FUTURELAB+

AG/ENVIRONMENTAL

Solution Seeking Microbes

Detecting *Wolbachia*: A Microbe to Control Disease

Laboratory Investigation

Developed in partnership with:

Bay Area Bioscience Education Community

In this Lesson Plan:

Print the **Teacher Section** →

Cover Image
Lactobacillus casei is one of many friendly bacteria in your gut microbiome.

Print the **Student Section** →

Teachers [T] *and* Student Resources [S] *can be printed independently. Select the appropriate printer icon above to print either section in its entirety.*

Follow the tips below in the Range field of your Print panel to print single or a range of pages:

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AG/ENVIRONMENTAL / SOLUTION SEEKING MICROBES

Lab: Detecting *Wolbachia*: A Microbe to Control Disease

DRIVING QUESTION

How can infection with Wolbachia bacteria impact insect populations and be used to solve problems, such as insect-borne disease?

ACTIVITY DURATION

Five class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

How can the Wolbachia bacteria affect insect populations?

How can we use microbes to control insect populations to solve problems, such as insect-borne disease?

How can we use DNA extraction, PCR (Polymerase Chain Reaction), and gel electrophoresis to analyze DNA?

OVERVIEW

Wolbachia is a fascinating genus of bacteria that lives symbiotically, often parasitically, within the reproductive cells of insects. Up to 60 percent of insects worldwide are predicted to be infected with *Wolbachia* (Zhang and Lui, 2020). Through a variety of mechanisms, infection with *Wolbachia* results in the feminization of host populations (an increase in the number of female individuals), which in turn increases the transmission of *Wolbachia* in the population. There is also evidence that *Wolbachia* inhibits certain viruses' ability to replicate inside mosquitos, and therefore is now being explored as a potential avenue to help prevent the spread of mosquito-borne diseases such as dengue and Zika.

In this lab, students first collect an insect from their local environment and extract its DNA. They then use PCR (Polymerase Chain Reaction) to detect the presence or absence of the Wspec gene of *Wolbachia* in the DNA sample. After visualizing their PCR results using gel electrophoresis, they collect class data to determine the *Wolbachia* infection rates among the local insect populations they sampled. Finally, they conduct further research to explain how *Wolbachia* impacts insect populations and how it might be used to mitigate an insect-associated problem.

BACKGROUND INFORMATION

This lesson introduces the technique of PCR (Polymerase Chain Reaction), which is used to amplify and detect segments of DNA. Experience with micropipetting and gel electrophoresis is recommended before conducting this lab. It is also helpful for students to have a basic understanding of bacterial cell structure and the definition of insect biological sex in relation to genotype and phenotype (XX=female, XY=male).

The idea and methods for this lab are based on the materials developed by Dr. Seth Bordenstein and other scientists of the Wolbachia Project at Woods Hole Marine Biological Laboratory (MBL). We gratefully acknowledge Dr. Bordenstein, Michael Clark, Michele Bahr, and others at MBL for their generous support in developing this curriculum.

Source: *Releasing Wolbachia-infected Aedes aegypti to prevent the spread of dengue virus: A mathematical study*

Have you ever wondered…

If insects can be infected with bacteria like people can?

Wolbachia is a ubiquitous bacteria that infects numerous insect and arthropod species in a symbiotic, often parasitic, way. *Wolbachia* cells live inside the cells of their hosts and cannot replicate on their own. To increase its own chances of survival, it causes the insect population to become more female in a variety of different ways.

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

An important part of using microbes to solve real-world problems is to first understand their role in nature. This allows us to identify mechanisms that we can then exploit for a particular purpose. This lab provides students with an opportunity to detect a particular microbe in their environment (*Wolbachia*) and to explore how it might be used to solve a problem.

How does this connect to careers?

Microbiologists study microscopic life forms, such as bacteria. They design and perform experiments to answer questions about the cellular processes and mechanisms governing microbe behavior.

Research associates use basic lab techniques, such as PCR and gel electrophoresis. They follow written protocols as they collect samples and perform tests to analyze specimen DNA and other substances. They maintain clear records of their findings.

Entomologists study all aspects of insects, including behavior, population dynamics, and classification. Many entomologists focus exclusively on insects that spread diseases, such as mosquitoes or ticks.

How does this relate to the product development life cycle?

Before developing a new product or process, scientists often first look in nature to identify and research potentially useful organisms and compounds.

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students work cooperatively with a partner and group of four throughout the lab, helping them develop relationship skills. They also build social awareness by considering mosquito-borne diseases that affect populations around the world.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lab provides students with an authentic science experience that gives them ownership over the analysis of a specimen they collect themselves. Positioning all students as scientists and asking them to collect insects from their own environment helps make the activity relevant to them. The lesson also sheds light on mosquito-borne illnesses, such as dengue, which affect much of the developing world.

COMPUTATIONAL THINKING PRACTICES

After performing PCR and gel electrophoresis using their insect tissue DNA, students will collect and analyze data showing the presence or absence of genes specific to insects and *Wolbachia* bacteria. Students will then find patterns in their class data to identify relationships between the insects tested and the prevalence of *Wolbachia* infection, as well as limitations of the investigation.

OBJECTIVES

Students will be able to:

Describe the reproductive effects of *Wolbachia* bacteria on insects using scientific text.

Extract, amplify, and visualize DNA from an insect to determine if it is infected with *Wolbachia* using scientific protocols.

Identify patterns and describe limitations of an investigation using experimental results.

Explain how *Wolbachia* impacts insect populations and can be used to solve problems, such as a mosquito-borne disease, using outside research and experimental results.

Materials

Documents
Lab Preparation (for teacher)
Thermal Cycler Grid (1 per class)
Background Reading: *Wolbachia* (Jigsaw) (1 per student)
Background Reading: What is Polymerase Chain Reaction (PCR)? (1 per student)
Background Reading: Analyzing *Wolbachia* PCR Results (1 per student)
Career Profile: Dr. Rusty Lowe (1 per student)
Vocabulary Tool (1 per student)
Student Protocol, Part 1: Insect DNA Extraction and PCR (1 per pair)
Student Protocol, Part 2: Gel Electrophoresis (1 per pair)
Student Guide (1 per student)

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Materials

Reagents

Lab Part 1:
DNA Extraction and PCR

- *Lysis Buffer (1 mL per student)*
- *5M NaCl (80 µL per student)*
- *TE/RNase (100 µL per student)*
- *91–100% Isopropanol (3 mL per pair)*
- *Wolbachia Master Mix (20 µL per student)*
- *Wolbachia Primer Mix (20 µL per student)*
- *Positive control DNA (15 µL PCR product per gel)*
- *dH_2O (15 µL PCR product per gel)*

Lab Part 2: Gel Electrophoresis

- *1X TAE running buffer (150–300 mL per group of four students—depending on which gel electrophoresis system you are using)*
- *2% agarose gel with DNA stain (1 per group of four)*
- *10X loading dye (1 per group)*
- *100 bp ladder (1 per group)*

Equipment and Consumables

Lab Part 1:
DNA Extraction

- *P1000 micropipettes (1 per pair)*
- *P1000 tips (1 box per pair)*
- *P200 micropipettes (1 per pair)*
- *P200 tips (1 box per pair)*
- *P20 micropipettes (1 per pair)*
- *P20 tips (1 box per pair)*
- *1.5 mL microtubes (3 per student)*
- *PCR tubes (1 per student + controls)*
- *Microtube rack (1 per pair)*
- *PCR tube rack (1 per pair)*
- *Centrifuge (1–2 per class)*
- *Thermal Cycler (1 per class)*
- *Heat block set at 99˚C (1 per class)*
- *Cap locks (1 per student)*
- *Permanent marker (1 per pair)*
- *Dry waste beaker (1 per group of four)*
- *Sink or wet waste beaker (1 per group of four)*
- *Ruler with millimeters (1 per group of four)*
- *Crushed ice (1 per group of four)*
- *Plastic micropestles (1 per student)*

Lab Part 2: Gel Electrophoresis

- *P20 micropipettes (1 per pair)*
- *P20 tips (1 box per pair)*
- *Microtube rack (1 per pair)*
- *Dry waste beaker (1 per group of four)*
- *Sink or wet waste beaker (1 per group of four)*
- *Electrophoresis gel setup (1 per group of four)*
- *UV light source and UV safety goggles (if needed for electrophoresis equipment)*

Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

Ask questions and make observations about how *Wolbachia* bacteria can be used as a tool to control mosquito-borne disease using a video.

Describe the reproductive effects of *Wolbachia* on insects using scientific text.

Teacher Note > *Before class, prepare an insect collection tube with isopropanol for each student (or have them prepare their own). See Lab Preparation.*

Whole Group (10 minutes)	
1	Warm up: What is an example of a disease or illness that is caused by an insect?
2	Call on students to share with the class and have a student record the responses on the board.
3	Show students the following two video clips and ask them to record three observations on question #1 in the *Student Guide, Part 1: Pre-Lab*. Afterward, give students a couple minutes to generate questions about this topic.
	a. Clip 1: Search for 'Mosquitoes with *Wolbachia* bacteria released to fight dengue fever' and play the full video on mute.
	b. Clip 2: Search for 'Evolution of Male Killing in Bacteria' and play only the first 45 seconds of the video with sound.
4	Ask students to share their observations and questions with a partner, and randomly select a few students to share with the class.
5	Introduce the lab to students by explaining that they will be testing their local insect population for *Wolbachia*.

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Day 1

Continued

Procedure

Small Group (30 minutes)

1 In working groups of four, ask students to complete the *Background Reading: Wolbachia (Jigsaw)*.

- **a.** In *Background Reading: Wolbachia (Jigsaw), Parts 1 and 2*, students can take turns reading the information out loud to their groups and then answer the corresponding questions.
- **b.** For, have each student in the group choose (or assign) one section to read about one of the reproductive effects of *Wolbachia*. Students should then take turns teaching their groups about the effect they read and answer the corresponding questions.

2 Option to show the following *video* from the World Mosquito Program.

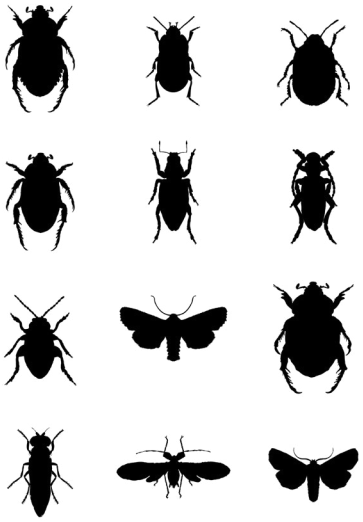
Individual (5 minutes)

1 Exit Ticket: Why should people learn about *Wolbachia* and what it does?

2 Pass out collection tubes with isopropanol and instruct students to collect an insect of their choosing for homework. Remind students to take care not to touch the isopropanol as it can irritate the skin and eyes. Ask them to also complete the *Student Guide, Part 1: Pre-Lab* question #2 to describe and identify their insects. Helpful websites include:

- **a.** *BugFinder*
- **b.** *Insect Orders: Identification Guide*

Teacher Note > *Wolbachia has a feminization effect on some insects (causing genetic males to be phenotypically female), this lab provides an opportunity to discuss how DNA relates (and does not relate) to sex and gender.*



Day 2

Procedure

LEARNING OUTCOMES

Students will be able to:

Extract DNA from insect tissue using scientific protocols.

Predict how *Wolbachia* infection may impact insect populations and ecosystems using their understanding of ecosystem dynamics.

Teacher Note > *Before class, aliquot reagents, set up heat block and centrifuge station(s) around the room, and set up lab stations using Lab Preparation.*

Whole Group (5 minutes)

1. Instruct students to take out their tubes with the insects they collected to compare their descriptions from *Student Guide, Part 1: Pre-Lab* question #2 with a partner.

2. Point out where the heat block and centrifuge station(s) are and share the following safety precautions and guidelines:

   a. Do not touch heated blocks (they may be extremely hot, even if off).

   b. Always make sure that the centrifuge is balanced with a counter weight before running.

   c. Wash your hands before and after the lab.

   d. Do not eat or drink during the lab.

   e. Keep your lab station clean and clear of clutter.

