“Life Within the Flame...

...Using PCR to Determine the Presence of the Symbiotic Bacterium – *Wolbachia* in the Red Imported Fire Ant”

*Wolbachia* and *S. invicta* bands on gel.

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INTRODUCTION

Fire ants are a diverse group that originated in South America about 65 million years ago. Fire ants are a member of the order of insects known as Hymenoptera. From the human standpoint, this order is probably the most beneficial in the entire insect class. It contains a great many species that are of value as parasites or predators of insect pests, and it contains the most important pollinators of plants, the bees.

The Hymenoptera are a very interesting group in terms of their biology. They exhibit a great diversity of habits and complexity of behavior culminating in the social organization of the wasps, bees and ants.

Fire ants belong to the family, Formicidae, which is a very common and widespread group well known to most people. The ants are probably the most successful of all of the insect groups. They occur practically everywhere in terrestrial habitats and out number in individuals that of most terrestrial animals.

The imported fire ants Solenopsis invicta and Solenopsis richteri are important pests in the southeast. The red imported fire ant, Solenopsis invicta is the most common forms. The black fire ant Solenopsis richteri has a rather limited distribution in a few of the southeastern states.

Ants of the genus Solenopsis may be distinguished by the fact that the antennae are ten segmented with a two (2) segmented club.

Wolbachia is a bacterium that inhabits the abdomens of numerous insects and can affect these populations. Insects are among the most diverse and abundant animals on earth. They represent 85% of all described animal species and there an estimated 1,000,000,000,000,000,000 (1018) insects alive on the planet any given day. If the services insects provide everyday (for free) disappeared suddenly, humans would soon disappear. Insects clean water, pollinate flowers that produce about 1/3 of the food we eat, breakdown waste and decompose plants and animals. Wolbachia has been found in over 20% of insects. Therefore its frequency in any insect population is of interest. This module will focus solely on determining the presence of Wolbachia in Solenopsis invicta (RIFA = Red Imported Fire Ant).

The RIFA has two social forms: monogyne, which have a single queen, and polygyne, which have multiple queens. The social form of a colony affects many behaviors, including territoriality, and it also affects the colony’s ability to survive infections. In these experiments, you will determine the social form of colonies and also whether Wolbachia infects one or both social forms. Combined with data from students and teachers from other schools, your data will be an important part of understanding the relationship between RIFA social form and infection with Wolbachia. You will gather RIFA samples from a variety of fire ant mounds, grind them to get the DNA (and the DNA of any Wolbachia in them) out of them, and run a set of reactions called polymerase chain reactions followed by agarose gel electrophoresis to determine if the fire ant mounds you collected have one queen (monogyne) or multiple queens (polygyne) and if they have Wolbachia in them. You will determine the frequencies of infection and if there are any correlations with colony social form. If any Wolbachia DNA is detected, you will send the DNA sample to Woods Hole Marine Biological Laboratory for sequencing and entry into the Wolbachia database as part of the HHMI “Discover the Microbes Within: The Wolbachia project” (http://discover.mbl.edu/).
**Eight weeks prior** to starting this investigation you should contact Michele Bahr mbahr@mbl.edu in order to obtain the following:

- Primers to identify Wolbachia and insect DNA  
- and – Nasonia controls

Wolbachia DNA control

This module will refocus the “Discover the Microbes Within: The Wolbachia Project”, funded by HHMI and created in the laboratory of S. Bordenstein, Ph.D. at Woods Hole Marine Biology Laboratory, to compare the presence of Wolbachia bacterial symbionts between monogyne (single queen) and polygyne (multiple queens) colonies. The research plan will focus on identification of monogyne vs. polygyne nests, DNA extraction, polymerase chain reaction, agarose gel electrophoresis, and analysis and documentation of experimental results. Awesome PowerPoints for each module are found at [http://discover.mbl.edu/](http://discover.mbl.edu/) Please review these before starting this project and use them as teaching tools.

Introductory reference material:


Science influences everything we do and there is no better way to teach science than to experience it. Experience leads to empowerment and empowerment creates the foundation for critical thinking skills and ultimately a scientifically-literate public.

Discover the Microbes Within: The Wolbachia Project is designed for high school biology educators in an effort to bring real-world scientific research into biology labs and lesson plans with inquiry, discovery, biotechnology, and a culture of excellence. The goals include engaging high school students in real-world research and showing them what it is like to be a scientist.

