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The *Wolbachia* Project

Discover the Microbes Within!

Lab 4: Gel Electrophoresis

Standard

Project Guide

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The *Wolbachia* Project: Discover the Microbes Within! was developed by a collaboration of scientists, educators, and outreach specialists. It is directed by the Bordenstein Lab at Vanderbilt University.
https://www.vanderbilt.edu/wolbachiaproject

Some figures created with BioRender.com

Introduction

This lab will determine the presence or absence of amplified DNA in your samples by visualization on an agarose gel. Arthropod and *Wolbachia* DNA, if present, will be distinguishable based on the size, or base pair (bp) length, of the DNA molecule.

Gel electrophoresis

Gel electrophoresis is a method of separating DNA fragments by movement through a Jello-like substance called agarose. Derived from a seaweed polysaccharide, agarose gels form small pores that act as sieves to separate DNA based on size; whereby smaller DNA molecules move through the pores faster and easier than larger molecules. **Loading wells** are oriented at the top of the gel to allow for precise insertion of PCR products into the gel. An electrical current is applied to move negatively charged DNA molecules away from a negative electrode (-) and toward a positive electrode (+). DNA migrates through the gel in a single, vertical **lane**. Three factors influence the speed of movement: (i) the voltage of the electrical field, (ii) the concentration of agarose, and (iii) most importantly, the size of the DNA molecule.

DNA Visualization

DNA itself is not visible within an agarose gel. Therefore, a fluorescent stain is added to the gel that binds DNA and fluoresces under UV or blue light. DNA will appear as a horizontal line, or **band**, on the agarose gel.

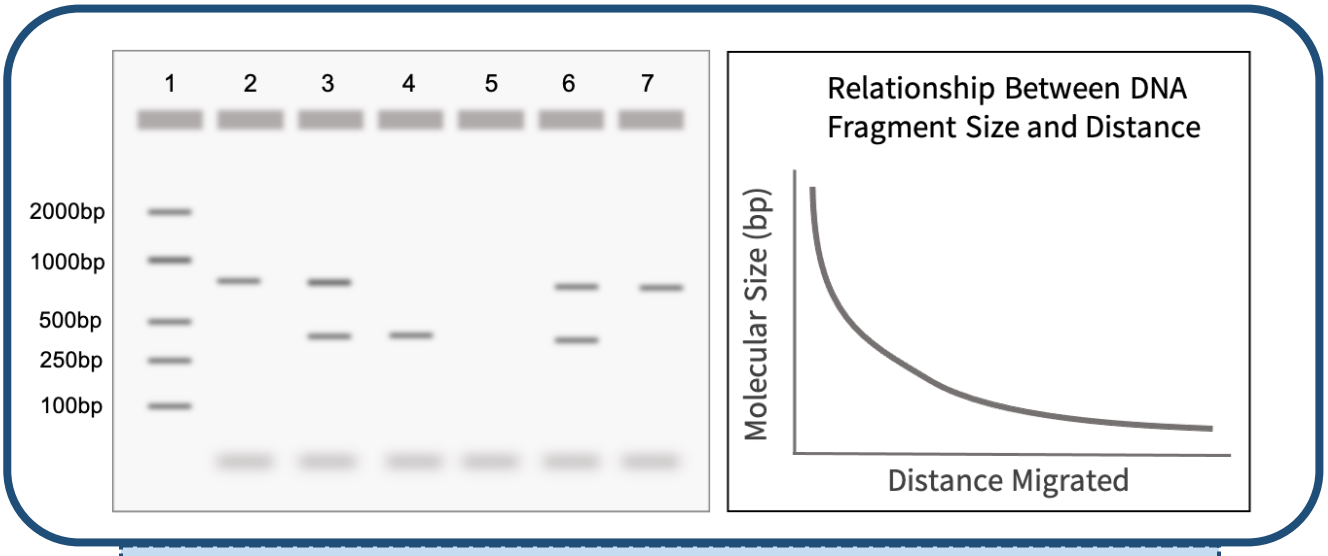


Figure 4.1 Gel electrophoresis allows for differentiation of DNA by size. Larger fragments do not move as fast as smaller fragments, and there is a nonlinear relationship between the size of the DNA fragments and the distance migrated. A DNA ladder, a sample with known fragment sizes, should always be run to identify the size of experimental bands.

Key Elements for Gel Electrophoresis

PCR Products (DNA)

The purpose of this lab is to visualize the PCR products, or amplified DNA, from your arthropod samples.

DNA Ladder

A DNA ladder is a cocktail of DNA fragments with pre-determined sizes. The ladder, also called a DNA marker, is loaded alongside experimental samples as a reference tool for estimating band size.

