

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Overview and Purpose

In this series of labs, students use biotechnology methods including DNA extraction, the Polymerase Chain Reaction and gel electrophoresis to test insects they have collected and identified for the presence of *Wolbachia*, a bacterial endosymbiont that lives with the cells of the reproductive tracts of over 20% of insect species. *Wolbachia* can cause male offspring to develop into females, can cause females to reproduce by parthenogenesis, so that offspring are clones of the female, and can prevent production of offspring by uninfected females. Because *Wolbachia* affects the reproduction of insects and other arthropods, it has significantly affected their evolution.

Innovation

This project is innovative because it involves students in authentic scientific research that generates data on the infection rate, prevalence among different insect orders, and geographic distribution of the bacterial endosymbiont *Wolbachia*. In addition, students will soon be able to submit their *Wolbachia* samples for DNA sequencing and publication in a national databank and will be able to compare their own *Wolbachia* DNA sequences to those found by others. Thus, students will be contributing to the scientific community's understanding of this important microbial endosymbiont.

Goals of the Unit

To engage high school science students in authentic scientific research on insect bacterial endosymbionts, to contribute to the collection and reporting of new scientific data on these endosymbionts, and to enhance student interest in and understanding of a range of concepts including biodiversity, systematics, symbiosis, evolution, molecular biology, biotechnology and bioinformatics.

Educational Standards

This unit addresses the following themes of AP Biology as set by the College Board: evolution, structure and function, continuity and change, interdependence in nature, science as process, and science, technology and society. In the Massachusetts Biology frameworks, it addresses standards **2.2** (prokaryote and eukaryote cells), **3.2** DNA replication and transmission, **5.1**, **5.2**, **5.3** evolution through natural selection and biodiversity, and **6.3** relationships among organisms.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Objectives

As a result of this unit, students will be able to:

1. use an online taxonomic key to identify insects they have collected and prepared for DNA analysis;
2. extract and isolate genomic DNA from insects and a bacterial endosymbiont;
3. screen for the presence of *Wolbachia* DNA using the Polymerase Chain Reaction;
4. determine the presence or absence of *Wolbachia* DNA in the PCR product and the size of the DNA molecule using gel electrophoresis; and
5. identify an unknown nucleotide sequence from an insect endosymbiont using the NCBI search tool BLAST.

Research Question

What percentage of insects and what orders of insects contain *Wolbachia*?

Materials and Procedures

The labs described in the following pages are adapted from the *Wolbachia* Project lab series originally developed by Dr. Seth Bordenstein of the Marine Biological Laboratory and are submitted here with his permission. Last year I was one of three pilot teachers that tested this lab series with students. For - and + *Nasonia* wasp controls and W-spec primers, contact Dr. Bordenstein at sbordenstein@mbl.edu.

Students can work in groups of 2 or 3 depending on class size and available materials. Students should wear gloves and safety glasses. Introduce the lab series by showing the online video (43 minutes) about *Wolbachia* available at <http://jbpc.mbl.edu/~sbordenstein/workshop.html>.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Insect Identification Lab

(1 or 2 periods)

Materials

Insect specimens, bent probe, forceps, 3 Petri dishes, transfer pipette (eye dropper), squirt bottle of 95% ethanol, 6 glass vials or film canisters, marking pen, labeling tape, colored pencils, 3 microcentrifuge tubes, dissecting microscope, computer. Prior to the lab, have each student collect two or more of three different insects from their yard or school. Insects can be collected in vials or film canisters. Fill each insect collection container with 95% ethanol and store in the freezer.

Procedures

1. Place a Petri dish on the stage of the dissecting scope and add enough ethanol to the dish to cover the insects. Ethanol preserves DNA. Using forceps, place the insects into the Petri dish. Use a pipette to wash the insects, making sure they stay in the ethanol. All insects can be put in the same dish.
2. Sort similar looking specimens into groups of morphospecies, individuals that have similar morphology and are probably members of the same species.
3. Choose 3 different morphospecies to be further analyzed for the presence of *Wolbachia*. It is best to use smaller species because they are easier to dissect and preserve better. Pick two insects that are the same; one will be used for the *Wolbachia* testing and one for vouchering.
4. Make a written observation of the three morphospecies chosen for testing. Carefully draw the three morphospecies using colored pencils. Voucher insects can also be photographed using a digital camera.
5. On the computer, log on to: http://pick4.pick.uga.edu/mp/20q?guide=Insect_orders. Use the online taxonomic key to identify the insects to Order. Click on the pictures for a more detailed explanation. After answering several questions, click on any of the “Search” buttons to narrow down the list of Orders that have the characteristics selected. Next, click on the “Simplify” button in the upper left menu column to eliminate unimportant characteristics from the question list. The new list will show only the characteristics that might help distinguish the specimen. Label the drawings made in step 4.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Insect Identification Lab (Cont'd)