Biotechnology has revolutionized medicine, agriculture, taxonomy, ecological studies, archeological studies, and many, many more areas of science. It has led to the development of new drugs, to better understandings of diseases from cancer to the common cold, to better diagnosis of diseases; it has led
to the generation of genetically modified crops which are drought-resistant and pesticide-resistant; it has led to the generation of farm animals with a higher protein content in milk and a lower fat content in meat. We are in the midst of a biotechnology revolution—which means we all need to understand how biotechnology works. These explorations will introduce you to three specific and essential techniques used in all medical testing labs: DNA extraction, polymerase chain reaction, and agarose gel electrophoresis.

Some bacteria cannot be cultured outside the host. Therefore, the only way to determine its presence inside a host is by total genomic extraction of both host and parasite. Using PCR, the DNA of both can be amplified and studied. *Wolbachia* cannot be cultured outside the host.

The **polymerase chain reaction (PCR)** is a technique widely used in molecular biology. It derives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by *in vitro* enzymatic replication. As PCR progresses, the DNA thus generated is itself used as template for replication. This sets in motion a chain reaction in which the DNA template is exponentially amplified. With PCR it is possible to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece. PCR can be extensively modified to perform a wide array of genetic manipulations.

Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquaticus*. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, using single-stranded DNA as template and DNA *oligonucleotides* (also called DNA primers) required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary to physically separate the strands (at high temperatures) in a DNA double helix (DNA melting) used as template during DNA synthesis (at lower temperatures) by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions.

There are **three steps in PCR** cycle:

1. **Denature** – hydrogen bonds are broken so DNA helix unzip; the highest temperature in the cycle.
2. **Anneal** – primers attach to target DNA; the coolest temperature of cycle.
3. **Extension** – Taq polymerase initiates extension of complement strand.

Developed in 1983 by **Kary Mullis**, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensics and paternity testing); and the detection and diagnosis of infectious diseases. In **1993 Mullis** won the Nobel Prize for his work on PCR.
**EARLY HISTORY**

*Solenopsis richteri* and *Solenopsis invicta* originally inhabited different parts of the world’s largest wetland. *S. richteri* lived on the periphery of the wetland and then expanded its range edging into the Pampas. *S. invicta* can be found throughout the marshy river basins. The area is characterized by frequent disturbances. During the dry season, thick grasses clog the riverbeds. When the rains come, the water is forced to cut new channels, eventually overflowing and flooding the landscape. The river’s vagrancy caused a wealth of microclimates and the area is dominated by a rich area of plants. In 1929 a geographer noted, “The most striking feature in the natural vegetation is its lack of uniformity.” The ants have adapted to this situation by exploiting the disturbances. They are opportunistic- entomologist called them “weeds” infiltrating disrupted areas and growing quickly. A single queen gives birth to 250,000 workers in three years, but is forced out when the ecology matures.

*S. richteri* was the first of the two species to break from the wetland and travel north. In the late nineteenth and early twentieth century the cattle industry was flourishing. The ants which lived near major points of distribution stowed away on ships and reached Mobile, Alabama around 1918. In the new world the insects faced climatically similar conditions as in South America but ecologically very different. Approximately eighty percent of the land within a hundred miles radius was thick forest. Some two decades later, after the cattle industry reached deeper into the South American interior where *S. invicta* lived, the red ants also reached Mobile. At this time the South was on the brink of agricultural revolution that would lead to the complete alteration to the ecology of the region opening many new spaces for the fire ants to colonize. This change in ecology allowed *S. invicta* to spread rapidly because of the vast area of disrupted habitats to exploit.

The spread and the success of *S. invicta* were also enhanced by a biological quirk. In their native homeland *S. invicta* live in two social forms: monogynne and polygynne colonies. Monogynne colonies have a single queen which mates in aerial swarms and founds her colony independently. Polygynne colonies contain several, sometimes, several hundred queens, most of which mate within the nest and form new colonies by recruiting workers from the mother colony and re-locating to an nearby area to build a new nest. Both forms arrived in America. Bigger and stronger, the monogamous queens initially predominated. They could spread widely and colonize many disturbed habitats quickly. But, as the environment became saturated with ants, the polygynne colonies came to dominate. Young queens were protected in the nest and were subsidized by the mother colony when founding their own nests. The increasing prevalence of polygynne allowed more and more ants to be packed into the same area.