Agarose Gel

Agarose powder is dissolved into running buffer and boiled until the solution becomes clear. After slightly cooling, it is poured into a casting tray with combs to solidify and form the agarose gel. DNA will migrate through the gel and form separate bands based on size (correlating to length in bp).

DNA Stain

A DNA stain is added to the agarose gel to visualize DNA under a UV or blue light. There are two primary methods of DNA Staining:

- **Pre-stain**: DNA stain is added to the agarose gel mixture after melting, but before pouring, the gel.
- **Post-stain**: The gel is incubated in a stain solution after gel electrophoresis.

Running Buffer

Running buffer is a conductive liquid that allows the DNA to migrate through the agarose gel. It is important that the agarose gel be made using the same buffer. Tris/Borate/ETDA (TBE) or Tris/Acetic Acid/EDTA (TAE) are commonly used.

Loading Dye

Loading dye has two primary components: (i) a visible dye indicates how far the DNA has run on the gel and (ii) glycerol, which is denser than the buffer, ensures that samples fall into the loading wells rather than float back out. Some Taq Master Mixes (e.g., Promega GoTaq) already contain a pre-mixed loading dye.

Electrophoresis System

Running buffer and the agarose gel will be placed into the chamber of an electrophoresis system. After loading the samples, an electric current is applied to move the negatively charged DNA towards the positive end of the system. Without this electric field, the DNA will not migrate through the agarose gel. If the electric field is reversed, the DNA will run in the opposite direction, towards the top of the gel, and eventually exit the gel.

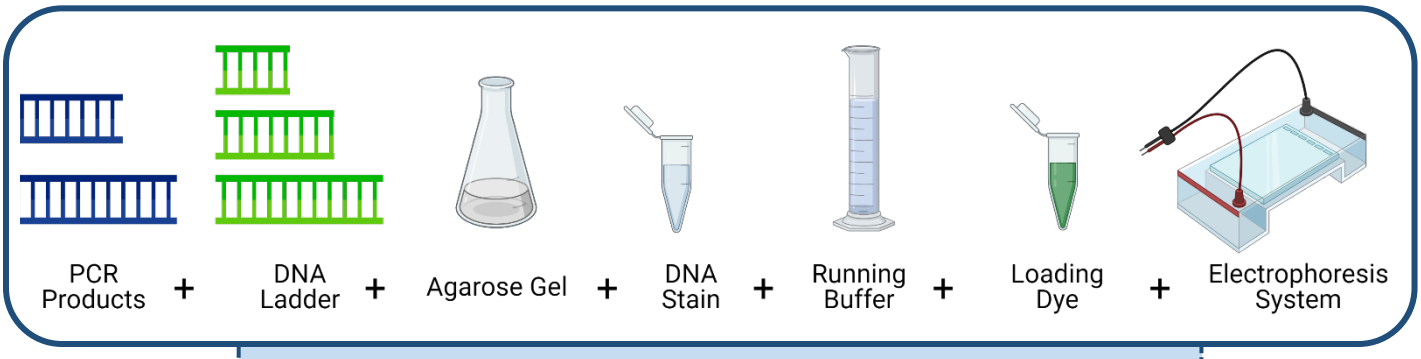


Figure 3.4. Pictograph of all necessary components for gel electrophoresis.

How to Read a Gel

Lanes

DNA that was loaded into each well will migrate in a single, vertical lane towards the (+) charge.

Bands

When DNA becomes separated by size due to gel electrophoresis, they appear as bands in the gel. These are clearly defined, bright lines in the gel.

DNA Ladder

The DNA ladder will contain multiple bands in one lane. Each band represents a pre-determined length of DNA and can be used as a reference tool to estimate DNA size for each of the experimental samples. Refer to the product information for specific band sizes.

Primer Dimers

PCR reactions are set up with an excess of primers. In addition, some primers bind to each other instead of binding to the DNA, creating primer dimers. Primers are ~25bp long, so excess primers appear as fuzzy bands on the bottom of the gel ~25-50bp. This is normal and to be expected.

Reading a Single PCR vs. Duplex PCR Gel

A single PCR gel will contain only one amplified PCR product, either *Wolbachia* or arthropod, in each lane. A separate gel will need to be run for each DNA type.

A duplex PCR means that both the arthropod barcoding gene and the 16S rRNA fragment from *Wolbachia* were amplified in the same PCR reaction. When visualizing this PCR reaction, two bands should appear in the same lane if *Wolbachia* is present, and only one band will appear if the arthropod is uninfected.