Small Group (35 minutes)

1. Break students into lab groups of four, assign lab stations, and ask them to complete steps #1–18 of *Student Protocol, Part 1: Insect DNA Extraction and PCR*.

2. During wait times, instruct students to discuss and predict the possible cascading effects of a *Wolbachia* infection in a population and in their local ecosystem in question #3 of the *Student Guide, Part 1: Pre-Lab*.

3. Have students clean up and give you their tubes of isolated insect DNA to store in the freezer until next class.

Individual (5 minutes)

1. Exit Ticket: Describe the process of DNA extraction. What do you think should be the next step in the lab?

2. Ask students to write their own sentences for the words in the *Vocabulary Tool* for homework.

Day 3

LEARNING OUTCOMES

Students will be able to:

Amplify the *COI* and *Wspec* genes using Polymerase Chain Reaction (PCR).

Describe how PCR can be used to amplify specific regions of DNA using scientific text.

Procedure

Teacher Note > *Before class, set up lab stations using Lab Preparation and remove students' partially completed DNA extractions from the freezer. Place a Thermal Cycler Grid next to the thermal cycler for students to record PCR tube placement. You will also need to set up the positive and negative control PCR reactions today. Once the PCR reaction is complete, store the tubes in the freezer.*

Whole Group (10 minutes)	
1	Warm-Up: List all the things you think could be done to a DNA sample to help analyze it.
2	Ask students to share with a partner, and share one idea each partner said with the whole group.
3	Show students this *video* of PCR from DNALC on mute and ask them to record two observations and two questions about what they see.
4	Share that today students will be completing their insect DNA extraction and performing the technique they saw in the video (called PCR) to amplify a particular region of insect and *Wolbachia* DNA.

Small Group (30 minutes)	
1	Pass out one copy per pair of *Student Protocol, Part 1: Insect DNA Extraction and PCR* and one copy of *Background Reading: What is Polymerase Chain Reaction (PCR)?* per student.
2	Ask students to retrieve their insect DNA samples and go to their lab stations (four students per group).

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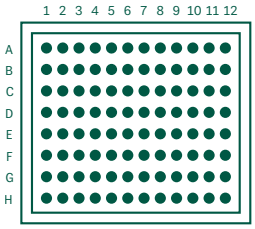
Day 3

Continued

Procedure



3	Share the following safety reminders with students and ask them to complete steps #19–30 of *Student Protocol, Part 1: Insect DNA Extraction and PCR*. During wait times they should complete the background reading and questions, and then check their answers with the key. • Always make sure that the centrifuge is balanced with a counter weight before running. • Wash your hands before and after the lab. • Do not eat or drink during the lab. • Keep your lab station clean and clear of clutter.
4	Once ALL students have placed their PCR tube in the Thermal Cycler and recorded the position of their tube in the Thermal Cycler Grid, run the program described in *Lab Preparation* (do not forget the positive and negative control reactions). PCR will take approximately 2 hours to run. (Do NOT save tubes to run later.)

Individual (5 minutes)

1	Exit Ticket: Look at the questions you recorded while watching the PCR video. Which can you now answer? What is a new question you have?
2	Pass out *Career Profile: Dr. Rusty Lowe* and ask students to read and annotate for homework. Option to watch the *video interview*.

Day 4

Procedure

LEARNING OUTCOMES

Students will be able to:

Visualize the amplified DNA from their PCR reactions to determine if their insects are infected with *Wolbachia* using gel electrophoresis.

Describe how gel electrophoresis can be used to determine if an insect is infected with *Wolbachia* using scientific text.

Teacher Note > *Before class, set up lab stations using Lab Preparation and remove students' PCR reactions from the freezer. Each group of four students will need one agarose gel with at least seven wells for loading their samples. You may pre-pour gels for students or have each group pour their own. It is suggested to prepare additional pre-poured gels in case students puncture the wells.*

Whole Group (5 minutes)	
1	Warm-up: Jot down all the things you remember about gel electrophoresis. *(e.g., What is it used for? What are the important components? How do you load and run a gel?)*
2	Call on students and have a student record responses on the board.
3	Share that today students will use electrophoresis to visualize their PCR products from the last class to determine whether or not their insect is infected with *Wolbachia*.

Small Group (30 minutes)	
1	Pass out one copy of *Student Protocol, Part 2: Gel Electrophoresis* per pair and *Background Reading: Analyzing Wolbachia PCR Results* per student.
2	Ask students to retrieve their insect DNA samples and go to their lab stations (four students per group).

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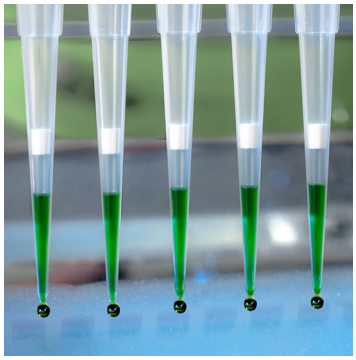
Day 4

Continued

Procedure



3	Share the following safety reminders with students and ask them to complete the steps of *Student Protocol, Part 2: Gel Electrophoresis*. • Be careful with liquid agarose—it is extremely hot! • If using non-minione electrophoresis equipment, turn off the power supply before opening the gel box. • If using non-minione electrophoresis equipment, wear UV goggles when looking at the gel under UV light.
4	While the gel is running, students can complete the *Background Reading: Analyzing Wolbachia PCR Results* and questions.
5	After the gel has run, ask students to take a picture with their phones (or take a picture for them) and attach it to their *Student Guide, Part 2: Lab* and label the lanes.

Individual (5 minutes)

1	Ask students to complete *Student Guide, Part 3: Data Analysis* question #1.
2	Ask students to complete #30–32 in their unit **Toolkit** using their understanding of *Wolbachia* for homework.

Day 5

Procedure

LEARNING OUTCOMES

Students will be able to:

Identify patterns and describe limitations of the investigation using experimental results.

Conduct research on how *Wolbachia* is being used to control mosquito-borne disease using online sources.

Explain how *Wolbachia* can be used to solve problems, such as mosquito-borne disease, using outside research and experimental results.

Teacher Note > *If time permits, it is strongly suggested to break this into two days: one for data analysis and one for further research and writing.*

Whole Group (10 minutes)

1 Warm-up: J Jot down all the problems you can think of that are related to insects. (For example, environmental problems, health problems, etc.)

2 Ask students to share with a partner and share one of their partner's responses to the class.

3 Share that today students will collect and analyze class data for *Wolbachia* infection rates as well as research how *Wolbachia* could be used to solve a problem.

Whole Group (10 minutes)

1 Collect class data using a spreadsheet or on the board (only collect data from successful DNA extractions and amplifications—lanes that show a band at 709 bp). *Sample class data is also included under the digital option in Lab Preparation.*

	Insect Scientific Name (common name)	Collection Location (city)	Number Collected	Number Infected with *Wolbachia*	% Infected (infected/ collected x 100)
1					
2					
3					
4					
		Totals			

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Day 5

Continued

Procedure

2	Ask students to identify three to five patterns in the data and record their answers under *Student Guide, Part 3: Data Analysis* question #2. Optional (if time): Ask students to consider limitations of the investigation and describe a follow-up experiment under *Student Guide, Part 3: Data Analysis* questions #4–5.

Optional	If time allows, ask students to work with their partner or group of four to make a graph or other visualization of the class data on a whiteboard or poster paper. Then have students view each others' graphs, choose one they think is best, and share why they think it is the best representation of the data.

Small Group (20 minutes)

1 Instruct students to find and choose an article about how *Wolbachia* can be used to control mosquito-borne disease. After reading, students can complete the organizer in *Student Guide, Part 4: Construct an Explanation* question #1 to summarize. Examples of searches that bring up relevant articles are listed below:

- **a.** Bacteria-Infected Mosquitoes Could Slow Spread of Zika Virus
- **b.** Flipping the switch on Controlling Disease-carrying Insects
- **c.** United States Government Approves 'Killer' Mosquitoes to Fight Disease

Individual (5 minutes)

Have students begin writing an explanation that answers the question "How can infection with *Wolbachia* bacteria impact insect populations and be used to solve problems, such as insect-borne disease?" in *Student Guide, Part 4: Construct an Explanation* question #2 and complete it for homework.

National Standards

Next Generation Science Standards

LS2.A: Interdependent Relationships in Ecosystems
Ecosystems have carrying capacities, which are limits to the numbers of organisms and populations they can support. These limits result from such factors as the availability of living and nonliving resources and from such challenges such as predation, competition, and disease. Organisms would have the capacity to produce populations of great size were it not for the fact that environments and resources are finite. This fundamental tension affects the abundance (number of individuals) of species in any given ecosystem.

Science and Engineering Practices
Constructing Explanations
Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects.

Crosscutting Concepts
Patterns
Using the concept of orders of magnitude.

Math

MP.4 Model with mathematics.
Calculate *Wolbachia* infection rate of insect populations, considering possible sources of error from unsuccessful DNA extraction.

Career and Technical Education (CTE)

A3.5
Predict outcomes of DNA and protein separation protocols.

A3.3
Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.

A8.1
Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.

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National Standards

CTE *Continued*	**A8.6** Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances.
	A8.7 Determine which equipment is appropriate to use for a given task and the units of measurement used.
	A8.8 Perform specimen collection, label samples, and prepare samples for testing.
	A8.9 Handle, transport and store samples safely.

Lab

Preparation

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Quick Tips	
1	Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab.
2	Before the lab, every student needs an insect (or a 2 mm piece of an insect). Insects can be stored in alcohol for an indefinite amount of time before the lab. When collecting an insect, the more recently deceased it is, the greater your chances of successfully extracting DNA for PCR.
3	We recommend having students complete this in a group of four with each student individually extracting DNA from their own insect, setting up their own PCR reaction, and visualizing their own sample on the gel.
4	*Virtual Learning Options* for this lab, including digital-only resources, are provided.

Preparation, Part 1: DNA Extraction and PCR

1	Any time before the lab	30 min
	Prepare for collection of insects:	
☐	Aliquot *500 µL isopropanol* into a 1.5 mL tube for each student (or have students prepare their own tubes).	
☐	Ask students to collect an insect and store it in the alcohol until the class is ready for the DNA extraction (the more recently deceased, the greater your chances of successfully extracting DNA for PCR).	
☐	Clean micropestles with ethanol if they have been used before.	

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Lab

Continued

Preparation

2 1–2 days before the lab — 30 min

☐ *Aliquot reagents for DNA extraction:*

Example for a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
Lysis Buffer	4 students X 1 mL X 1.1 (overage)	4.4 mL
5M NaCl	4 students X 80 µL X 1.1 (overage)	352 µL
TE/RNase	4 students X 100 µL X 1.1 (overage)	440 µL
Isopropanol	4 students X 800 µL X 1.1 (overage)	—

Note > *Suggest setting up 3–8 beakers with ~50 mL of alcohol in ice buckets near group lab stations (day of lab).*

3 1–2 days before the lab — 30 min

☐ *Aliquot reagents for PCR:*

Example for a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
Wolbachia Master Mix	4 students X 20 µL X 1.1 (overage)	88 µL
Wolbachia Primer Mix	4 students X 20 µL X 1.1 (overage)	88 µL
Water	In PCR tube as “reference tube” for students to check their volume	50 µL

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Lab

Continued

Preparation

3	During the lab	5 min
	Prepare Positive and Negative PCR Controls:	
☐	Each PCR reaction with loading dye yields *55 µL* total.	
☐	At 15 µL per gel, there is enough PCR product for 3 gels in one reaction.	
☐	To determine number of control reactions needed, divide the number of gels by 3 and round up to the nearest whole number: — e.g., 8 groups ÷ 3 = 2.67 reactions → *3 PCR reactions* — *Teacher sets up 3 Positive Control and 3 Negative Control PCR reactions for the 8 gels in the class.* **Note** > *The positive control DNA includes both insect and Wolbachia genes and the negative control is water.*	