*S. invicta* can and did adapt to different habitats during its long history, first being found in wetlands and then moving into drier areas. The fire ant mound consists of a series of interlocking galleries, tunnels and chambers that extend downward into the ground. Galleries can extend anywhere from 1-3 feet into the soil, usually forming an inverted cone. Foraging tunnels just below the soil surface extend several yards out from the mound with periodic exits from which workers emerge to search for food on the soil surface.
LIFE WITHIN THE FLAME....

THE WOLBACHIA MODULE AT A GLANCE

Goal:
To introduce students to cutting-edge biotechnology techniques by searching for evidence of the endosymbiont, Wolbachia, in a familiar insect, the red imported fire ant. This bacterium is of interest because it has the power to manipulate DNA. In some species it has skewed the male/female ratio. Students will learn how to organize the variety of reagents used, extract DNA, use PCR and analyze DNA results using gel electrophoresis. **Prerequisite Skills:** Prior practice with micropipettors.

Learning Objectives:
Upon completion of this module, students will have knowledge of how DNA can be used as a diagnostic tool to discover unseen microbes and understand the process of inquiry and discovery-based research. They will isolate total genomic DNA from ants and possibly, a symbiotic bacterium if it is present. See page 28 for State Science Objectives.

Teaching Time:
7 Class Periods – 50 minutes each

**Timeline for Teaching Living within the Flame**

National Science Standards:

*Science as Inquiry* – Students develop scientific inquiry skills in posing scientific questions and predictions, taking ownership of their research, and using varied tools and evidence to characterize the insect samples that they chose to study.

*Science and Technology* – The students integrate field work with taxonomic classification in the lab to determine insect Order using a computerized key

*Life Science* – In this activity, students use field work, classification, and basic content on symbiosis to learn about evolution and ecology of insects, the interdependence of microbes and animals, and the organization of living systems including insect body structure, life cycle, and associations with microbes.
LAB-1: DISPENSING REAGENTS & INSECT PREPARATION

ACTIVITY AT A GLANCE

Goal:
To introduce students to the time efficient method of dispensing reagents by calculating the total amount of reagent required for each group and assigning students to aliquot designated reagent. Students will also select and prepare the ants they will use for this module and place in microtubes.

Learning Objectives:
Upon completion of this activity, students will have knowledge of how to correctly manage and organize a large variety of reagents.

OVERVIEW

Collected ants should be stored in rubbing alcohol (isopropyl) or 95-100% ethanol.

It is important that students document two pieces of information: 1) identify their ant as virgin male, virgin female, or worker. Virgins have wings. The virgin males are solid black and virgin females are red like the workers. Workers are smaller and will not have wing. 2) Write the location of the ant’s nest.

MATERIALS:

- 2 ants from different nests
- + and – Nasonia controls (Woods Hole)
- Tweezers
- Petri dish – to pour ants in for selection
- 1 microtube rack
- 1 sheet of Bounty paper towel or Kimwipe
- 1 box of P20 pipet tips
- 1 box of P200 pipet tips
- P20 and P200 pipets/group
- P1000 or 1.0 ml graduated disposable pipet (used to aliquot reagents)
- 1 waste cup for tips & tubes
- PBS Buffer (1 ml) - NOT included in Qiagen kit
- Ethanol (95-100% - 1ml) or isopropyl alcohol - NOT included in Qiagen kit
- Qiagen DNeasy Kit (#69504 from Qiagen)
  - Proteinase K (100 μl) - Qiagen kit
  - Buffer AL (1 ml) - Qiagen kit
  - Buffer AW1 (2.0 ml) - Qiagen kit
  - Buffer AW2 (2.0 ml) - Qiagen kit
  - Buffer AE (500 μl) - Qiagen kit
- 4 empty 1.5 ml microcentrifuge tubes – for insects (color code for groups if possible)
- 9 empty 1.5 ml microcentrifuge tubes – for reagents (color code for reagent if possible)
Teacher Preparation

1. Use this website for PowerPoint and additional lab information: http://discover.mbl.edu/labs.htm
2. Make copies of student activity sheet.
3. Keep Qiagen kit at teacher station. Place pipettes, tips, waste cup, sharpie, paper towel, microtube rack, and microtubes at each student activity station.

Activity Procedure

1) Hand out student activity sheet. Go over each reagent and its purpose (the purpose is found in overview of Lab 2). Have students complete the table on their sheet. An example for 4 samples is included below. You can modify this lab for a different sample size, but the total volumes needed for each group will differ from the example table.