Procedures (Cont'd)

6. Fill three vials halfway with ethanol and make labels for each vial with the tape and marking pen. Record student names, the date, and the voucher number (#1, 2, or 3). Label three microcentrifuge tubes with the same information.
7. Using forceps, transfer one of the specimens of the morphospecies into the glass vial, the other into the microcentrifuge tube. Repeat this step for the other two morphospecies. If the insect is large, remove the abdomen and use the forceps to obtain a “fruit fly-sized” amount of the abdomen. *Wolbachia* is concentrated in the reproductive organs in the abdomen. If the insect is small, the entire body can be placed in the tube. Cover the insects with ethanol and place vials and tubes in the freezer.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

DNA Isolation Lab

(1 or 2 periods)

Materials

Incubator or water bath set at 70°C, vortexer, centrifuge, insect specimens, + and - *Nasonia* wasp controls, Qiagen DNeasy Kit (#69504), P200 and P1000 pipette and tips, float racks, waste container, 1X Phosphate Buffer Saline (10X PBS from Fisher BP399-500 diluted to 1X), marking pen, tweezers, Kimwipes, 95% ethanol, microcentrifuge tube racks, 1.5 ml microcentrifuge tubes.

Procedure

The procedure includes lysing the cells, removing cellular debris and eluting DNA.

Cell Lysis:

1. Collect five 1.5 ml microcentrifuge tubes. Label these with a marking pen with student initials and the specimen number, 1, 2, or 3, +control, and -control.
2. Place 180 microliters (μl) of PBS buffer into each tube to macerate the insects in.
3. Place the small insect or abdomen of a larger insect into the buffer of tube 1 with tweezers. If the insect or *Nasonia* wasp control is in ethanol, briefly blot it dry on a Kimwipe.
4. It is important to do this step as rapidly as possible. Macerated tissue releases DNases which rapidly break down DNA. Macerate the insect in tube 1 thoroughly using a microtube pestle. Immediately add 20 μl of Proteinase K (destroys the DNase enzymes that break down DNA). Add 200 μl of buffer AL (to break open cells). Mix by vortexing for 10 seconds. Do not pre-mix Proteinase K and Buffer AL.
5. Repeat steps 2–4 with the other four samples. Use a different pestle and pipette tip for each tube.
6. Incubate all five tubes for at least 10 minutes at 70°C in the water bath or incubator.
7. Add 200 μl of ethanol (95–100%) to each tube then vortex. This will precipitate DNA from the extracted material. Tubes can be stored in a 4°C refrigerator overnight.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

DNA Isolation Lab (Cont'd)

Cellular Debris Removal:

1. Fit five DNeasy spin columns into five 2.0 ml collection tubes and label both the lids of the columns and the collection tubes 1–5.
2. Pipette the liquid from tube 1 of the cell lysis steps into the DNeasy Mini spin column #1. Using a new pipette tip for each transfer, repeat this process with the other four tubes.
3. Centrifuge all tubes for 1 minute at 8,000 rpm. The DNA is caught in the filter of the spin column.
4. Discard the flow through waste in the 2.0ml collection tubes into the waste container.
5. Place the spin columns containing the DNA into their respective emptied 2.0ml collection tube.
6. To each tube, add 500 μ l of Buffer AW1. This washes the DNA. Centrifuge for 1 minute at 8,000 rpm.
7. Again, discard the flow through waste in the 2.0ml collection tubes into the waste container and place the DNeasy Mini spin columns from tube into their respective 2 ml collection tube.
8. Add 500 μ l of Buffer AW2 (a second wash buffer) to each of the five 5 tubes and centrifuge for 3 minutes at 13,000 rpm (or max speed if the centrifuge doesn't go that high, then let the tubes air dry for five minutes). Discard flow-through and collection tubes. This step is also removing the ethanol.
9. Place the spin columns into 1.5 ml microcentrifuge tubes. Label the lids of each tube 1–5 and include students' initials. After elution with Buffer AE, these tubes will contain the purified DNA samples.