Expected Band Sizes

Arthropod Barcoding Gene (CO1): **708bp**
Wolbachia Specific Gene (16S rRNA): **438bp**

How to Interpret Gel Electrophoresis Results

To interpret gel electrophoresis results, first ensure that all controls are correct. The DNA ladder, (+) Arthropod control, (-) Arthropod control, and (+) DNA control should produce bands of expected size, whereas the water lane should be empty.

1. **DNA Ladder**
   To accurately read the gel, confirm the band size of experimental samples by comparing their location in the gel to reference bands in the DNA Ladder. Refer to the information sheet accompanying your DNA ladder for specific band sizes as the bands, or rungs, vary by product. Below is the DNA Marker (M3104) from MiniOne.
2. **Positive Controls**
   The (+) Arthropod control and (+) DNA control should have both the CO1 and *Wolbachia* band present. In a duplex PCR, as shown below, these will appear in the same lane. In a standard (single) PCR, these will be loaded into separate gels. The DNA ladder bands should be clearly present and separated.
3. **Negative Controls**
   The (-) Arthropod control should have a CO1 band, but no *Wolbachia* band. The negative water control should not have any band or smudge.

If all controls worked, the results of your experiment are valid, and the experimental bands can be analyzed. If the controls have unexpected results, or if there is a band in the water lane, refer to the Troubleshooting guide on page 16.

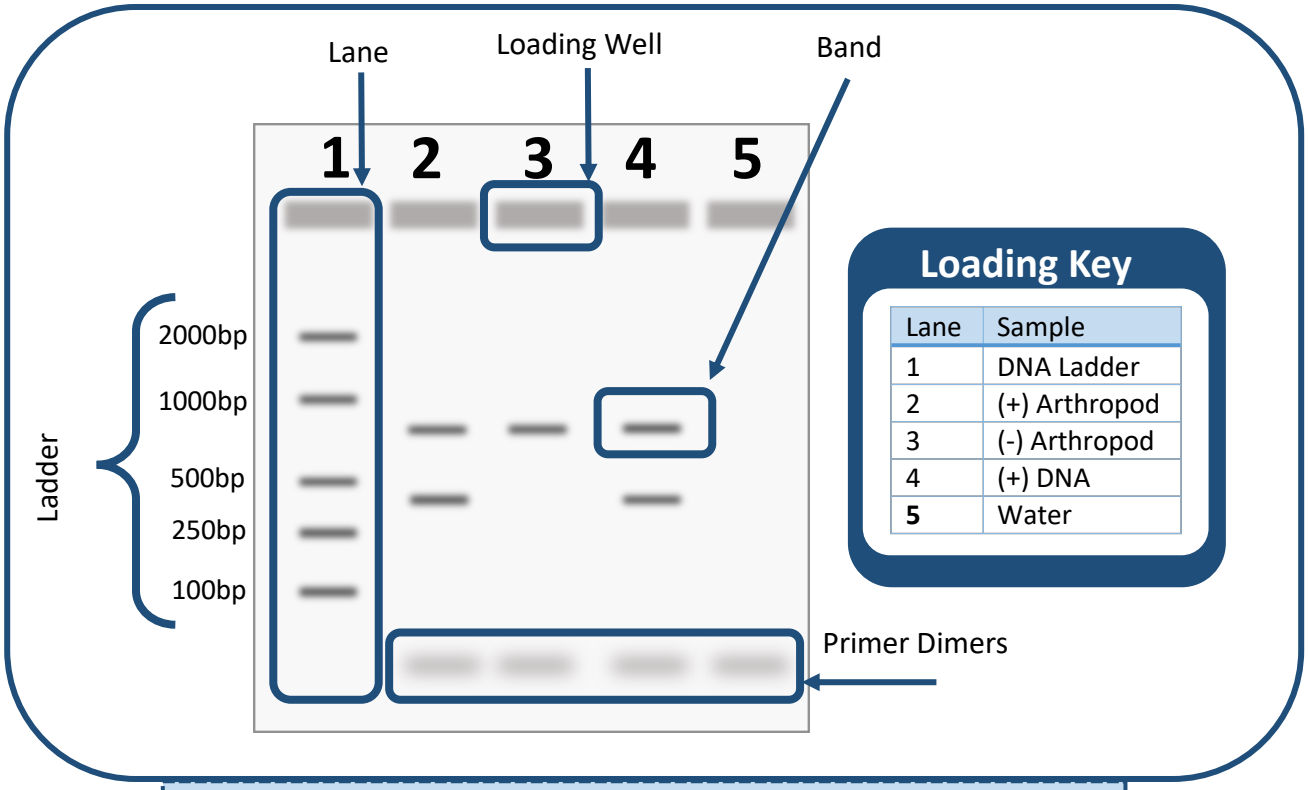


Figure 4.3. Schematic of a duplex PCR gel where both Arthropod and *Wolbachia* PCR products are loaded on the same gel. Common vocabulary terms are labeled on the gel, and the loading key is labeled according to each lane.