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Lab

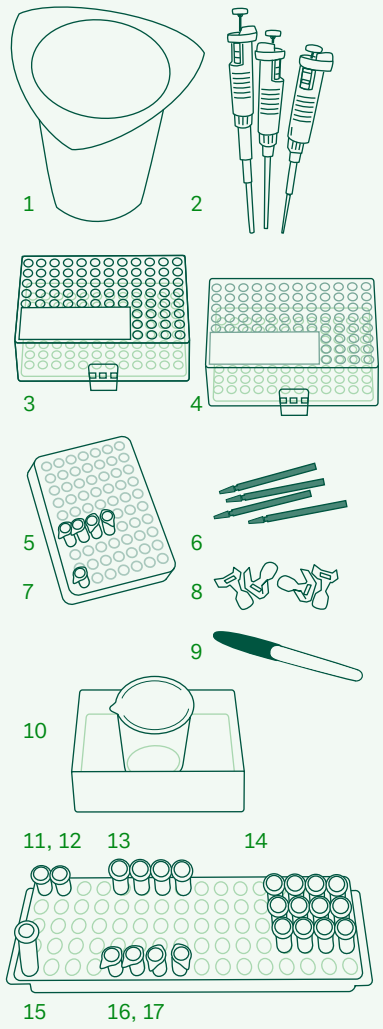
Continued

Preparation

4 | 1–2 days before the lab | 30 min

☐ Set up lab stations (1 per group of four):

1	Waste bucket
2	P20, P200, P1000
3	P1000 blue tips
4	P20 and P200 yellow tips
5	4 PCR Tubes and Rack
6	4 micropestles
7	50 µL reference tube
8	4 cap locks
9	Permanent marker
10	Isopropanol on ice
11	440 µL TE/RNAse
12	325 µL 5M NaCl
13	4 insects in isopropanol
14	12 empty tubes
15	4.4 mL Lysis Buffer
16	88 µL Master Mix
17	88 µL Primer Mix



Note > *Cap locks prevent tubes from opening due to pressure and allow for easier handling. If you do not have cap locks available, you may cover all the tubes with a sheet of foil after all students have placed their tubes in the heat block.*

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Lab
Continued

Preparation

5	Class Equipment Notes
	Micropestles
☐	Clean the micropestles with soap and water, and soak in ethanol to sterilize. This will reduce cross-contamination when they are used by the next class.
	Centrifuge
☐	You will need one to complete this lab with your class, but we recommend having more than one in the room to prevent bottleneck situations.
☐	If you only have one, we recommend doing the DNA extraction part of the lab in lock step to allow for easier management of the centrifuge.
☐	Never start the centrifuge with an uneven number of tubes—every tube must be counterbalanced with another tube, otherwise it can damage the equipment.
	Heat block
☐	Allow to preheat prior to the start or at the beginning of class to 99 ˚C.
☐	We recommend leaving a note next to the heat block to caution students from touching the heated blocks because it may not be apparent that the unit is on.
☐	If you do not have a heat block available, set up a water bath at 99 ˚C—take similar precautions for safety.
	Thermal Cycler
☐	Place a Thermal Cycler Grid next to the thermal cycler for students to record PCR tube placement—*place all tubes as close to the center as possible*.
☐	Use the following program (it will take ~1.5 hours to complete).

1	94 ˚C for 2 min
2	29 cycles of — 94 ˚C for 30 s — 55 ˚C for 45 s — 72 ˚C for 1 min
3	72 ˚C for 10 min
4	4 ˚C hold

Lab

Continued

Preparation

Preparation, Part 2: Gel Electrophoresis

1 — 1–2 days before the lab — 30 min

☐ *Aliquot reagents for Gel Electrophoresis:*

Example for a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
Loading Dye	4 students X 5 µL X 1.1 (overage)	22 µL
100 bp ladder	15 µL per gel	15 µL
Positive PCR Control	15 µL per gel	15 µL
Negative PCR Control	15 µL per gel	15 µL

Note > *Control reactions should have been set up during the PCR. Before aliquoting, add 5 µL loading dye per 50 µL reaction.*

2 — 1–2 days before the lab or day of lab — 30 min

☐ Prepare 1X TAE and 2% Agarose gels. See *Preparing 2% Agarose Gels* resource. (1 gel with at least 7 wells per group of four).

Options for pouring gels:

Students pour during lab	Give each group a 50 mL beaker with 20 mL* liquid agarose**. Gels typically take 15–20 minutes to solidify. *Amount of liquid agarose needed may vary between gel boxes. **Keep melted agarose in 65 ˚C water bath to prevent solidifying.
Teacher pours day before lab	Gels can be stored in the refrigerator wrapped in plastic with 1–2 mL of 1X TAE buffer to keep moist.
Teacher pours day of lab	You can reuse gels by remelting in the microwave.

Continues next page >

Lab

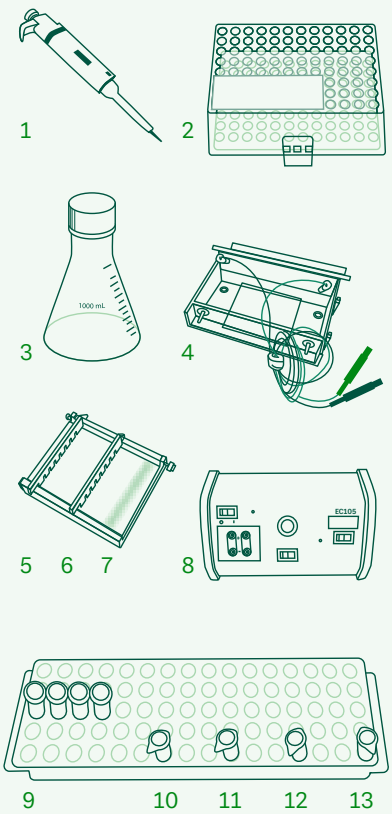
Continued

Preparation

3 | 1–2 days before the lab | 30 min

☐ Set up lab stations (1 per group of four):

1	P20 micropipette
2	P20 Micropipette tips
3	1X TAE Buffer (to fill gel box after gel is cast)
4	Gel box with lid
5	Gel casting tray and gates
6	Comb (add middle comb if two groups sharing one gel)
7	2% agarose in 1X TAE + GelGreen (pour here to cast gel)
8	Power supply
9	4 student PCR products
10	22 µL loading dye
11	15 µL 100 bp ladder
12	15 µL Positive Control PCR product
13	15 µL Negative Control PCR product



3 | After the lab | 15 min

☐ Properly dispose of lab supplies:

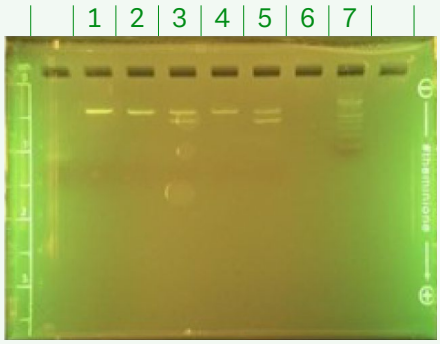
- Any excess solutions can go down the drain.
- Used micropipette tips and microtubes can go in the trash.
- Agarose Gels can also be discarded in the regular waste. GelGreen® DNA stain is not hazardous.
- Agarose gels can be reused. For best quality, do not reuse the agarose gel more than 5 times. To reuse gel, simply stuff the gel back into a bottle or a beaker. Make sure to keep different percentage gels in separate containers. Microwave gel until liquid to recast. You will need to re-add DNA stain if viewing DNA.

Lab

Continued

Virtual Learning Options

1	Anytime	30 min
☐	Students click through and answer the questions embedded in this *DNA Extraction Simulation* from Learn Genetics at the University of Utah.	
2	Anytime	30 min
☐	Students click through this *PCR animation* from DNALC and answer the following questions: — Why are samples heated to 94–96 °C at the start of PCR? — Describe "anneal" in your own words. — What molecule builds the new strand of DNA? — What do you notice after 5 cycles? — How many copies of the target DNA sequence do you have after 30 PCR cycles	
3	Anytime	30 min
	Students click through and answer the questions embedded in one of the following Gel Electrophoresis simulations: — *DNALC* — *University of Utah* — *LabXchange*	
4	Anytime	30 min
	Give students the following example gel, sample class data, and have them complete the Student Guide:	



1	Fly in a bottle
2	Mosquito
3	Large ant
4	Positive control bug
5	Positive PCR control
6	Negative PCR control
7	Ladder

Continues next page >

Lab

Continued

Virtual Learning Options

5 Sample Class Data

	Insect Scientific Name (common name)	Collection Location (city)	Number Collected	Number Infected with *Wolbachia*	% Infected (infected/ collected x 100)
1	Diptera (fly)	San Francisco	4	2	50%
2	Formicidae (ant)	San Francisco	12	5	42%
3	Drosophila melanogaster (fruit fly)	San Francisco	7	5	71%
4	Grylloidea (cricket)	San Francisco	1	0	0%
5	Blattodea (cockroach)	San Francisco	2	1	50%
6	Coleoptera (beetle)	San Francisco	4	3	75%

Skills

Preparing 2% Agarose Gels

Teacher Note > *Watch this video from the University of Leicester for an overview, however, be sure to follow the instructions below: Making an Agarose Gel—University of Leicester.*

Procedure	
1	Prepare 1X TAE by adding 20 mL 50X TAE to 980 mL of distilled water.
2	Add 6 g agarose powder to a 500 mL or larger glass bottle, flask, or beaker.
3	Add 1X TAE to the agarose powder to a final volume of 300 mL.

Note > *For volumes of agarose less than 300 mL, make sure to pour the amount of liquefied agarose you need to a clean beaker and use the corresponding amount of GelGreen® for the volume, e.g. for 100 mL of agarose, use 100 µL of 1000X GelGreen®*

4	Microwave on a low power setting (such as 50% or on "defrost") until liquid is translucent. Check every 5 minutes by removing the bottle with an oven mitt and swirling until melted. • *Caution:* Agarose can be superheated and let off steam explosively. Microwaving at a low power setting for longer reduces this possibility. • After making sure there are no visible lumps, microwave at full power for 20 seconds to dissolve any remaining solute.
5	Let the agarose cool slightly on the benchtop for 5–10 minutes (until you can touch the bottle without burning your hand and the agarose is still liquid or 50–60 ˚C).
6	While the agarose is cooling, prepare 10 gel trays with combs (this lab requires at least 7 wells).
7	To the 300 mL of cooling agarose, add 300 µL of GelGreen® DNA stain (provided at a 1,000X concentration). Swirl thoroughly to mix.
8	Immediately, pour approximately 15 mL (if using the miniPCR system) of agarose with GelGreen® and 25–30 mL (if using another system such as Fotodyne) to each of the prepared gel trays (work quickly to avoid agarose solidifying).

Note > *If the agarose has solidified after adding the GelGreen®, you can still microwave the gel to liquefy. The GelGreen® will lose optimal activity after microwaving, however.*

9	Do not move the tray until the gel has completely cooled and solidified.
10	Carefully pull the combs out to create the wells (pull straight up).

Background Reading: *Wolbachia* Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions after closely reading the background material with your group. Complete Parts 1 and 2 together, and divide Part 3 amongst your group members.

Part 1: *Wolbachia* and Mosquito-borne Diseases

1. What is *Wolbachia*?

Wolbachia is a type of bacteria that infects up to 60 percent of all insect species.

2. How and why are researchers, such as the World Mosquito Program, using *Wolbachia*?

Researchers are infecting Aedes aegypti mosquitoes in the lab with *Wolbachia* and releasing them to local mosquito populations. They are doing this because these mosquitoes spread diseases, such as dengue, and *Wolbachia* prevents the virus from replicating and infecting new hosts.

Part 2: *Wolbachia* Symbiosis

3. Describe "symbiosis" in your own words.

Symbiosis describes a type of close relationship between different organisms and can benefit one or both parties, or benefit one and harm the other.

4. What type of symbiosis does *Wolbachia* have with its hosts (mutualism, commensalism, parasitism, or a mix)? Explain.

Wolbachia bacteria live inside insect cells and form both parasitic and mutualistic relationships because, in many insects, it acts as a parasite, whereas other insect species rely on *Wolbachia* for survival and reproduction.

5. In what way do *Wolbachia* alter the reproduction of its host and how is it beneficial for them?

Wolbachia changes the reproduction of its host to increase the number of females in the population. This is beneficial because it reduces dead end transmission in the males and increases efficiency of transmission the next generation.