*a) Make sure students have correct answers for Total Volume.
*b) Note that AW1 and AW2 require 2 ml, therefore, two microtubes are required to hold their total volumes.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>1)PBS</th>
<th>2)Proteinase K</th>
<th>3)AL</th>
<th>4)Ethanol</th>
<th>5) AW-1</th>
<th>6) AW-2</th>
<th>7) AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (µL)</td>
<td>180</td>
<td>20</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td># Samples</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total Volume</td>
<td>720</td>
<td>80</td>
<td>800</td>
<td>800</td>
<td>2,000</td>
<td>2,000</td>
<td>400</td>
</tr>
<tr>
<td>Purpose</td>
<td>Denature proteins (DNAses)</td>
<td>Lysis of membranes</td>
<td>Precipitate DNA</td>
<td>Wash away things other than DNA &amp; removes ethanol</td>
<td>Elution of DNA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2) Students can select their ants and Nasonia controls. Place in the color coded microtubes. Add just enough ethanol (or isopropyl) to cover them in the tube. This alcohol will be discarded in the next lab.

Answers to Activity Sheet:
1) It reduces chances of contamination because only one person handles the reagent. It also saves time and reduces lab traffic because group members do not have to leave their station.
3) The total volume is 2 ml and one tube only holds 1.5 ml.
LAB-1: DISPENSING REAGENTS & INSECT PREP – Activity Sheet
Student:______________________________________________Date:_________________

1) Why is it important to aliquot the total amounts needed by each group before starting the lab?
_______________________________________________________________________________

2) Complete the table for the number of samples from which you will extract DNA.

3) You will be using 1.5 ml tubes. Why do you need two tubes for AW1 and AW2?
_______________________________________________________________________________

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<tr>
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<th>3)AL</th>
<th>4)Ethanol</th>
<th>5) AW-1</th>
<th>6) AW-2</th>
<th>7) AE</th>
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<tbody>
<tr>
<td>Volume (µL)</td>
<td>180</td>
<td>20</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td># Samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color of Tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purpose</td>
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<td>Wash away things other than DNA &amp; removes ethanol</td>
<td>Elution of DNA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4) Label all reagent tubes.
5) Assign a student to dispense each reagent and record their names:
   - PBS ___________________________, Prot K ___________________________, AL ___________________________,
   - Ethanol _______________________, AW1 ____________________________, AW2 _______________________,
   - AE ___________________________*Ethanol should be cold when used so it is recommended to place the microtube rack with reagents and ants in the fridge until next class meeting.

6) Group color of specimen tubes (if applicable): ___________________________

7) Label the 4 specimen sample tubes: Ant1, Ant 2, Nas -, and Nas +.

8) After selecting 2 ants, record the following: (virgin male or female, or worker) and (nest location)
   a) Ant-1 = ___________________________ and ___________________________
   b) Ant-2 = ___________________________ and ___________________________
LAB-2: DNA EXTRACTION & PURIFICATION

OVERVIEW

Total Genomic DNA can be isolated from tissues using a variety of protocols, but all involve two steps: Cell lysis, or breaking open of the cells and nuclei, and DNA purification, which purifies the DNA from the rest of the cellular debris. Cell lysis is often accomplished by physical force (grinding or blending) in a detergent and salt solution to penetrate lipid membranes, by use of enzymes that degrade cell walls (for fungi and bacteria), or a combination of both. DNA is usually purified by centrifugation to remove large cellular debris, followed by alcohol treatment to precipitate DNA. We will use a commercially available kit, the Qiagen DNEasy Blood and Tissue Kit, which uses physical force for cell lysis and a spin column (a small column with a membrane that retains the precipitated DNA—it helps purify it away from cellular proteins, lipids, and carbohydrates). The total genomic DNA isolated will include the genomic DNA of your samples, as well as DNA from any symbiotic bacteria (Wolbachia) that are present.

The extraction of total genomic DNA involves three distinct steps:

1. **Cell Lysis**: Students will begin by washing their insect specimens in phosphate buffered saline (PBS) a salty solution of constant pH to keep tissues, cells, and proteins intact during maceration. Then add a cell lysis solution (Buffer AL). This basically breaks open cell and nuclear membranes. The dilemma here is that it also exposes DNA to proteins in the insect tissue. Therefore, the enzyme Proteinase K must be added to denature the proteins and keep the DNA intact. Finally, they will add ethanol to precipitate the DNA.