Pre-Lab Questions

Read through the entire protocol and answer the questions below.

1. Assume that single PCR reactions were loaded into two separate gels for arthropod and *Wolbachia* DNA analysis. Fill in the expected bands for lanes 2-7 using the table below.

Arthropod (COI) gel

Wolbachia (16S rRNA) gel

Lane	Sample
1	DNA Ladder (bands already shown)
2	An arthropod sample positive for *Wolbachia*
3	An arthropod sample negative for *Wolbachia*
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water

2. This experiment included five controls. In the table below, list the following for each lab activity:
   - (+) for positive control
   - (-) for negative control
   - N/A for not applicable

Control	DNA Extraction	PCR	Gel Electrophoresis
DNA Ladder			
(+) Arthropod Control			
(-) Arthropod Control			
(+) DNA Control			
Water			

Getting Started

Introduction

In this activity, you will use agarose gel electrophoresis to determine the presence and size of two different gene fragments (arthropod COI, and *Wolbachia* 16S rRNA) previously amplified by PCR. If you ran two separate PCR reactions, arthropod and *Wolbachia*, you should prepare and run two gels. If you set up a duplex reaction (both primer sets in one PCR tube), you will only need one gel.

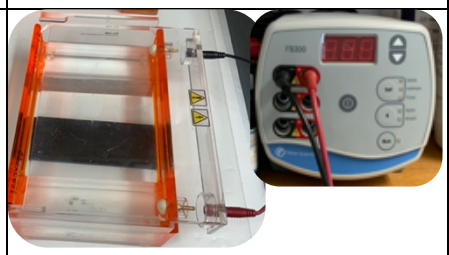
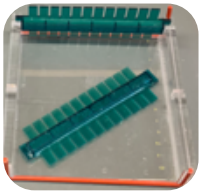
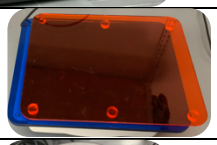
Standard Gel Electrophoresis System

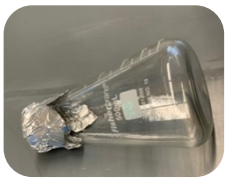
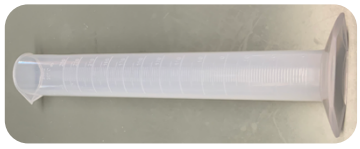
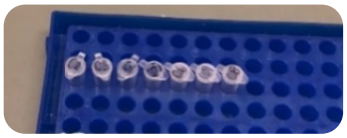
This protocol uses a standard electrophoresis system. The agarose gel will be made by adding agarose powder (or tablets) to running buffer, boiling the mixture, then letting it cool into a gelatin-like slab. The agarose gel is run in a standard electrophoresis system, then visualized with a transilluminator.

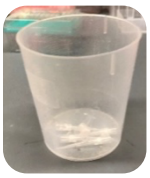
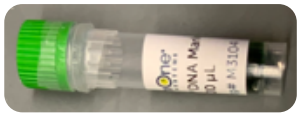
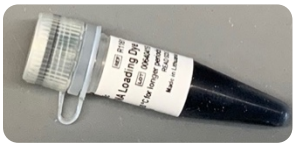
Pre-Lab Preparation

Make sure your running buffer is made up and diluted to 1X. Running buffer (commonly TBE or TAE) is usually concentrated in a 10X or 20X solution. Dilute the buffer following manufacturer's specifications using deionized (DI) or distilled water, not tap water.

Refer to your specific equipment manuals and reagent instructions before moving on.

Visual Supplies Checklist			
✓	**Name**	**Picture**	**Purpose**
	Electrophoresis tank & power supply		*Equipment* A standard electrophoresis system includes a tank to hold buffer, and (-) and (+) electrodes that are plugged into a power supply.
	Standard electrophoresis casting tray and comb		*Equipment* Used to cast gels. Before pouring the gel, the ends should be leak-proof, and a comb should be added.
	Microwave or hot water bath		*Equipment* The microwave is used to melt the agar solution before casting into a gel.
	Imager		*Equipment* A transilluminator, geldoc, or other imaging system to image and photograph the gel.
	Electronic balance		*Equipment* A balance is needed to weigh the amount of agarose powder to make a 1% gel.
	Squirt bottle or spray bottle with 70% ethanol		*Cleaning* 70% ethanol is used to clean the workspace before and after experiments.
	Gloves		*Personal Protective Equipment (PPE)* Gloves are used to protect both the scientist and sample from contamination.
	Oven mitt		*Personal Protective Equipment (PPE)* A waterproof, heat-proof oven mitt is used to protect the scientist from hot glass and steam after microwaving the agarose.