Continues next page >

Background Reading: *Wolbachia* Questions

ANSWER KEY **Do not share with students**

Continued

Part 3: Reproductive Effects of *Wolbachia*

6. Complete the table below.

Reproductive Effect	Description/Picture
Cytoplasmic Incompatibility	*Wolbachia*-infected males can only successfully reproduce with females that are infected with the same strain of *Wolbachia* bacteria that they are. If a *Wolbachia*-infected male mates with an uninfected female, all the offspring will die.
Parthenogenesis	Infected females reproduce on their own, without males. Offspring are genetically identical to the mother; not only are they all female, they also all carry the *Wolbachia* infection.
Feminization	Infection with *Wolbachia* causes fertilized eggs to develop as female, regardless of the genotype by suppressing production of masculinizing hormones.
Male Killing	*Wolbachia* infection causes male embryos to abort in early development, allowing only genetically female embryos to hatch.

Background Reading:
What is Polymerase Chain Reaction (PCR)? Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions after closely reading the background material.

1. Why is PCR a useful tool for analyzing DNA?

Often, the starting amount of DNA in a sample is too small to be analyzed or used as it is. PCR can then be used to make a large enough amount of the particular DNA sequence of interest so that it can be studied.

2. What might happen if you do not include a buffer or magnesium chloride in your PCR master mix?

Both a buffer and magnesium chloride are essential for Taq polymerase activity. Without them, the enzyme could not function and new strands of DNA would not be built.

3. Describe the structure and function of a PCR primer.

Structure: A single strand of DNA about 20 nucleotides long.

Function: Binds to a sequence of DNA flanking the target sequence to be amplified.

4. Describe each of the three steps in one PCR cycle using non-scientific language.

a. The two DNA strands separate under high temperature (denaturation).
b. The primers stick to the separated DNA strands on either side of the target sequence (annealing).
c. The Taq molecule reads the target DNA sequence starting at the primer and builds a new DNA strand (extension).

Background Reading: Analyzing *Wolbachia* PCR Results Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions after closely reading the background material.

1. Will the fragment of insect DNA appear as the top or bottom band of the PCR product in the gel? Explain how you know.

The fragment of insect DNA will appear as the top band in the gel because it moves more slowly through the agar than the *Wolbachia* fragment. This is because it is longer than the *Wolbachia* fragment by 271 base pairs.

2. In each case (one band, two bands, or no bands), determine whether or not the insect is infected with *Wolbachia*. Fill in this information in the "What it means" column of the data table. How do you know?

# Bands	What it means	How you know
1	The insect is NOT infected with *Wolbachia*.	It is most likely the COI band, indicating the DNA was successfully amplified from the insect but *Wolbachia* DNA was not present.
2	The insect IS infected with *Wolbachia*.	These are most likely the COI and Wspec bands, indicating the DNA was successfully amplified from the insect and *Wolbachia* DNA was also present.
0	DNA was not successfully extracted and/or amplified from the insect—you cannot determine whether or not it was infected.	If the ladder and/or other DNA samples are visible on the gel, then the DNA extraction and/or PCR were likely unsuccessful.

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Directions

In this lab, you will play the role of an entomologist exploring how Wolbachia *can be used as a tool to control mosquito-borne disease. To begin, watch the video clips your teacher shares and record at least three observations. After watching the videos, write down two questions that you have about this topic.*

1. Phenomenon:

Observations *I see...*	Questions *I wonder...Could it be that?*
All relevant notes and questions to the videos are acceptable.	

2. Sketch and describe your insect in the table below. Then, use an insect identification app or online search to find the scientific and common name of your insect (ex. Drosophila melanogaster / fruit fly).

Sketch	
Description *wings, number of legs, etc.*	Dependent on insects collected.
Scientific Name / common name	
Source of information	

Continues next page >

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Continued

3. Imagine that 10 females in the insect population that you or your partner sampled from are infected with *Wolbachia*. Work with your partner to discuss what might happen to that particular insect population and the ecosystem as a whole over time as a result.
There is no one correct answer!

Cause and Effect Sentence Frames

If...then...because...	Since...
...could lead to...because...	Consequently...
As a result of..., then...	Therefore...
...would cause...	Due to the fact that...

A particular insect population over time.	Any reasonable and logical predictions are acceptable as this is meant to be a thought exercise. Sample student response: If *Wolbachia* infects insects in a particular population... → An infected individuals are unable to mate successfully → Did individuals increase in number until entire population is infected or → The number of male offspring will decrease because asexual reproduction → Females are able to reproduce by themselves so population stays same or decreases slightly or
The ecosystem you live in over time.	Any reasonable and logical predictions are acceptable as this is meant to be a thought exercise.

Student Guide, Part 2: Lab

ANSWER KEY — **Do not share with students**

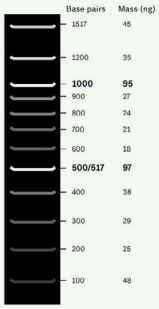
Directions

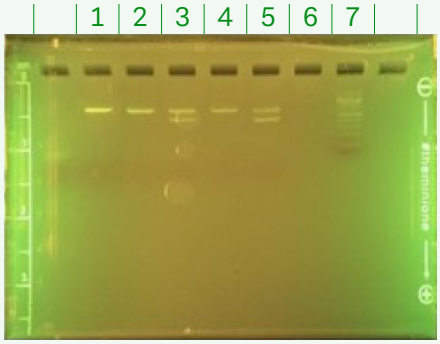
Draw or paste the picture of your gel below after completing the lab. Label each well and the DNA ladder (without writing directly on the picture).

The gel shown below has the lanes in reverse:

Lane 2 should be empty (negative PCR control).

Lane 3 should have two bands (positive PCR control).





1	Fly in a bottle
2	Mosquito
3	Large ant
4	Positive control bug
5	Positive PCR control
6	Negative PCR control
7	Ladder

Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Directions
Analyze your results from the lab by answering the questions below.

1. PCR Results: *(Example)*

   a. Were you able to successfully amplify DNA from your insect?

   Yes

   b. Is your insect infected with *Wolbachia*?

   Yes

   c. Explain how you know
   The gel shows…which means…:

   The lane of the gel where I loaded my PCR reaction shows two bands—one around the 700 bp DNA fragment in the ladder and one in between 400 and 500 bp fragments in the ladder. This means the PCR reaction amplified the 709pb segment of the COI gene found in all insects as well as the 438 bp segment of the Wspec gene found in *Wolbachia* bacteria.

2. Collect class data to determine what proportion of insects from each order are infected with *Wolbachia* (do not include data from unsuccessful DNA isolations where no bands are present on the gel).

   Identify three to five patterns in the class data.

Examples:

There is a higher percentage of *Wolbachia* infection in _____ than in _____ population.

There were more _____ specimens collected than _____ specimens.

Most of the infected insects were collected around _____.

3. Optional: How accurately do you think our class data reflects the *Wolbachia* infection rate of insects in our area (in other words, how reliable do you think our class data is)? Consider the limitations of our investigation including sample size, how samples were collected, etc.

The class data is not very reliable. The data set is very small both within an insect population and across populations. Only a few individuals from a population and from a species are represented out of thousands (or more).

It is also likely that many students did not successfully extract and/or amplify DNA from their insect (as indicated by an empty lane in a gel with a successful positive control).

4. Optional: If you were to conduct another investigation, what could you do to increase the reliability of our data?

   Describe the investigation.

Examples:

— Narrow the investigation to one type of insect.
— Compare distinct locations.
— Collect multiple specimens of the same species.

Student Guide, Part 4: Construct an Explanation

ANSWER KEY **Do not share with students**

Directions
Conduct research and find an article to read about how mosquitoes are being used to control insect-borne disease. Use this information along with information from background readings to explain how Wolbachia *bacteria impact insect populations and be used to solve problems, such as insect-borne disease.*

1. Summarize the article in the table below.

Title All articles and notes relevant to this topic are acceptable.	
	Main Idea	**Supporting Detail**
1		
2		
3		

Summary of article

Source

Continues next page >

Student Guide, Part 4: Construct an Explanation

ANSWER KEY **Do not share with students**

Continued

2. Construct an explanation that answers the question: How can infection with *Wolbachia* bacteria impact insect populations and be used to solve problems, such as insect-borne disease?

Sample student response:

Wolbachia is a commonly occurring bacteria that impacts insects by living and reproducing within its hosts' cells. When the cells reproduce, the bacteria is able to reproduce as well. Since *Wolbachia* can only be transferred from generation to generation through female insects, *Wolbachia* has developed a multitude of strategies to increase the number of females in an insect population to increase its reproductive efficiency. For example, some *Wolbachia* will kill males before birth, some will prevent male insects from producing male hormones, some will not allow noninfected males to reproduce, and some will allow females to reproduce asexually.

Wolbachia has many different relationships with its hosts. While there are some mutualistic relationships between *Wolbachia* and its hosts, *Wolbachia* is usually parasitic, meaning that it may harm its host in order to reproduce. These mechanisms result in an increase of females infected with *Wolbachia* in a given insect population.

When *Wolbachia* is introduced to the A aegypti mosquito and released into the natural population, it has the desired effect of mitigating insect-borne diseases such as dengue. When the mosquitoes are infected with *Wolbachia*, it proliferates in the female mosquitoes, which typically bite and transmit the dengue virus. *Wolbachia* has been shown to compete with other viruses in host cells and block their replication—thereby reducing transmission of a disease.

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Thermal Cycler Grid

Directions
Write your initials in the box that matches where you placed your PCR tube in the thermal cycler. Fill in the middle spots of the thermal cycler first.

Teacher:

_____

Period:

_____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Background Reading References

Wolbachia (Jigsaw)

WerrenLab-WolbachiaBiology

The mosquito strategy that could eliminate dengue

World Mosquito Program

What is Polymerase Chain Reaction (PCR)?

Polymerase Chain Reaction (PCR) Fact Sheet

Function of Taq DNA Polymerase in PCR

Polymerase Chain Reaction

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Background Reading: *Wolbachia* (Jigsaw)

Part 1: *Wolbachia* and Mosquito-borne Diseases

Wolbachia is a common type of bacteria found in the reproductive cells of insects. Up to 60% of insects worldwide are predicted to be infected with *Wolbachia* (Zhang and Lui, 2020). *Wolbachia* is non-pathogenic for humans (it cannot make us sick). In mosquito cells, the *Wolbachia* bacterium is able to prevent viruses, such as dengue, from replicating. If the virus cannot produce copies of itself inside the mosquito, it cannot be passed on to a new host through a bite. As a result, *Wolbachia* continues to be studied for its ability to stop the spread of viruses transmitted by insects.

Mosquitoes are hosts to multiple viruses that infect humans. Since they are not naturally infected by these viruses, mosquitos only pick them up if they bite an infected person. And since only female mosquitoes bite humans, viruses are only transmitted by female mosquitoes. The viruses that cause dengue, yellow fever, Zika, and chikungunya are all transmitted mainly by the *Aedes aegypti* mosquito. Recently, the *Aedes aegypti* population has increased in size and range as a result of increased human population growth and global travel as well as climate change. This in turn is leading to a rapidly growing number of people who are affected by mosquito-borne diseases. Dengue in particular has seen a rapid increase in recent decades, and is estimated to affect up to 400 million people every year worldwide. 25,000 are killed each year by the disease, mostly in low- and middle-income countries in Asia, the Pacific, and Latin America (Callaway, 2020).

In the 1990s and early 2000s, scientists at the World Mosquito Program developed populations of *Wolbachia*-infected *A. aegypti* in the lab and demonstrated that they were not able to transmit the dengue virus. They began releasing these mosquitoes in Australia, where *Wolbachia* quickly spread throughout local mosquito populations. Dengue rates plummeted, but the study did not include control areas. In a following study during 2016, mosquitos that carried *Wolbachia* were released in parts of Yogyakarta, Indonesia. The results showed that rates of dengue in these areas were 77 percent lower than areas that did not receive the mosquitoes (Callaway, 2020).

