electrodes and turn on your gel electrophoresis system**
   - If using a Bandit™ STEM electrophoresis kit, refer to page 33 for detailed assembly instructions.

4. **Conduct electrophoresis for 15-25 minutes**
   - Longer electrophoresis times will result in better separation.
   - Placing the gel over a white background will make it easier to see your results.

Post-lab study questions

Interpreting results

1. Use the image on the right to draw the bands that you see on your gel. There are six lanes on the gel: one for each dye sample.



Charge:

2. Which lane(s) in the gel contain bands made up of negatively charged molecules? Explain how you can tell.

3. Which lanes(s) in the gel contain bands made up of positively charged molecules? Explain how you can tell.

Composition of samples:

4. Which sample(s) contained a single type of dye molecule? Explain how you can tell.

5. Which sample(s) were mixtures and contained more than one type of dye molecule? Explain how you can tell.

Size:

6. Let's focus on the negatively charged dyes. If you assume all the molecules have the same net charge, which color band(s) in the gel seem to be made up of the smallest molecules? Explain how you can tell.

7. If you assume all the negatively charged molecules have the same net charge, which color band(s) in the gel seem to be made up of the largest molecules? Explain how you can tell.

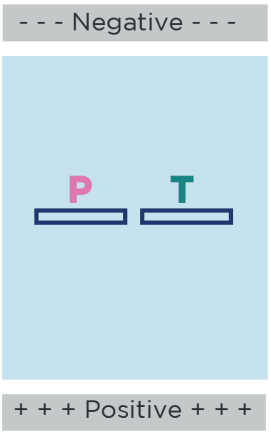
Critical thinking

8. Were the same types of molecules present in more than one sample? Explain how you can tell.

9. In questions 6 and 7 above, you were asked to assume the molecules you were comparing had the same net charge. Imagine you run another gel where one sample contains pink molecules and the other sample contains teal molecules. The pink and teal molecules are the same shape and size, but the pink molecules have a net charge of +3 while the teal molecules have a net charge of +1.

   A. Use the image on the right to draw the expected results from this experiment.

   B. In the gel you ran, all of the molecules had a similar net charge. Why was that important for interpreting the relative size of the molecules? Use the example given here in your answer.



CER Table

Fill in the table based on your results from the lab. Use the rubric on the next page to guide your answers.

Question:

Which dye(s) contained negatively charged molecules and which dye(s) contained positively charged molecules?

Claim Make a clear statement that answers the above question.	
Evidence Provide data from the lab that supports your claim *(hint: you may want to consult other lab groups' results, in order to have more data to evaluate)*	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100