	Eye protection		*Personal Protective Equipment (PPE)* If you are using a UV light to observe and image the gel, eye proteciton is REQUIRED. UV light can damage the scientist's eyes.
	Weigh Dish		*Supplies* For weighing agarose powder on the electronic balance.
	1X Running Buffer (TAE or TBE)		*Supplies* Running Buffer allows the gel fragments to migrate through the gel. It is a concentrated solution and needs to be diluted prior to use.
	500mL flask		*Supplies* Used to boil the agarose powder and running buffer in the microwave to make molten agarose gel.
	Graduated cylinder		*Supplies* A graduated cylinder is used to dilute the running buffer, and to transfer buffer to the electrophoresis tank.
	Rack for 0.2mL PCR tubes		*Organization* PCR tubes are small, it is necessary to have a tube rack so they are not lost.
	20 µL pipette		*Liquid Management* Pipettes are used to move accurate and precise amounts of liquid from one place to another.
	20 µl pipette tips		*Liquid Management* 20 µL tips are used to move 2-20 µL of liquid. Tips should be changed between each sample to avoid contamination.

	Waste cup for tips		*Disposal* Keeping all waste in one area until the end of the experiment increases efficiency.
	Sharpie		*Organization* It is extremely important to label all tubes and samples.
	Parafilm (optional)		*Supplies* Can be used to cover the agarose mixture in the flask while melting to prevent evaporation.It can also be used to mix PCR products with loading dye.
	Lab tape (optional)		*Supplies* Can be used to seal off the ends of a casting tray before adding warm agarose.
Visual Reagents Checklist			
	Agarose powder		*Supplies* Agarose powder is measured and combined with running buffer to make an agarose gel. The powder dissolves into solution after boiling, or ~80 seconds in the microwave.
	DNA stain		*Reagent* DNA stain binds to DNA, making it visible in UV or blue light.
	PCR products from Lab 3		*Samples* These contain amplified DNA from the collected arthropod samples, control arthropods, control DNA, and water.
	DNA ladder		*Reagent* The DNA ladder is made up of pre-determined fragment sizes of DNA and serves as a reference for band size determination.
	Loading dye (optional)		*Reagent* If PCR products are colorless (i.e., no loading dye in the Taq Master Mix), this should be added to each sample prior to loading the gel. It helps to visualize movement through the agarose.

Gel Electrophoresis Protocol

Standard

Class Preparation: Running Buffer Working Solution

1. Together with your class, prepare a working solution of 1X electrophoresis running buffer.

Prepare Lab Space

2. Remove all unnecessary items from your lab station.
3. Put on nitrile gloves and clean all surfaces by wiping down with 70% Ethanol.

Prepare the Gel with DNA Stain

4. Measure 1g agarose powder and add it to a 500mL flask.
5. Add 100mL running buffer to the flask. (1% solution; note the total gel volume will vary depending on the size of the casting tray; refer to manufacturer instructions)
6. Melt the agarose in a microwave or hot water bath until the solution becomes clear. Look for "lenses" in the liquid by holding the flask up to the light. If using a microwave, place a ball of Kimwipe tissue in the flask opening, or cover with parafilm and poke a hole for venting. Heat the solution for several short (~20 second) intervals, do not let the solution boil over.
7. Let the solution cool to about 50-55°C, swirling the flask occasionally so it cools evenly. The flask should be warm, but not too hot to touch.
8. Seal the ends of the casting tray by placing it into the gel electrophoresis tank, or by sealing the ends with wide lab tape. Refer to the manual of your electrophoresis system for more information.
9. Add 10uL GelRed (Biotium), or comparable DNA stain, to the agarose. Swirl to mix.
10. Select a comb that will accommodate all samples. Place the comb(s) in the gel casting tray.
11. Slowly pour the melted agarose solution into the casting tray. Gently pop any bubbles with a pipette tip. *Note: Fill the gel in the tray so the teeth of the comb are immersed under the gel, but the base of the comb is above the gel. You do not have to use all the melted agarose.*
12. Let gel cool undisturbed on a solid, flat surface until it is opaque and solid. This may take at least 15-30 minutes. Moving the tray or not waiting until the gel is fully set may affect your results.
13. Carefully pull out the combs and remove the tape or lift casting tray out of the electrophoresis chamber.
14. Place the gel in the electrophoresis chamber with the wells oriented near the (-) electrode.
15. Add enough running buffer so there is 2-3mm of buffer over the gel.