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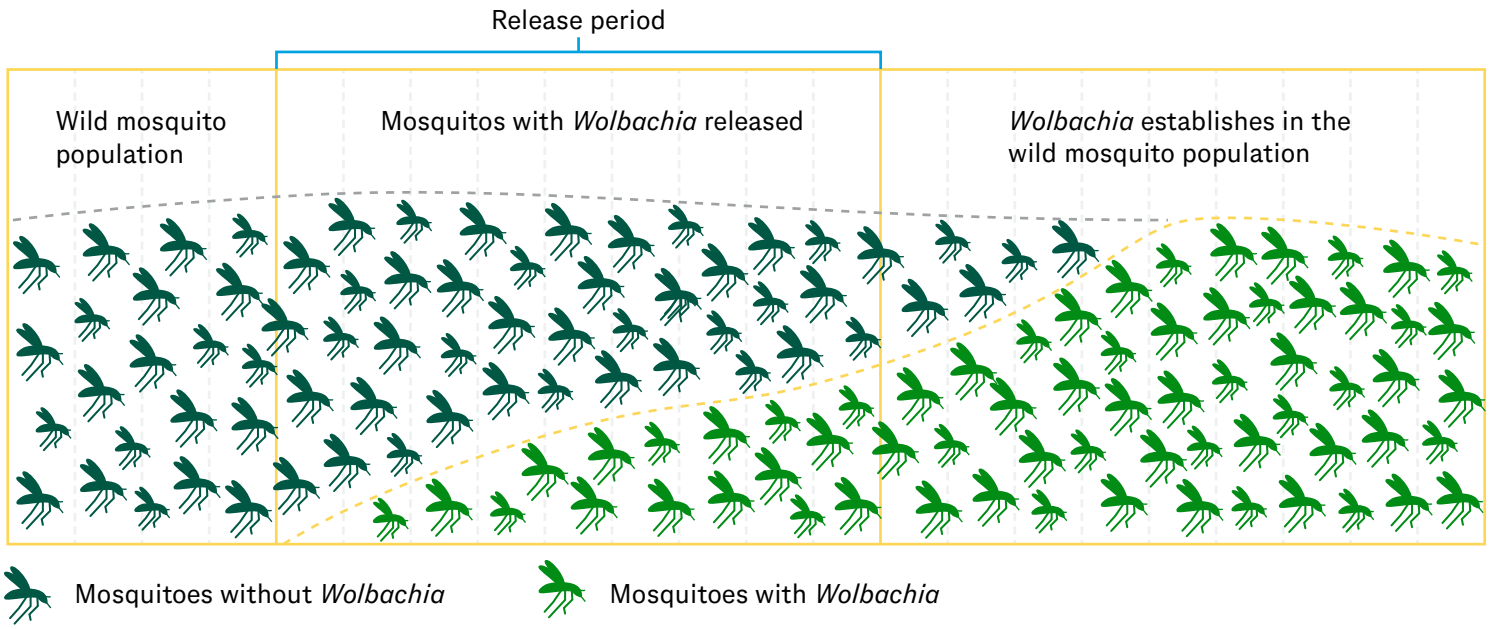
Using *Wolbachia* -infected mosquitoes to combat dengue



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Background Reading: *Wolbachia* (Jigsaw)

Continued

Part 2: *Wolbachia* Symbiosis

There are many different ways in which organisms in an ecosystem interact with each other. If the relationship between two organisms of different species is close and long-term, it is symbiotic. There are three types of symbiotic relationships: *mutualism* (both organisms benefit), *commensalism* (one organism benefits and the other does not but is not harmed), and *parasitism* (one organism benefits and the other is harmed).

Wolbachia bacteria form both parasitic and mutualistic relationships with a host depending on the species it infects. In many insects it is characterized as a parasite, whereas other insect species rely on *Wolbachia* for survival and reproduction.*Wolbachia* is an endosymbiotic parasite, meaning that it lives inside insect cells. It cannot reproduce outside of a host, though it has been shown to survive short periods of time outside of a host cell.

Unlike many parasitic organisms, which are only transmitted to other hosts through contact with another organism or the environment (horizontal transmission), *Wolbachia* can also be transmitted from parent to offspring (vertical transmission) through the female's eggs. Males do not play a role in passing the bacteria to their offspring.

Wolbachia has evolved several mechanisms that help it spread within an insect population. It alters the reproduction of its host to increase the number of females in the population. By reducing or eliminating the number of males in the population, it prevents "dead end" transmission. Since only females can pass *Wolbachia* to offspring, this ensures that a high percentage of insects in the next generation are infected.

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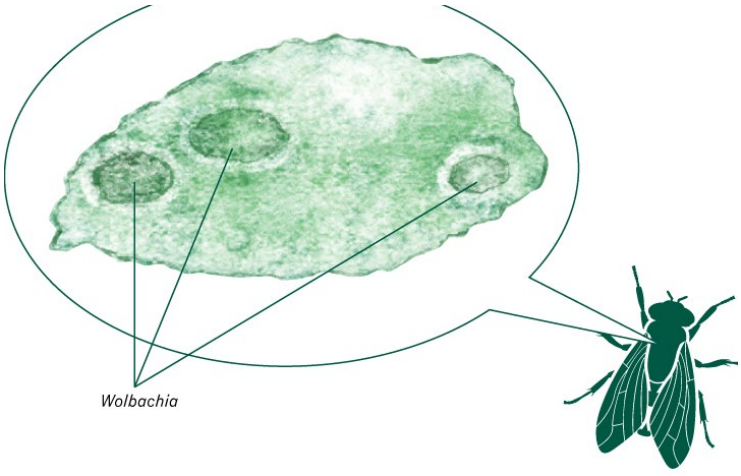
Wolbachia Symbiosis



Wolbachia bacteria inside an insect cell.

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Background Reading: *Wolbachia* (Jigsaw)

Continued

Part 3: Reproductive Effects of *Wolbachia*

○ = Healthy ○ = infected ○ = dead

A. Cytoplasmic Incompatibility

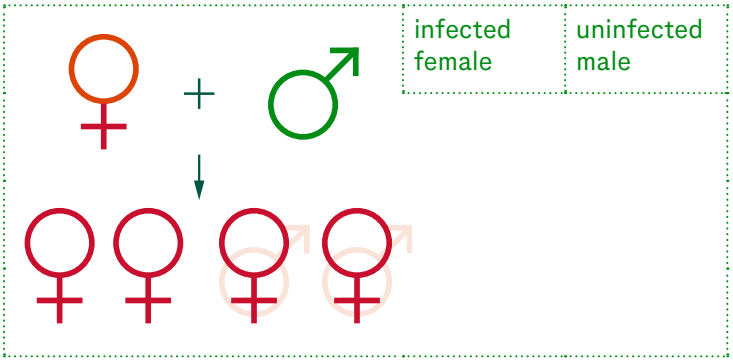
Wolbachia-infected males only successfully reproduce with females infected with the same strain of *Wolbachia* bacteria. All offspring die if a *Wolbachia*-infected male mates with an uninfected female as the sperm and egg are incompatible. Since *Wolbachia*-infected females are more reproductively fit, the bacteria increases within the population. This is the most common reproductive effect of *Wolbachia* infection.

♀ + ♂ = ♀♀♂♂	both unifected	uninfected offspring
♀ + ♂ = ♀♀♂♂	both infected	infected offspring
♀ + ♂ = ♀♀♂♂	uninfected male	infected offspring
♀ + ♂ = ♀♀♂♂	uninfected female	dead offspring

Infected males are incompatible with uninfected females

C. Feminization

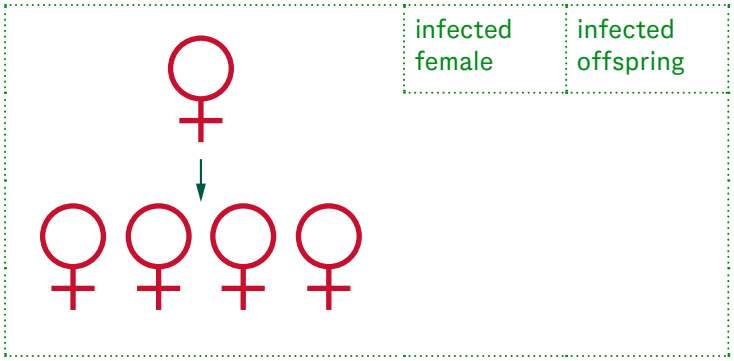
In some species, the offspring of *Wolbachia*-infected females develop as females, regardless of their genotype. (Even if their genes provide the instructions for them to become males, they will develop all the structures and characteristics of females.) Not only will these *feminized* embryos inherit the *Wolbachia* bacteria, but they will also pass it on to their own offspring because only females pass on *Wolbachia*.



Wolbachia causes males to develop as females

B. Parthenogenesis

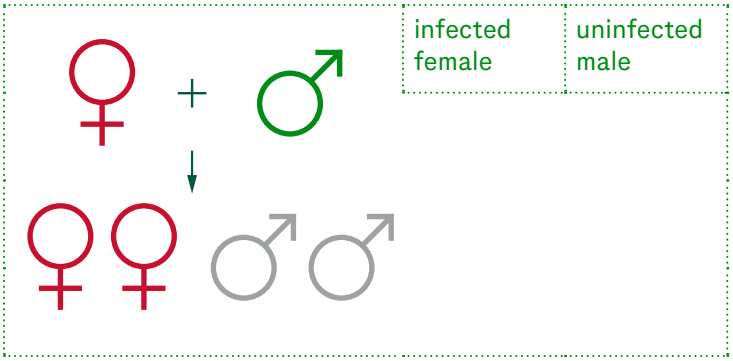
In some species, *Wolbachia*-infected females are able to reproduce on their own, without males. In parthenogenetic reproduction, eggs can develop without being fertilized by sperm. This results in all the offspring being genetically identical to the mother, but also carrying *Wolbachia*.



Wolbachia causes female reproduction without males

D. Male Killing

In some species, infection with *Wolbachia* causes male embryos to abort in early development. As a result of this *male killing*, the only offspring of *Wolbachia*-infected females are female. Not only will they all inherit the *Wolbachia* bacteria, they will also pass it on to their own offspring because only females pass on *Wolbachia*.



Wolbachia causes only female embryos to hatch

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Background Reading: *Wolbachia* Questions

Directions
Answer the questions after closely reading the background material with your group. Complete Parts 1 and 2 together, and divide Part 3 amongst your group members.

Part 1: *Wolbachia* and Mosquito-borne Diseases

1. What is *Wolbachia*?

2. How and why are researchers, such as the World Mosquito Program, using *Wolbachia*?

Part 2: *Wolbachia* Symbiosis

3. Describe "symbiosis" in your own words.

4. What type of symbiosis does *Wolbachia* have with its hosts (mutualism, commensalism, parasitism, or a mix)? Explain.

5. In what way do *Wolbachia* alter the reproduction of its host and how is it beneficial for them?

Continues next page >

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Background Reading: *Wolbachia* Questions

Continued

Part 3: Reproductive Effects of *Wolbachia*

6. Complete the table below.

Reproductive Effect	Description/Picture
Cytoplasmic Incompatibility	
Parthenogenesis	
Feminization	
Male Killing	

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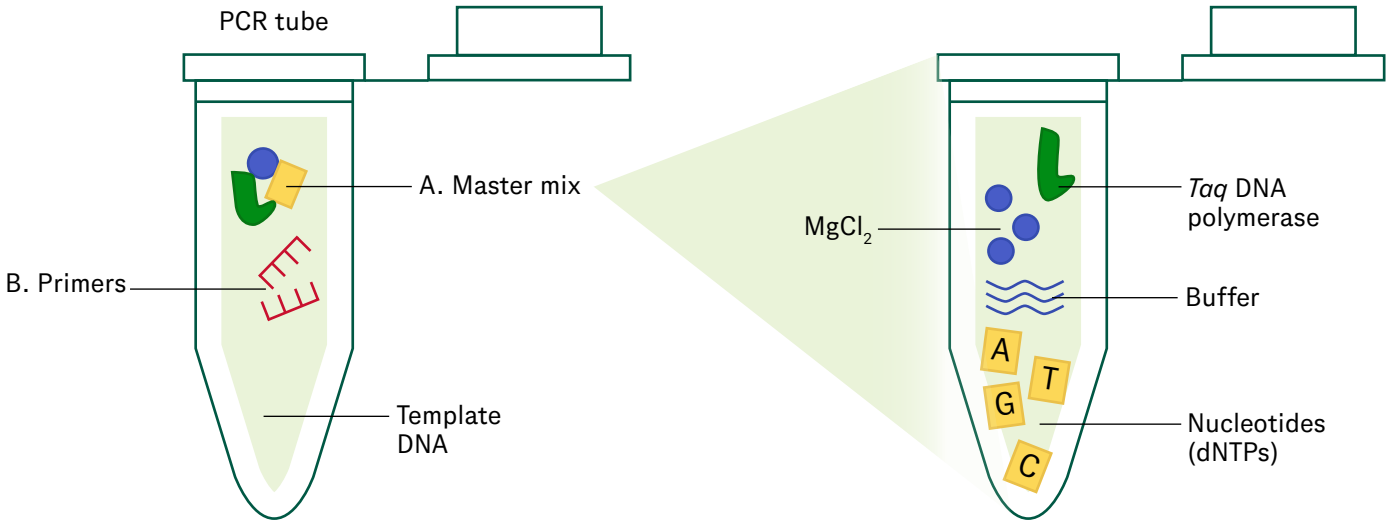
Background Reading: What is Polymerase Chain Reaction (PCR)?