2. **Elimination of Cellular Debris**: Once students have destroyed the hydrolytic enzymes and precipitated DNA, they will begin the DNA purification process. In essence they will place the cellular components, including DNA, into a spin column and wash the spin column of all components except DNA. Upon centrifugation the material will pass through the filter, which attracts DNA and allows debris to pass through. This will be followed by two wash steps with two buffers (AW1 and AW2).

3. **DNA Elution**: Students will complete the activity by removing the DNA from the filter. This is done by adding the elution buffer (AE). Spinning the tube with the DNA embedded in the filter will pull the elution buffer through the matrix, thus pulling the DNA into the collection tube.
Materials Needed / Group:

- 1 microtube rack
- Sharpie
- 1 paper towel or kimwipe to blot dry insect
- 1 box of P20 pipet tips
- 1 box of P200 pipet tips
- P20 and P200 pipets/group
- 1 waste cup for tips & tubes
- 4 Microtube pestles or sealed mL microtips (preferred)
- 4 spin columns – Qiagen kit
- The following items prepared in Lab 1:
  - *1 tube PBS Buffer
  - *1 tube Proteinase K (100 μl)
  - *1 tube AL (1 ml)
  - *2 tubes AW1 (2.0 ml)
  - *2 tubes AW2 (2.0 ml)
  - *1 tube AE (500 μl)
  - *1 tube cold ethanol (800 μl)
  - *2 tubes ants
  - *2 tubes of Nasonia controls
- 8 empty 1.5 ml microcentrifuge tubes – for insects (color code for groups if possible)

Activity Procedure

In this activity, you will:
- Isolate total genomic DNA from ants.
- Isolate DNA from infected (+) and uninfected (-) Nasonia controls.
- Learn about DNA as the molecular basis of heredity and the interactions of bacterial and animal organisms.
- Learn about cell structures and the nature of scientific knowledge.

Teaching Time: two class periods (50 minutes each)

In this activity, you will extract total genomic DNA from each of their insect samples using Qiagen’s DNeasy Blood and Tissue Culture Kit. Total genomic DNA includes DNA of the insect host as well as any symbiotic bacteria Wolbachia, if present. In addition to the 2 unknown ant samples, you will also prepare positive and negative controls using Nasonia vitripennis wasps that are infected and uninfected with Wolbachia, respectively. Review the activity flow-chart (page 15). Read through the procedure prior to beginning an activity in order to identify and understand the purpose of each reagent.
Lab 2: DNA Extraction & Purification

PROCEDURE

1. In the chart below note the contents of what you will put in each tube.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Contents / Location of Nest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>- <em>Nasonia</em> control</td>
</tr>
<tr>
<td>4</td>
<td>+ <em>Nasonia</em> control</td>
</tr>
</tbody>
</table>

2. Collect four clean 1.5 ml microcentrifuge tubes matching your group color code (if applicable). Using a Sharpie™ marker, number them 1-4 along with your initials or Group #.

3. Obtain your microtube rack with your specimens. These tubes contain an alcohol that has been preserving your insect and now must be removed. Using a paper towel, open and invert your tubes, tapping them on the paper towel to remove your insect. Blot the ethanol away from your + and – *Nasonia* controls as well. Once insect is dry, continue to next step.

Cell Lysis

1. Place 180 microliters (µl) of PBS buffer into each of the 4 clean tubes.
2. Place the small insect or abodmen of a larger insect into the buffer (no larger than 2 mm²) of Tube 1 with tweezers. May use 1 or 2 ants per sample tube but each tube should represent a different nest. Begin with tube 1 and macerate *THEROUgLHY* using a microtube pestle (or sealed microtip). Hemolymph, a whitish substance, may be visible after maceration.

3. **IT IS IMPORTANT TO DO STEP 3 AS RAPIDLY AS POSSIBLE!**
   
   MACERATED TISSUE RELEASES DNases WHICH LEAD TO A RAPID BREAKDOWN OF DNA.

   IMMEDIATELY add 20 µl of Proteinase K (destroys Dnases that break down DNA), and 200 µl of buffer AL (lysis buffer to break open cells). Mix by vortexing for 10 sec or inverting 25 times.