Prepare to Load the Gel

16. Fill out the Loading Key on the next page.
17. If your PCR Master Mix has no loading dye (your PCR products are clear), follow 17a. If your PCR products are colored, move to *Load the Gel*.
    (a) Pipette 2uL drops of 5X loading buffer onto a piece of Parafilm or wax paper. Add 10uL from each of your PCR reactions 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.

Load the Gel

18. Pipette 10uL of the DNA ladder in the first well. Hover your pipette above the well, and slowly empty your pipette. Do not press to the second stop.

19. Continue in this manner, carefully pipetting 10uL of each sample/sample loading buffer mixture into separate wells in the gel. Change tips between each sample and store remaining PCR products in the freezer.

Run the Gel

20. Place the lid on the gel box, connecting the electrodes appropriately (positive is red, negative is black). The negative electrode should be near the wells of the gel, your DNA should "run to red". Also connect your electrodes to your power supply.
21. Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on size of the electrophoresis chamber, and will be printed on the label.

Gel #1 Loading Key: Arthropod

Lane	Sample
1	DNA Ladder
2	
3	
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water

22. Check to make sure current is running through the buffer by looking for bubbles that form on each electrode.
23. Check to make sure the current is moving in the correct direction by observing the movement of the loading dye. This may take a few minutes.
24. Let the power run until the yellow (or bottom) band in the loading dye is ¾ down the gel. Then, turn off the power, disconnect the electrodes, and remove the lid and the gel using gloves.

Obtain an Image of the Gel

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

25. Place the gel on the transilluminator or other imaging equipment. Put on eye protection before turning on the transilluminator. Use the equipment as directed, following the manual.
26. Note the presence or absence of bands in each lane. Use the DNA ladder, with fragments of a known size, to determine the size of each of the PCR products.
27. Document your results on the next page.

Repeat Steps 4-27 for the Wolbachia Gel Electrophoresis

Clean your Work Station

28. Discard used tips and wipe down the bench with 70% ethanol.
29. Refer to your equipment's manual to clean, dry, and store the electrophoresis system.

Label the Gel(s)

30. Transfer the gel images to a computer and use a program such as Powerpoint, Google Slides or Preview to label each gel (Arthropod/*Wolbachia*) and corresponding lanes.

Gel #2 Loading Key: *Wolbachia*

Lane	Sample
1	DNA Ladder
2	
3	
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water

Results

Use the table below to record presence (+) or absence (-) of bands.

Lane	DNA Source	Arthropod CO1 Band?	Wolbachia 16S Band?
1	DNA Ladder	N/A	N/A
2			
3			
4	(+) Arthropod Control		
5	(-) Arthropod Control		
6	(+) DNA Control		
7	Water		

Do your control bands match the expected results you drew in Pre-Lab Question 1?

Based on your answer, how confident are you that the experimental results are valid? Why?

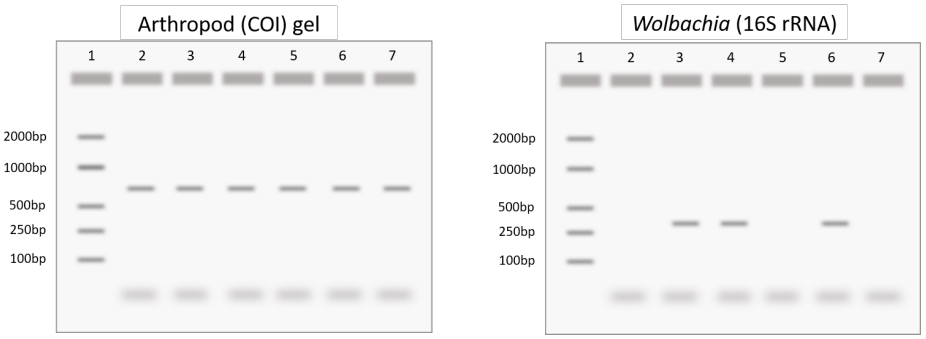
Complete the table below:

Tube label	Arthropod ID	*Wolbachia*-infected? (Yes, No, Unknown)	Confidence (High, Low)

Post-Lab Questions

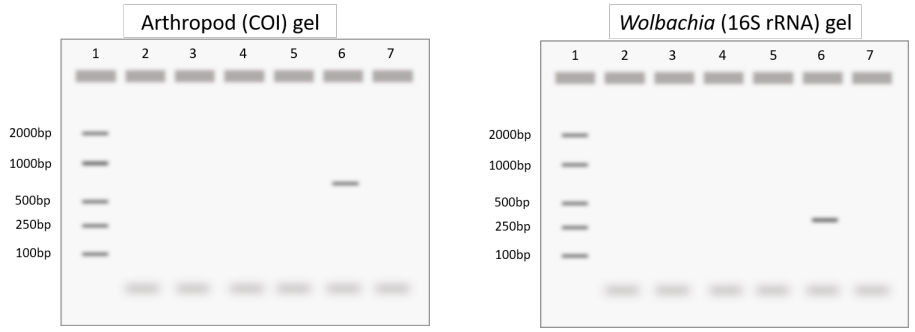
1. In gel electrophoresis, pore size depends on agarose content. Higher % gels have smaller pores whereas lower % gels have larger pores. In this lab, you used a 1% agarose gel. What would happen if you used a 2% agarose gel, but ran it for the same amount of time?