Polymerase Chain Reaction (PCR) is a technique used in biotechnology to make millions of copies of a small segment of DNA. Often, the starting amount of DNA in a sample is too small to be analyzed or used as it is. PCR can then be used to make a large enough amount of the particular DNA sequence of interest so that it can be studied. For example, PCR is used to detect whether someone is infected with SARS-CoV-2 (the virus that causes COVID-19). The amount of virus genetic material in the person's tissue sample is too small to be detected as is, so PCR is performed to amplify a segment of the virus' genome. This allows the lab to determine if the virus is present or absent in the patient sample.

Components of PCR

A. Master Mix



The new DNA molecules are built by a DNA polymerase that has been isolated from a bacteria called *Thermus aquaticus (Taq)*. This bacteria is a thermophile, meaning it thrives in extremely hot temperatures (it was discovered in hot springs in Yellowstone National Park). This means its polymerase remains functional at the high temperatures necessary for PCR.

The buffer keeps the pH in an optimal range and magnesium chloride increases enzyme activity for Taq polymerase. An equal mix of adenine, guanine, cystine, and thymine are also included in the reaction as building blocks for the new DNA molecules.

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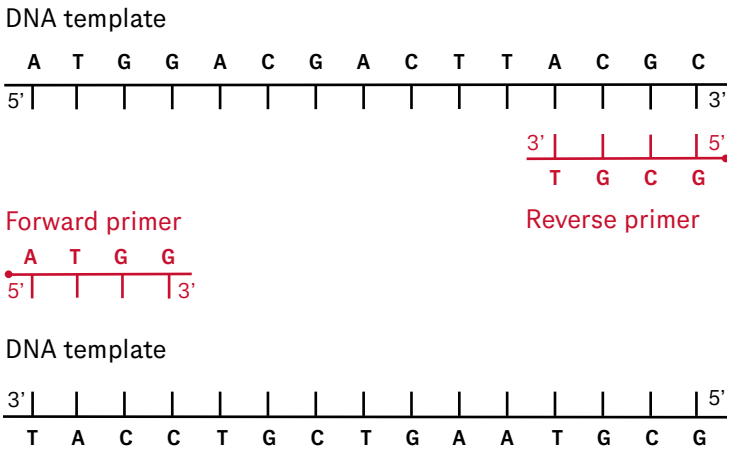
Background Reading: What is Polymerase Chain Reaction (PCR)?

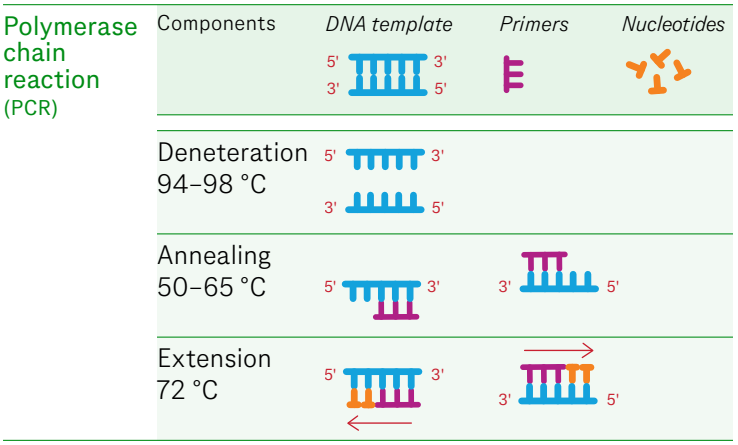
Continued

B. Primers



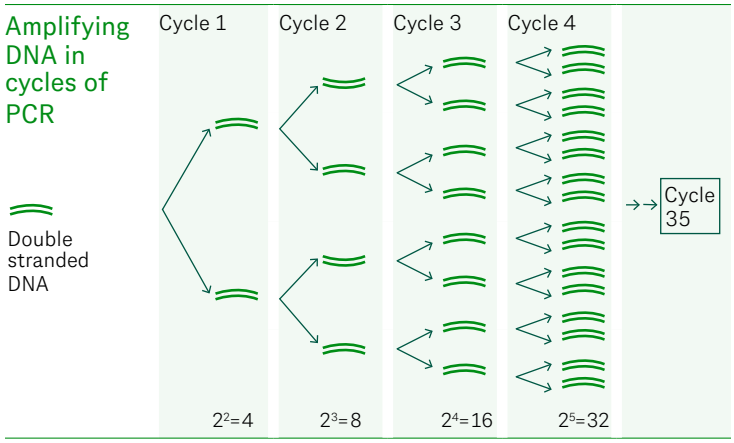
Primers allow the PCR reaction to amplify (make lots of copies of) a very specific region of DNA. Each primer is a small single-stranded DNA (~20 nucleotides) that is complementary to the DNA sequence. One PCR reaction requires two primers (one forward and one reverse) so that only the DNA segment in which you are interested is copied. *Taq* DNA Polymerase binds to the primers and begins building a new DNA strand that is complementary to the template in the 5' to 3' direction.

Temperature Cycle



The first step of PCR is to heat the reaction to 95 °C. This high temperature breaks the hydrogen bonds between the base pairs, allowing the primers and Taq polymerase access to the nucleotides. Therefore, it is called the *Denaturation* step. Next, the temperature is cooled to around 60 °C, which causes the primers to bind to their complementary sequences in the DNA template. This process is called *Annealing*. After the primers are bound, the temperature rises to 72 °C, which is the optimal temperature for Taq polymerase to build a new DNA molecule. The polymerase "reads" the target DNA sequence and adds nucleotides to the primer that are complementary to the sequence. This is the *Extension* step. When the cycle is complete, there are now two copies of the original DNA.

This process repeats to complete 25–40 total cycles, resulting in exponential growth in the number of copies of the original DNA template. At the end of the cycles, there is sufficient DNA to be analyzed.



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Background Reading:
What is Polymerase Chain Reaction (PCR)? Questions

Directions
Answer the questions after closely reading the background material.

1. Why is PCR a useful tool for analyzing DNA?

2. What might happen if you do not include a buffer or magnesium chloride in your PCR master mix?

3. Describe the structure and function of a PCR primer.

4. Describe each of the three steps in one PCR cycle using non-scientific language.

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Background Reading: Analyzing *Wolbachia* PCR Results

To determine whether or not an insect is infected with *Wolbachia*, you will need to visualize the products of your PCR reactions. This will be done using agarose gel electrophoresis—a technique for separating and observing DNA fragments.

PCR Reactions

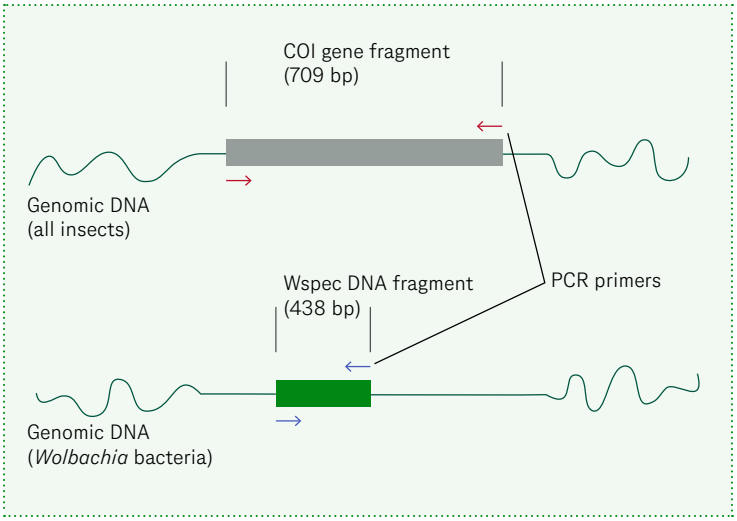
The primer mix you used when setting up your PCR contained two sets of primers. One set amplified the *Cytochrome C Oxidase (COI)* gene found in all insects. This gene plays a role in cellular respiration and is part of the mitochondrial DNA. If the DNA from your insect was isolated successfully, PCR amplification of this gene from the insect genome will produce a DNA fragment *709 bp* in length. This PCR reaction is used to verify that you had a successful DNA extraction and the fragment should be present in all samples, regardless of whether or not the insect is infected with the *Wolbachia* bacteria. In other words, it serves as an internal control, or *reference gene*. The second set of primers amplifies a *Wolbachia*-specific region of *bacterial ribosomal DNA (Wspec)*. This gene is only found in *Wolbachia* bacteria and will produce a DNA fragment *438 bp* in length.

Gel Electrophoresis Results

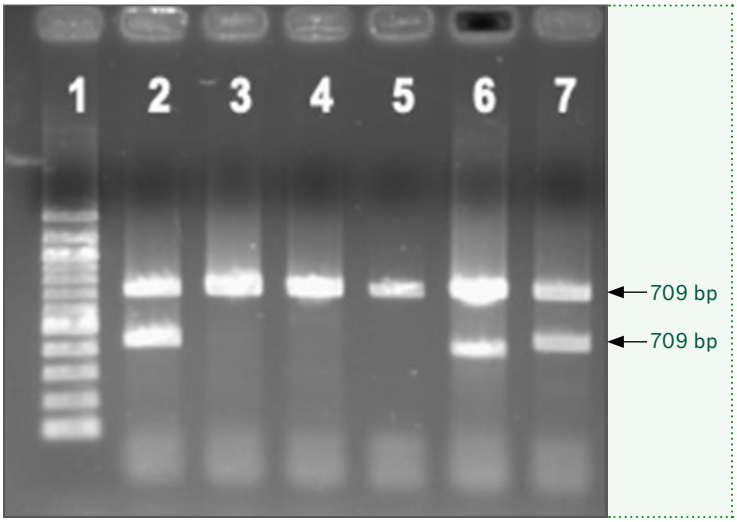
Since DNA is negatively charged, it will migrate toward the positive electrode of a gel and shorter fragments of DNA move at a faster rate than longer ones. This means the COI gene fragment will migrate more slowly during electrophoresis and produce a band closer to the top of the gel. Because the *Wolbachia* DNA fragment is smaller, it will move more quickly through the gel and appear closer to the bottom. Therefore, if the insect is infected with *Wolbachia* bacteria, you will see *two bands in the same lane* on the gel—one at 709 bp and one at 438pb.

By examining your agarose gel, you will determine 1) whether or not you successfully extracted and amplified DNA from the insect you identified and 2) whether or not it is infected with the *Wolbachia* bacteria. In the example below, lane 1 is the DNA ladder and lanes 2, 6, and 7 show insects that are infected with *Wolbachia*.

PCR Amplification



Example Gel Electrophoresis Results



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Background Reading: Analyzing *Wolbachia* PCR Results Questions

Directions
Answer the questions after closely reading the background material.

