

Agarose Gel Electrophoresis Lab

ACTIVITY AT A GLANCE

Goal:

This lab will determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the products.

Learning Objectives:

Upon completion of this activity, students will have integrated scientific discovery, inquiry, and biotechnology. Students will understand that DNA contains hereditary information in the form of genes, how DNA samples separate based upon different sizes, learn how to visualize DNA samples. We will be using agarose gel electrophoresis to determine the presence and size of two different gene fragments amplified by PCR. The two genes we are amplifying are a mitochondrial gene (*Cytochrome Oxidase I*) found in all eukaryotes, and a version of a *16S rDNA* found only in *Wolbachia*.

Assessed Outcomes:

Assess the student's ability to use and understand the technique of gel electrophoresis. Assess their ability to explain the importance of agarose gel electrophoresis as part of the *Wolbachia* Project.

Assess the student's ability to describe how gel electrophoresis separates DNA and its application to identifying PCR products.

Teaching Time:

90 minutes or two class periods of 45 minutes each

National Science Education Standards Addressed:

Unifying Concepts and Processes in Science, Science as Inquiry, Science and Technology, Life Science, History and Nature of Science

MATERIALS (per group of two students)

- | | |
|---|---|
| <input type="checkbox"/> Your PCR products | <input type="checkbox"/> GelRed fluorescent DNA stain |
| <input type="checkbox"/> Gloves | <input type="checkbox"/> Parafilm or wax paper |
| <input type="checkbox"/> 1 sharpie | <input type="checkbox"/> Electronic balance for class |
| <input type="checkbox"/> 1 box of P200 pipet tips | <input type="checkbox"/> Weighing dishes or paper |
| <input type="checkbox"/> 1 box of P20 pipet tips | <input type="checkbox"/> Spatula |
| <input type="checkbox"/> P200 and P20 pipets | <input type="checkbox"/> 500 ml flask |
| <input type="checkbox"/> 1 rack for holding PCR tubes | <input type="checkbox"/> 100 ml graduated cylinder |
| <input type="checkbox"/> 5X Loading Buffer | <input type="checkbox"/> Microwave or hot plate for class |
| <input type="checkbox"/> DNA ladder Bio-Rad EZ Load | <input type="checkbox"/> Plate or water bath for class |
| <input type="checkbox"/> Precision Molecular Mass Ruler | <input type="checkbox"/> Oven mitt or tongs for class |
| <input type="checkbox"/> SeaKem Agarose | <input type="checkbox"/> Masking tape for class |
| <input type="checkbox"/> Gel casting tray and combs | <input type="checkbox"/> Safety goggles |
| <input type="checkbox"/> SB (Sodium Borate) Buffer 1x | |

OVERVIEW

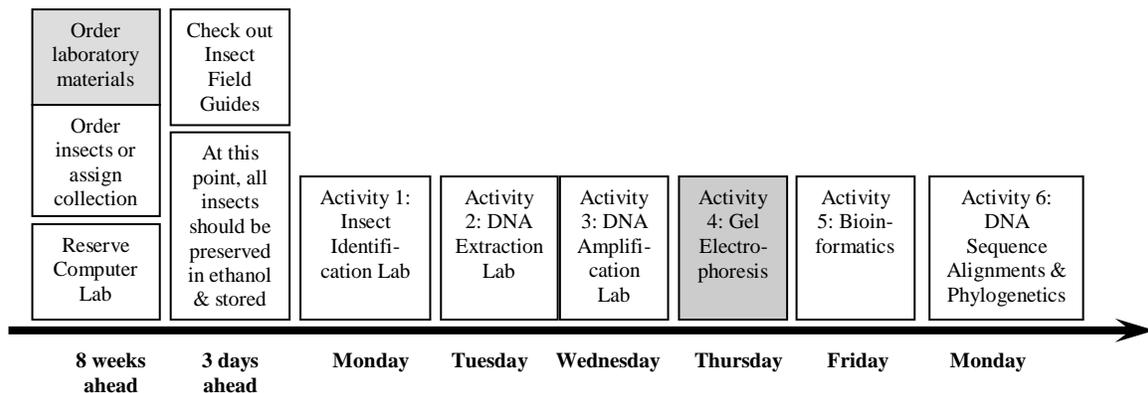


Introduction:

Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab.

During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is pulled through the pores of the gel by the electrical current. Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose, and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

Timeline for Teaching *Discover the Microbes Within: The Wolbachia Project*



Agarose Gel Electrophoresis Lab

Goal:

The methods used during this lab will determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product.