DNA Elution:

1. Pipette 100 μ l of Buffer AE directly onto the membrane of the spin column. This is an elution buffer that rinses the DNA off the spin column filter and into the 1.5 ml tube.
2. Incubate all 5 tubes at room temperature for 1 minute then centrifuge at 8,000 rpm for 1 minute.
3. Discard the spin column and keep the labeled 1.5 ml tube containing DNA. Store in a 4°C refrigerator.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

PCR Lab

(one period, can also pre-make gels this period)

Materials

Thermalcycler for the class, 3 DNA samples from insects, 2 DNA samples from + and - *Nasonia* controls, + DNA control, 6 PCR Ready Bead tubes, P20 and P200 pipettes and tips, 1 rack for PCR tubes, 1 tube each of Wspec-F primer (5 micromolar, 20 μ l) and Wspec-R primer (5 micromolar, 20 μ l), 1 tube of sterile distilled water (200 μ l), 1 waste container, marking pen.

Procedure

1. Collect 5 PCR Ready tubes. Each of these contains a preformulated, pellet of “master mix” containing Taq polymerase, MgCl₂, Buffer, and dNTPs. Label the tubes with initials and the voucher # 1, 2, 3, +control, -control.
2. To each tube, add the materials in the sequence below. Use a new tip for each step. Add 19 μ l of sterile distilled water, 2 μ l of Primer W-spec forward, 2 μ l of Primer W-spec reverse, and 2 μ l of DNA from each sample to its corresponding tube. Be sure to change the pipette tips for each DNA template!
3. Cap and gently tap the bottom of each tube to mix the components. Place the five tubes into the thermalcycler. The thermalcycler should be programmed and run for the following optimum settings:

<u>1 cycle</u>	<u>38 cycles</u>	<u>1 cycle</u>
2 min @ 95 C	30 sec @ 94 C	10 min @ 72 C
	45 sec @ 55 C	
	90 sec @ 72 C	

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Gel Electrophoresis Lab

(one period, running the gel, staining and destaining may take longer)

Materials

5 tubes of PCR products, rack for PCR tubes, P200 and P20 pipettes and tips, marking pen, 6X loading buffer (Fisher TAK-9156), DNA ladder (Fisher PR-G3 161), 500 ml TAE buffer (Fisher PRV4271) agarose, casting tray and combs, QUIKView DNA stain (Ward's 38 V 9014), staining tray, weighing paper, spatula, 500 ml flask, 100 ml graduated cylinder, oven mitt, masking tape, microwave oven, electronic balance, electrophoresis box, power supply, and cables.

Procedure

Preparing the Gel:

(1% solution, gel volume will vary depending on casting tray size)

1. Measure 1.25 g agarose powder and add it to a 500 ml flask.
2. Add 125 ml TAE Buffer to the flask.
3. Melt the agarose in a microwave heating for several short intervals until the solution becomes clear. Do not let the solution boil for long periods as it may boil out of the flask.
4. Let the solution cool to about 50–55°C, swirling the flask occasionally so it cools evenly.
5. Seal the ends of the casting tray with two layers of masking tape. Place the combs in the tray.
6. Pour the melted agarose solution into the casting tray and let cool until it is solid (milky blue).
7. Carefully pull out the combs and remove the tape. Place the tray in the electrophoresis box.
8. Add enough TAE Buffer so that there is about 2–3 mm of buffer over the gel.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Gel Electrophoresis Lab (Cont'd)

Procedure (Cont'd)

Loading and Running the Gel:

- Record the order each sample will be loaded in the gel, including who prepared the sample, what organism the DNA samples came from, + and - controls and DNA ladder.
- Carefully pipette 20 μ l of each sample/loading buffer mixture into separate wells in the gel.
- Pipette 10 μ l of the DNA ladder standard into at least one well of each row on the gel.
- Place the lid on the gel box, connecting the electrodes appropriately. Turn on the power supply to about 100 volts. Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode. Let the power run until the blue dye approaches the end of the gel, then turn off the power, disconnect the electrodes and remove the lid and the gel using gloves.

Gel Staining:

1. Place the gel into the staining dish. Add warmed (50–55°) staining mix.
2. Allow gel to stain for at least 25–30 minutes until dark blue, then pour off the stain (can be reused).
3. Rinse the gel and staining tray with warm water to remove stain. Change the water several times. The gel will become lighter, leaving only dark blue DNA bands. Destain overnight for best results.
4. View the gel against a light box or bright surface. Record and discuss the data while the gel is fresh.