2. (a) Based on these results, which step of the experiment likely went wrong? Explain. (b) Are the arthropods tested here confidently infected with *Wolbachia?*



Lane	Sample
1	DNA Ladder
2	Sample A
3	Sample B
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water

3. (a) Based on these results, which step of the experiment likely went wrong? Explain. (b) Are the arthropods tested here confidently uninfected?



Lane	Sample
1	DNA Ladder
2	Sample A
3	Sample B
4	(+) Arthropod Control
5	(-) Arthropod Control
6	(+) DNA Control
7	Water

Troubleshooting

	ISSUE	RECOMMENDATION
Agarose Gel Leaked	The agarose gel leaked out of the casting tray.	Your agarose mixture may be too hot. Wait for it to cool to about 50°C before pouring your gel. This may be at least 15 minutes after you melt your agarose gel in the microwave.
		If you set your casting tray inside the electrophoresis tank to seal the ends, make sure the rubber gaskets on the end of the casting tray are inside of the grooves and not degraded. Agarose can easily escape if these gaskets are not flush against the electrophoresis tank.
		If you seal your casting tray with lab tape, make sure the tape is secure against the edges of the casting tray.
Ladder	If bands are visible on your gel, but not the ladder, it was most likely misloaded.	When loading your gel, be careful and slowly pipette the ladder into the gel. If you pipette too quickly, or click past the second stop on the pipette, there is a chance of the ladder leaving the well and not running correctly.
		If your positive controls worked and produced a band, you can use these bands as an equivalent marker to gauge the size of your sample bands.
Gel is blank, no bands	If all bands are absent, including the ladder, the electrophoresis failed.	Can you see the loading dye? If not, the gel may have run too long and DNA will exit the gel.
		Confirm that the gel was oriented with loading wells near the negative charge. If reversed, the DNA will migrate in the opposite direction and out the top of the gel.
		Were the buffer and gel made with distilled or deionized water? Tap water is not recommended.
		Did the gel cool completely before applying the electrical current?
		Were the wells properly loaded? Tiny pin-like dots in a well indicate that it was punctured with the pipette tip during loading and DNA was lost. Foggy waves across the gel indicate that the samples might be floating in the buffer and did not fall into the wells.
		DNA visible in the wells, but not down the gel, indicates the gel was not run. Make sure the green LED near the power button is on to run your gel.
Water Control	A smudge or band in the water control indicates there was contamination in the PCR reaction.	Results cannot be trusted. If time allows, repeat the PCR. Use molecular-biology grade water and switch tips between each sample. Ensure the workspace is cleaned with 70% ethanol before and after each lab. If this is a persistent issue, all PCR reagents (primers, Taq master mix, water) should be discarded and replaced with new aliquots. However, a smudge around 25-50bp represents primer dimers and is to be expected.
(+) DNA Control	No band in the positive DNA control.	The PCR failed; both PCR and gel electrophoresis need to be repeated.
		If the (+) and (-) Arthropod controls worked, your (+) DNA control may be degraded. Replace the (+) DNA control.