Learning Objectives:

In this activity you will learn how DNA samples separate based upon different sizes and learn how to stain and visualize DNA samples. We will be using agarose gel electrophoresis to determine the presence and size of two different gene fragments (mitochondrial Cytochrome Oxidase I, and *Wolbachia 16S rDNA*) amplified by our PCR.

PROCEDURE:

Preparing the gel

1. Measure 2 g Agarose powder and add it to a 500 ml flask
2. Add 100 ml SB Buffer to the flask. (2% solution; note the total gel volume will vary depending on the size of the casting tray)
3. Melt the agarose in a microwave or hot water bath until the solution becomes clear. Look for “lenses” in the liquid by holding up against the light. If using a microwave, place a ball of Kimwipe tissue in the flask opening, or cover with film and poke a hole for venting, and heat the solution for several short intervals — do not let the solution boil over.
4. Let the solution cool to about 50–55°C, swirling the flask occasionally so it cools evenly. You will be able to hold it on your hand for an extended time.
5. Seal the ends of the casting tray with two layers of lab tape.
6. Add 10 µl GelRed (Phenix Research) stain to the agarose, swirl to mix.
7. Place the combs in the gel casting tray.
8. Pour the melted agarose solution into the casting tray. Note: fill the gel in the tray so that only the teeth of the comb are immersed under the gel, but the base of the comb is just above the gel. You do not have to use all the melted agarose. Let cool until it is solid (opaque).
9. Carefully pull out the combs and remove the tape.
10. Place the gel in the electrophoresis chamber.
11. Add enough SB Buffer so that there is about 2-3 mm of buffer over the gel.

Loading the gel

1. Pipet 2 µl drops of 5X Loading Buffer (BioRad) onto a piece of parafilm or wax paper. Add 10 µl from each of your PCR reaction tubes to a drop of loading buffer. Then mix well by gently pipetting up and down several times until the color of the liquid is homogenous. Note: if you do not intend to perform DNA sequencing, you can add loading buffer (5 µl) to the entire PCR reaction.

- Record the order each sample will be loaded on the gel, including who prepared the sample, the DNA template – the organism the DNA came from, controls and ladder.

<i>Lane #</i>	<i>Prepared by</i>	<i>DNA Template</i>	<i>notes</i>

- Carefully pipette 10 µl of each sample/Sample Loading Buffer mixture into separate wells in the gel. You can store the remaining solution in the freezer.
- Pipette 10 µl of the DNA ladder standard into at least one well of each row on the gel.

Running the gel

- Place the lid on the gel box, connecting the electrodes appropriately (positive (red) and negative (black))
- Turn on the power supply to about 200 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber, and will be printed on its label.
- Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
- Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes.
- Let the power run until the blue dye approaches the end of the gel, then turn off the power, disconnect the electrodes, remove the lid and the gel using gloves.

Viewing the gel

CAUTION; UV LIGHT CAN DAMAGE EYES!!!! EYE PROTECTION REQUIRED!

- Place the gel on the transilluminator. Note the presence or absence of bands in each lane. Use the DNA ladder of fragments of a known size to determine the size of the PCR products.

2. SUMMARY

Date _____

<i>Lane #</i>	<i>Prepared by</i>	<i>Insect Order</i>	<i>notes</i>	<i>Results (+/-) COI</i>	<i>Results (+/-) Wolbachia</i>
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Please answer the following questions:

1. Did your group's results demonstrate that *Wolbachia* can be found in insects? If they did or did not, explain why and use your data from the controls to validate your conclusions?

2. Which insect orders were collected by the class and what was the most commonly collected order by the class?

3. Which insect order is most commonly infected with *Wolbachia*? Explain the basis of why you chose this order.

4. Was the original hypothesis on the frequency of *Wolbachia* correct? Why or why not? Justify with the observed data.

5. What were the areas where errors could have occurred during your study and what does this teach you conducting your own science?

Sequencing positive PCR products

Freeze any samples that show a band on the gel for both the insect and *Wolbachia*; this DNA can be sent to Vanderbilt for sequencing.

Label the DNA extract and the corresponding PCR product tubes with student initials.

Wrap the caps in parafilm and place the samples in a Ziploc bag.

Include a list which gives teacher's name, student initials, insect order, any other identifying information that you have used, and a contact email address.

Contact *Wolbachia* Project (wolbachiproject@vanderbilt.edu) to coordinate the shipment of samples.

Ship samples FEDEX STANDARD OVERNIGHT to:

Bordenstein Lab
Vanderbilt University
465 21st Ave South
MRBIII, Room u7217
Nashville, TN 37232

Allow 4-6 weeks for your sequencing data (chromatograms and nucleotides).