Bioinformatics Lab

(one period)

Go online to <http://jbpc.mbl.edu/~sbordenstein/workshop.html> for the lab comparing *Wolbachia* DNA sequences to other DNA sequences in NCBI using the BLAST search tool.

Activities Outside of the Classroom

Students collect the insects used in the lab as a homework assignment. My students said this was both one of the hardest homework assignments they've ever had and one of the most fun. Having collected the insects themselves, they were very excited about testing them for *Wolbachia*. Students this year will participate in an online pre and post test and will be able to see what they learned through the unit by comparing their answers. Students also write a formal lab report. This gives me a clear idea of student understanding of the concepts and procedures learned. One student wrote,

“This lab made the science of DNA less of a mystery and allowed us to take knowledge we learned in the classroom and apply it in the lab. There was an actual scientific purpose to this lab which made it more significant and worthwhile to perform. It was exciting to participate in an actual scientific study and provide information that may be useful to the scientific community. It was a cutting-edge lab with techniques many undergraduates don't even get to do. I enjoyed it most out of all the labs performed this year.”

2008
Winning Lesson Plan
from Falmouth,
Massachusetts

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

**2008
Winning Lesson Plan
from Falmouth,
Massachusetts**

*Discover the Microbes
Within! The Wolbachia
Project*

by Christine Brothers
Falmouth High School

Subject: Advanced
Placement Biology

Grade Level: 11–12

Duration: Approximately 8
one-hour class periods.
These labs can be taught
as a two-week unit or the
labs can be taught
throughout the year as
related topics are taught in
the curriculum.

Dissemination Plan

This series of labs was originally developed by Dr. Seth Bordenstein at the Marine Biological Laboratory in Woods Hole, which is part of the Falmouth school district. I asked Seth permission to submit these labs as my lesson plan for the Amgen award and he enthusiastically supported my doing so as a way of disseminating the lessons. In 2005, I attended the first *Wolbachia* Project teacher workshop Seth organized. At the time, Seth was developing a funding proposal to the National Science Foundation to support additional teacher workshops. I served as the teacher advisor on this proposal and wrote a letter of support. Unfortunately, the project was not initially funded by NSF. In 2006, I received a grant for \$8,800 from the Massachusetts Biotechnology Council to purchase the biotechnology equipment for our school needed to implement the *Wolbachia* Project. I worked closely with Seth during this time to make these purchases, which we received in January, 2007, and later to test the labs before doing them with students. At this point, FHS was one of three schools nationwide piloting the labs with students.

I conducted the *Wolbachia* Project with my classes in May, 2007. Seth came to do an introductory lecture for my classes and returned subsequent days to observe my students working in the lab. For Seth, this was an exciting opportunity to see high school students actually doing the labs, something he had not previously had the chance to do. I had students write a formal lab report and provided copies of these to Seth which helped him evaluate what students had learned from the labs. We also provided him with feedback on how to revise the labs to make them more student-friendly. The current labs are greatly revised from their original 2005 form. In 2006, Seth wrote a proposal to the Howard Hughes Medical Institute, and once again, I acted as the teacher advisor in its development and wrote a letter of support. Happily, HHMI funded the project in the spring of 2007, and his /revised NSF proposal was then also funded in the fall of 2007. With substantial and solid funding for the next five years, the *Wolbachia* Project is now poised to go nationwide! Seth plans a number of teacher workshops hosted by *Wolbachia* research labs across the country. Based on my input, Seth included a thermalcycler loan program in his grant so that participating schools will be able to borrow this very expensive but vital piece of equipment needed to do the PCR lab. An exciting addition to the Project is that students across the country will be able to submit their *Wolbachia* DNA to Seth's lab to be sequenced and entered into a student generated database and will be able to compare their sequences with those discovered by others.

In November, I attended the National Association of Biology Teachers conference where I made a presentation to about 75 teachers promoting the Project and the next teacher workshop in April, 2008. I have helped Seth in spreading the word to teachers through my contacts at the MA Association of Biology Teachers, the MA Science Education Leadership Association and the MA Department of Education. At the workshop, I will give a presentation about how our school implemented the *Wolbachia* Project and some of my students will attend with me. I am now advising a teacher in NY who will be implementing the Project this year and my classes will be hosting a visit by another participating teacher when her classes visit Seth's lab in May. Finally, I am writing an article about the Project which I hope to publish in *American Biology Teacher*.