1. Will the fragment of insect DNA appear as the top or bottom band of the PCR product in the gel? Explain how you know.

2. In each case (one band, two bands, or no bands), determine whether or not the insect is infected with *Wolbachia*. Fill in this information in the "What it means" column of the data table. How do you know?

# Bands	What it means	How you know
1		
2		
0		

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Career Profile

Lead Scientist and
Globe Observer Mosquito
Habitat Mapper

Dr. Rusty Lowe
Institute for Global
Environmental Strategies

Where are you from?

I grew up in Maryland in the United States. I completed my BA at the University of Maine, did PhD work at the University of Munich and completed my dissertation at the University of Minnesota. My first job was as a professor at the University of Maryland, Munich. So you can see how easy it was for me—I have one "UM" tee shirt that describes most of where I've lived and most of my academic life!

What do you do?

I am a Senior Scientist at the Institute for Global Environmental Strategies in Arlington, VA. I work with scientists, communicators and teachers—some of the most dynamic people you will ever meet.

How do you use GLOBE Observer?

I am one of the lead scientists who developed the key used in the Mosquito Habitat Mapper app. I have used the app in an USAID project with teachers and students in Brazil and Peru, mapping mosquito habitats around their schools and in their communities. Reporting mosquito habitats and mitigating them so they can't be used for breeding sites can make a big difference and decrease disease transmission in a community. Where I live in Colorado, it is semi-arid so there are only container habitats near my home. I saw firsthand the importance of not storing my winter tires outside in the back of the barn—I had inadvertently created a perfect little mosquito nursery! Lots of larvae! Right now I am doing field research in Barbuda, a small island in the Caribbean, where we first tested the GO Mosquito Habitat Mapper app concept.

How do you plan to use GLOBE Observer in the future?

I am very interested in seeing how the GO Mosquito Habitat Mapper data correlates with GO Land Cover data. I have been working for two weeks describing the vegetation in Barbuda using a Land Cover app, as well as doing vegetation descriptions of floral formations. Once the snow melts in my home in the mountains of Boulder Colorado, I am planning to do a land cover survey of Lefthand Canyon, the canyon where I live in the Front Range of the Rocky Mountains. That is my "at home" citizen science project!

Why is citizen science important to you?

The essence of science is sharing data and discovery—for the greater good. I feel like every time I take a measurement, I am doing something that helps the world in a very small way. Not in a big, obvious, tangible way, like my husband who is a volunteer fireman and actually saves lives. But as one of millions of people reporting data, what can emerge is a better understanding of the world around us, an understanding whose meaning and utility is not yet known. I love that I am a tiny part of something much, much bigger than me.

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Career Profile

Continued

What advice do you have for people just getting into citizen science?

I'd say find a citizen science project that really excites you and matches your interests. For me, I studied plant taxonomy and forest ecology in graduate school as part of climate change studies and it's fun to return to this work as a volunteer citizen scientist, just for fun. It's especially exciting because my paid work is with the GO Mosquito Habitat Mapper, so I am interested in seeing how my hobby citizen science work (Land Cover citizen science) can help us understand patterns in the mosquito data.

What do you do for fun?

I live in the mountains and like to kayak, hike and cross-country ski. I'm always identifying plants when I walk. I have a mandolin and banjolin, and like to play music with friends. I used to foster rescue dogs, but I wanted to adopt too many of them, it just wasn't sustainable! We live with our "failed foster" dog, Harrold, a massive St. Bernard.

What inspires you?

Volunteers who work to make the world a better place. Every one of them. That includes luminaries such as Jane Goodall, the volunteer firefighters in my mountain district, and the citizen scientists and professional scientists working with them around the world.

Any favorite quote(s) that you would like to share?

"If you think you are too small to make a difference, try sleeping with a mosquito."

—The Dalai Lama

The following *video* interview is also available.

Source: *The Globe Observer*

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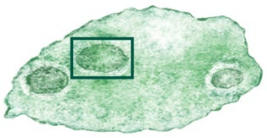
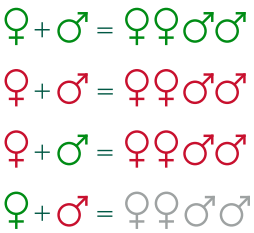
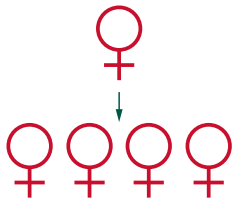
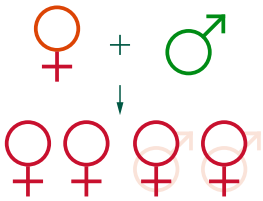
Vocabulary Tool

Directions
For each vocabulary word, write a new sentence that helps you practice using it.

Word	Image	Definition	Example Sentence	My Sentence
Wolbachia		A type of bacteria that infects arthropods, including many insects	Up to 60 percent of insect species are estimated to host *Wolbachia.*	
Symbiosis		A relationship between two different organisms in an ecosystem that is close and long-term	*Wolbachia* and its host have a *symbiotic* relationship that is on a spectrum between parasitism and mutualism.	
Cytoplasmic Incompatibility		*Wolbachia*-infected males can only successfully reproduce with females that are infected with the same strain of *Wolbachia* bacteria.	Mosquitoes that transmit dengue exhibit *cytoplasmic incompatibility* when they are infected with *Wolbachia*.	
Parthenogenesis		When infection with *Wolbachia* causes females to reproduce on their own	The offspring that result from *parthenogenesis* are clones of the mother.	
Feminization		When infection with *Wolbachia* causes fertilized eggs to develop as female, regardless of the genotype	In *feminization*, all offspring become phenotypically female even if they are genetically male.	

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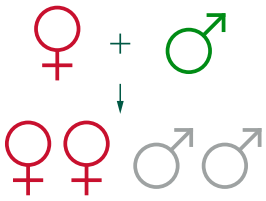
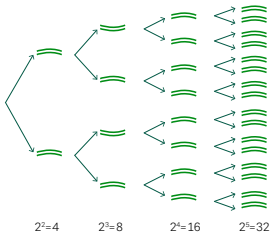
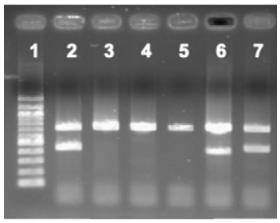
Vocabulary Tool

Continued

Word	Image	Definition	Example Sentence	My Sentence
Male Killing		When infection with *Wolbachia* causes male embryos to abort in early development	One way *Wolbachia* has evolved to increase the number of females in a population is by causing *male killing*.	
PCR *Polymerase Chain Reaction*		A technique used in biotechnology to make millions of copies of a small segment of DNA	In order to detect the presence of *Wolbachia* in your insect, you will amplify its DNA using *PCR*.	
Agarose Gel Electrophoresis		A technique used to separate molecules based on their mass and charge	After performing PCR, you will visualize the DNA using *gel electrophoresis*.	

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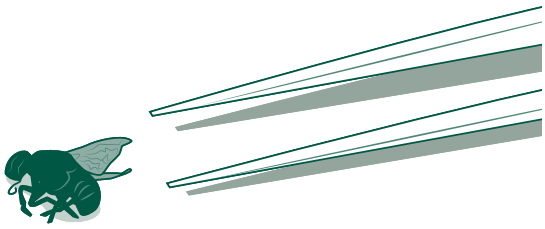
Student Protocol

Part 1: Insect DNA Extraction and PCR

Note > *Insects can be stored in alcohol for an indefinite amount of time before the lab. When collecting an insect, the more recently deceased it is, the greater your chances of successfully extracting DNA for PCR.*

A		Lyse the Insect
☐	1	Label a clean 1.5 mL microtube with your initials and group # on the lid and side of the tube. 
☐	2	Remove your insect from its collection tube with alcohol and place on a lab tissue or paper towel to wick away the moisture. 
☐	3	Using a ruler, measure the length of the insect's abdomen. If it is larger than 2 mm long/wide, you will need to *cut off a 2-mm portion from the posterior end of the abdomen and use that*. Any sharp edge, such as scissors or a scalpel, may be used to cut. If the insect is small enough, you can use the whole abdomen or even the whole insect.

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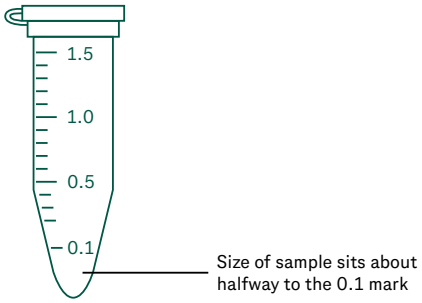
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

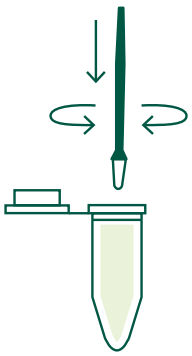
☐ 4 Place the insect sample into your labeled, clean microtube. The sample should sit about halfway to the 0.1 mL mark—if it is larger than that, *trim the sample* and try again. *(Too large of a sample can create excessive debris and inhibit PCR.)*



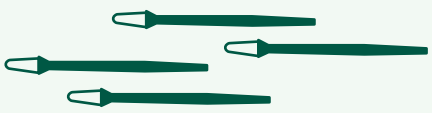
☐ 5 Add 200 µL of Lysis Buffer and close the lid tightly. This helps break up the cell to release the DNA into the solution.

☐ 6 Use a small plastic micropestle to crush your insect sample. Twist down and rotate with force to crush it as much as possible for at least 1 minute or until it has a "soupy" look.

Note > *If the insect sample gets stuck at the bottom of the tube, close and flick the tube with your finger to resuspend or use a clean pipette tip to dislodge it.*



Do not throw these away—clean thoroughly and reuse.



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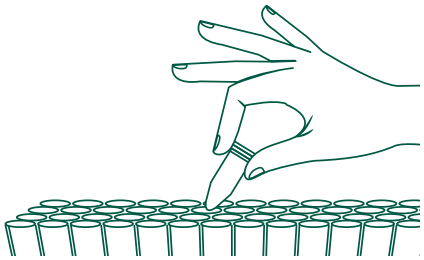
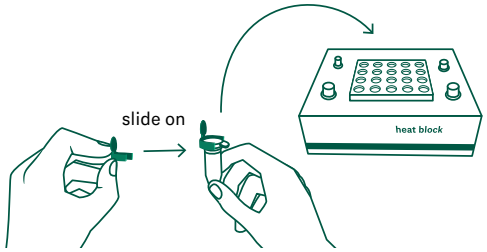
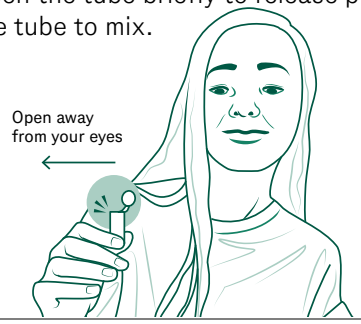
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

☐	7	Add 800 µL of Lysis Buffer and close the lid tightly.
☐	8	Mix by vortexing or "racking" (keep a finger on the top of the tube to prevent it from opening). **Note >** *Press down firmly and drag tube across rack to mix contents.* 
☐	9	Slide a cap lock onto your tube (opposite the tube hinge) to prevent it from popping open when heated. Place it in the 99 ˚C heat block or water bath for 5 minutes. 
☐	10	Open the tube briefly to release pressure, then close. Flick or "rack" the tube to mix. 
☐	11	In a centrifuge, spin your tube for 5 minutes at the highest speed, 12,000–14,000 rpm (at least 10,000 x g). Get your tube from the centrifuge and carefully place it in the rack without shaking it. You should see: — A pellet at the bottom of the tube (we do not want this) — Maybe an oily layer at the top of the tube (we do not want this)

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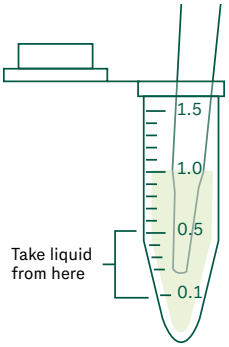
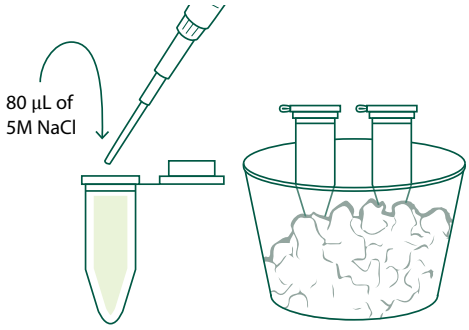
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

☐	12	Get another clean 1.5 mL microtube and label it with your initials and group # on the lid.
☐	13	Hold the tube you just centrifuged at eye level and use a P1000 to transfer about 800 µL of liquid from the middle of the insect lysate tube to the clean tube you just labeled in Step 12. **Note >** *Do this without disturbing the pellet and without getting a lot of the oily layer. If you do disturb the pellet, re-centrifuge the sample.* 
B		Remove Impurities from the Sample
☐	14	Add 80 µL of 5M NaCl to the tube with the clear insect lysate (the transparent product from breaking open the insect cells). Shake the tube a few times to mix and incubate on ice for 5–10 minutes. The solution may become cloudy. 
☐	15	Place the tube with NaCl into a balanced microfuge and spin again for 5 minutes at the highest rpm. After spinning, there may or may not be a noticeable pellet at the bottom of the tube.