No Arthropod (CO1) Band	If there is a *Wolbachia* band but no arthropod band, the PCR primers may not work for your specific arthropod.	The general primers used to amplify CO1 are designed to detect *most* arthropods, but there are some species that require specific primer sets. If you collected a butterfly, moth, or dragonfly, for example, this is most likely the case. Your sample can still be marked *Wolbachia* positive if it does not have a CO1 band.
Band Size	If bands are present, but the wrong size, the gels or PCR tubes may have been mixed.	Confirm that the unexpected band size matches the other PCR reaction (Arthropod: 708 bp / *Wolbachia*: 438 bp). If so, you can assume that the gel was misloaded or mislabeled. It is always best, but not necessary, to rerun the gel under optimal conditions.
Arthropod Controls	If the (+) DNA control has an arthropod band, but the Arthropod Controls do not, it is indicative that DNA extraction failed.	It is recommended that you repeat the DNA extraction with an individual from the same population. The most likely culprit is lack of appropriately lysed cells. Increase grinding time with the pestle. If using a pellet-based DNA extraction kit, the DNA pellet may have been lost. Practice refining your technique or try a column-based kit.
		It is possible that the DNA extraction kit or protocol may not be suitable for your specific arthropod. Some arthropods have thick exoskeletons that require additional lysis time/reagents. Avoid collecting the exoskeleton during DNA extraction. If necessary, extract DNA from a leg or other body part without much exoskeleton. Other arthropods contain proteins that inhibit PCR reactions. In this case, the DNA extraction is successful, but PCR will fail.
	A *Wolbachia* band in the (-) arthropod control, but not the water control.	This indicates contamination during the DNA extraction and results cannot be trusted. It is recommended that you repeat the DNA extraction with an individual from the same population. Use fresh aliquots for each reagent and change tips between each sample.
Smears and Curved Bands	Smears appear as faint smudges down the lane of a gel. Curved bands appear "smiley" or U-shaped.	Both smears and curved bands occur when the DNA concentration is too high. As long as the controls worked, results can be trusted. For publication-worthy results, rerun the gel with less DNA added to the well. If you have access to a spectrophotometer, measure the DNA concentration and load the exact amount based on product information for your specific gel and well size.
		Smearing can also occur if the gel was not mixed properly or allowed to fully solidify before applying the electrical current.
		Faint smears at the bottom of the gel (~25-50bp) are primer-dimers. PCR reactions are set up with an excess of primers. In addition, some primers bind to each other instead of binding to the DNA, this is normal and to be expected.
		If the wells are deformed, the gel was cast when it was too hot. Next time, let the gel cool until it is no longer steaming, but still liquid, before pouring and casting.

Lumps in the Gel	Lumps in the gel indicate that it was too cold when poured/cast.	After microwaving, swirl the agarose mixture around until everything is dissolved in solution. The agarose gel mixture is ready to pour when it is no longer steaming but before it over-cools and forms lumps. It should be warm to the touch, but not painful to hold. If lumps form, microwave the mixture to molten again.
Unexpected Bands	The presence of unexpected bands indicates that the primers amplified a different region of DNA.	Unexpected bands are most likely due to non-specific binding (i.e., there is an off-target sequence in the genome with similar primer-binding sites) or pseudogenes. In animals, for example, the COI gene is sometimes transferred from the mitochondrial DNA into the nuclear genome, termed nuclear mitochondrial DNA (NUMT). Evolutionary pressures induce mutations and truncations of the NUMT. If the primer-binding sites remain the same, they will still identify and amplify the target sequence, but the amplified product will be a different size than the original COI gene. If a band appears at the correct size, report the results accordingly: the lane is (+) but non-specific binding is present.

Database Entry

After completing the Gel Electrophoresis Lab, open your entries in The *Wolbachia* Project Database and record Methods and Results. A comprehensive guide is located under the Resources tab.

https://wolbachiaprojectdb.org/

Database Fields to Complete

Observations

Methods

- ✓ DNA extraction kit
- ✓ DNA extraction location
- ✓ Taq polymerase used
- ✓ Single/duplex PCR reaction
- ❑ Upload gel image
- ❑ Gel electrophoresis system
- ❑ Buffer
- ❑ DNA stain
- ❑ Update protocol notes

Results

- ❑ Wolbachia positive?
- ❑ Confidence level
- ❑ Explain confidence level

Glossary

Agarose: A polysaccharide purified from seaweed. When dry agarose is boiled in a buffer solution, it will harden into a flexible, gelatin-like slab when it cools.

Band: A clearly visible and defined mark on a gel, indicating DNA presence. Where the band is in relation to the ladder indicates the size of the DNA product amplified in PCR.

DNA Stain: A dye that binds to the DNA, making it visible in UV or blue light.

Electrophoresis: A method of separating substances based on the rate of movement while under the influence of an electric field.

Ladder: A mix of pre-cut DNA of defined sizes. Each ladder may have different "rungs" of different sizes; check the product information to find the sizes of DNA for your specific ladder.

Lane: The vertical path of DNA migration below each loading well.

Loading Dye: Loading dye is added to aid in loading of an agarose gel. It contains colored dye and glycerol.

Loading Well: An indentation in the agarose gel in which samples are loaded.

Negative Control: Ensures the process and samples are not contaminated, it is designed to produce a negative result.

Positive Control: A well-understood variable; should result in an expected positive result.

Primer Dimers: Primers that bind to each other instead of the target DNA during PCR. This is the "haze" seen at the bottom of the gel, ~25-50bp. This area may also include excess primers that were not used during PCR.

Running Buffer: A conductive liquid that allows the DNA to migrate through the agarose gel.