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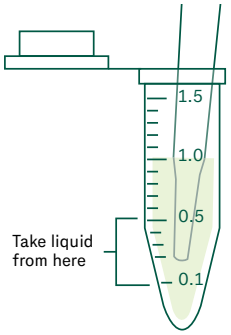
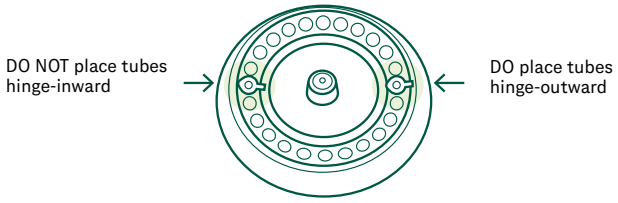
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

C		Isolate the DNA
☐	16	Get another clean 1.5 mL microtube. Label the tube "DNA" and your initials and group number.
☐	17	Hold the tube at eye level and use a P1,000 to transfer 600 µL of liquid from the top of the insect lysate/NaCl tube to the clean tube you just labeled "DNA". 
☐	18	Add 800 µL ice-cold isopropanol to your "DNA" tube. Mix contents by inverting your tube several times and then incubate on ice for at least 5 minutes or store in the freezer.

Note > *Potential Stopping Point: If there is not enough time to complete the DNA isolation, store samples in the freezer for up to 1 week.*

☐	19	Centrifuge the tube at top speed (12,000 x g) for 5 minutes. **VERY IMPORTANT >** *Orient the hinge of the tube to point outward and away from the middle of the microfuge. Nucleic acids (DNA) will pellet at the bottom-side of the tube under the hinge.* 

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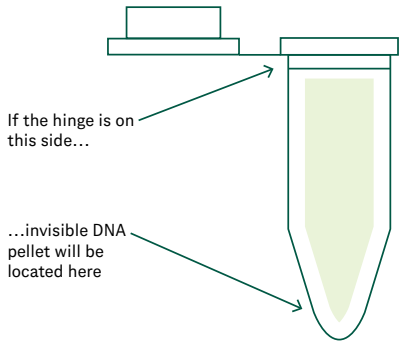
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

☐ 20 Carefully pour the liquid out of the tube (we only want the pellet) and tap the mouth of the tube hard, onto a clean paper towel to remove the liquid on the lip of the tube.

The pellet should be stuck to the bottom of the tube as a teardrop-shaped mark or may appear as minute speckles on the hinge-side of the tube. Do not worry if there is no visible pellet.



☐ 21 Open the cap and air-dry the pellet for about 5–10 minutes to evaporate all remaining isopropanol (residual alcohol will interfere with the PCR reaction).

Note > *To speed up the evaporation process, place tubes on a heat block set at about 50–70 °C. Keep caps open and monitor for evaporation. If most of the liquid has been removed from the tubes beforehand, this should take less than 5 minutes.*

☐ 22 Add 100 µL of TE/RNase buffer to your tube.

Scrape the side of the tube where the pellet is (or should be) with the micropipette tip to facilitate resuspension. Pipette up and down gently to collect DNA accumulated on the area underneath the hinge of the tube.

☐ 23 Centrifuge the tubes for 1 minute to pellet any particulates that did not dissolve in solution. This is your isolated insect (and possibly *Wolbachia*) DNA!

Note > *Potential Stopping Point: If there is not enough time to set up the PCR, store samples in the freezer.*

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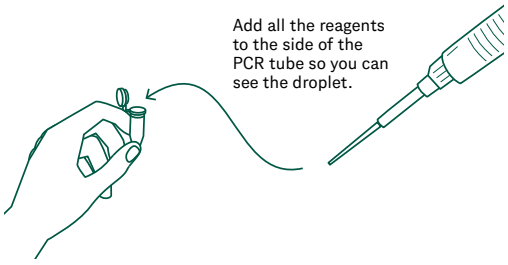
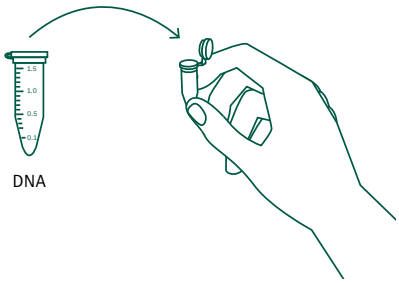
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

D		Set Up the PCR
☐	24	Label a PCR tube with your initials on the side and top.
☐	25	Pipette 20 µL of Master Mix into the PCR tube. 
☐	26	Add *20 µL of Primer Mix* into the PCR tube (use a new tip).
☐	27	*Add 10 µL of your extracted DNA* into your PCR tube (use a new tip). Your final volume should be 50 µL. 
☐	28	Make sure the cap is closed tight and *flick* the tube gently to *mix* the contents. Then *fling* the tube to move the liquid to the bottom. **Note >** *To fling: Hold the top of the tube firmly between your fingers and fling the tube, in a wide downward arc motion with force..*

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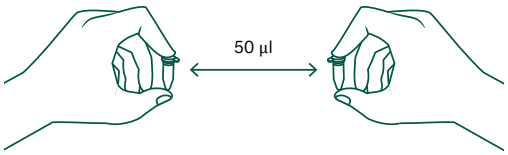
Student Protocol

Part 1: Insect DNA Extraction and PCR

Continued

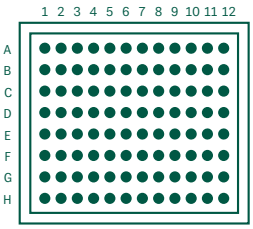
☐ 29 Compare the volume of your PCR tube with a reference PCR tube that has 50 µL in it.

Note > *If the volume of your tube does not match, set up a new PCR reaction.*



☐ 30 Place your PCR tube into the thermal cycler. Make sure to record the location of your tube on the Thermal Cycler Grid provided by your teacher (fill in the center first).

	Tube Location
1	Record your PCR tube location! Example: A11, A12



	Wolbachia PCR Thermal Cycler Parameters
1	94 °C hold for 2 minutes
2	29 cycles of:
	94 °C hold for 30 seconds
	55 °C hold for 45 seconds
	72 °C hold for 1 minute
3	72 °C hold for 2 minutes
4	4 °C hold for infinity

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Student Protocol

Part 2: Gel Electrophoresis

A		Prepare Agarose Gel
☐	1	Make sure you have all the materials needed to run your DNA on an agarose gel.
☐	2	Add 5 µL of loading dye to your 50 µL PCR reaction.
☐	3	Gently flick the tube to mix.
☐	4	Obtain a 2% agarose gel (teacher, see "Lab Preparation" for options on pouring gels). Make sure the gel is: a. Placed into the gel box with the wells oriented towards the negative (black) electrode. b. Covered in enough 1X TAE buffer to just cover the gel entirely.
☐	5	Use the P20 micropipette to load 15 µL of each sample into each of the following wells taking care not to puncture the gel. Make sure you load the gel from left to right with wells at the top of the gel box. a. Well 1 (far left): 100 bp ladder b. Well 2: Negative PCR control (water template) c. Well 3: Positive PCR control (insect and *Wolbachia* DNA template) d. Wells 4–7: PCR samples
B		Electrophoresis
☐	6	Plug your gel electrophoresis system into the power supply, cover with the lid, and run the gel at 150 volts for 10–20 minutes. **Note** > *Option to run on a lower voltage for a longer time to see better separation between bands. However, this may result in the DNA bands appearing dimmer.*
☐	7	Check that the gel is running by looking for small bubbles streaming off the electrodes.
☐	8	Turn the power supply off and take a picture of the gel through the hood (Minione equipment) or carefully remove the gel from the tray and visualize it on a UV transilluminator.
☐	9	Clean up by placing gel and used tubes and tips in the trash. Gently rinse the gel electrophoresis system with water and air dry.

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Student Guide, Part 1: Pre-Lab

Directions
In this lab, you will play the role of an entomologist exploring how Wolbachia *can be used as a tool to control mosquito-borne disease. To begin, watch the video clips your teacher shares and record at least three observations. After watching the videos, write down two questions that you have about this topic.*

1. Phenomenon:

Observations *I see...*	Questions *I wonder...Could it be that?*

2. Sketch and describe your insect in the table below. Then, use an insect identification app or online search to find the scientific and common name of your insect (ex. *Drosophila melanogaster*/fruit fly).

Sketch	
Description *wings, number of legs, etc.*	
Scientific Name / common name	
Source of information	

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Student Guide, Part 1: Pre-Lab

Continued

3. Imagine that 10 females in the insect population that you or your partner sampled from are infected with *Wolbachia*. Work with your partner to discuss what might happen to that particular insect population and the ecosystem as a whole over time as a result. There is no one correct answer!

Cause and Effect Sentence Frames

If...then...because...	Since...
...could lead to...because...	Consequently...
As a result of..., then...	Therefore...
...would cause...	Due to the fact that...

A particular insect population over time	
The ecosystem you live in over time	

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Student Guide, Part 2: Lab

Directions

Draw or paste the picture of your gel below after completing the lab. Label each well and the DNA ladder (without writing directly on the picture).

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Student Guide, Part 3: Data Analysis

Directions
Analyze your results from the lab by answering the questions below.

1. PCR Results:

   a. Were you able to successfully amplify DNA from your insect?

   b. Is your insect infected with *Wolbachia*?

   c. Explain how you know
   The gel shows…which means…:

2. Collect class data to determine what proportion of insects from each order are infected with *Wolbachia* (do not include data from unsuccessful DNA isolations where no bands are present on the gel).

   Identify three to five patterns in the class data.

3. Optional: How accurately do you think our class data reflects the *Wolbachia* infection rate of insects in our area (in other words, how reliable do you think our class data is)? Consider the limitations of our investigation including sample size, how samples were collected, etc.

4. Optional: If you were to conduct another investigation, what could you do to increase the reliability of our data?

   Describe the investigation.

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Student Guide, Part 4: Construct an Explanation

Directions
Conduct research and find an article to read about how mosquitoes are being used to control insect-borne disease. Use this information along with information from background readings to explain how Wolbachia *bacteria impact insect populations and be used to solve problems, such as insect-borne disease.*

1. Summarize the article in the table below.

Title	
Main Idea	**Supporting Detail**
1	
2	
3	
Summary of article	
Source	

Continues next page >

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Student Guide, Part 4: Construct an Explanation

Continued

2. Construct an explanation that answers the question:

   How can infection with *Wolbachia* bacteria impact insect populations and be used to solve problems, such as insect-borne disease?

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Constructing an Explanation Rubric

Score	4	3	2	1
Flow	Presents information and ideas in a complete, logical sequence to explain a question.	Presents information and ideas in a logical sequence to explain a question.	Presents information and ideas in a somewhat disjointed sequence to explain a question.	Presents a vague or incomplete explanation to a question.
Content	Shows a deep, thorough understanding of scientific content and vocabulary.	Shows a clear understanding of scientific content and vocabulary.	Shows some understanding of scientific content and vocabulary.	Shows little understanding of scientific content and vocabulary.
Final Score				