   *(Do not pre-mix Proteinase K and Buffer AL, they must be added separately)*

4. Repeat steps 2-4 with the other four samples. Be sure to use a different pestle and pipet tips for each tube.
5. Incubate for at least 10 minutes at 70°C.
6. Add 200 µl of Ethanol (96-100%) to each tube. This will precipitate DNA from the extracted material.
7. Vortex.
*Optional stopping point. Store tubes at 4°C overnight.

Cellular Debris Removal

1. Collect four DNeasy spin columns fitted in four 2.0 ml collection tubes and label the lids of the spin columns 1-4 with your initials or designated label.
2. Pipet the liquid from tube 1 of the above steps (with or without exoskeleton) into the DNeasy Mini spin column #1. Using a new pipet tip for each transfer, repeat this process with the three other tubes. Make sure to keep tube numbers consistent.
3. Centrifuge for 1 minute at 6000g or 8,000 rpm. The DNA is now caught in the filter of the spin column. Discard the flow through waste into the 2.0 ml collection tubes in the waste bucket.
4. Place the spin column containing the DNA from tube 1 in the same emptied 2.0 ml collection tube.
5. Repeat for your other 3 tubes, remembering to label.
6. To each, add 500 μl of Buffer AW1. This is a wash buffer that washes the DNA.
7. Centrifuge for 1 minute at 6000 g or 8000 rpm.
8. Again, discard the flow through waste in the 2.0 ml collection tubes in the waste bucket and place the DNeasy Mini spin column from tube 1 into the same emptied 2 ml collection tube labeled “1”; repeat for your other 4 tubes.
9. Add 500 ul of Buffer AW2 (a second wash buffer) to each of your 5 tubes and centrifuge for 3 minutes at 20,000 g or 13,000 rpm (or max speed if your centrifuge doesn’t go that high)*. Discard flow-through and collection tubes. This step is also removing the ethanol.
10. Place your spin columns into 1.5 ml microcentrifuge tubess

   Again, be sure to label the lids of each tube 1-4. These will contain your purified DNA samples.

   *Note-if the centrifuge you are using cannot attain this speed, you can allow the tube to air dry for 5 minutes. This will evaporate the ethanol.

DNA Elution and Dilution

1. Pipet 100 μl of Buffer AE directly onto the membrane. This is an elution buffer that rinses the DNA off the spin column filter and into the 1.5 ml tube.
2. Incubate at room temperature for 1 minute.
3. Centrifuge (6000g or 8,000 rpm) for 1 minute to elute.
4. Discard the spin column and KEEP the labeled 1.5 ml tube. Store in 4°C fridge until PCR.
DNA Isolation Flow Chart

1. Add PBS and Macerate
2. Add Proteinase K and AL
3. Vortex and Incubate @ 70°C for 10 minutes
4. Add Ethanol (Stopping point here and store in 4°C)
5. Spin through a filter and discard flow-through
6. Wash with Buffer AW1
7. Spin and discard flow-through
8. Wash With Buffer AW2
9. Spin and discard flow-through and collection tube

Place spin column in **clean microtube**, add Elution Buffer, spin, and keep flow-through (DNA sample)
LAB-2: DNA EXTRACTION – Activity Sheet

Student: ___________________________________________ Date: ________________

BEFORE YOU BEGIN

Note the purpose of each reagent:

- Phosphate Buffered Saline (PBS): ______________________________
- Proteinase K: _____________________________________________
- Buffer AL: _______________________________________________
- Ethanol: _________________________________________________
- Buffer AW1: ______________________________________________
- Buffer AW2: ______________________________________________
- Buffer AE: _______________________________________________

1) **Hypothesis**: Based on the background information, formulate a hypothesis about the frequency of *Wolbachia* endosymbionts in your specimens:

________________________________________________________________________________
________________________________________________________________________________

2) What is the purpose of the positive and negative Nasonia controls? __________________________

________________________________________________________________________________
________________________________________________________________________________

3) Which two membranes must be ruptured to release DNA? _________________________________

________________________________________________________________________________

4) What are these membranes composed of? _____________________________________________

5) Which reagent will help rupture these membranes? _________________________________
Lab-2: DNA Extraction & Purification

Student Checklist

Student: __________________________________________________________ Date: ________________

Protocol Day 1 (Cell Lysis):  *Blot insect dry if stored in ethanol before placing in tube. Label tubes in the following order: Ant1(1), Ant2(2), Nas-(3), Nas+(4)*

_____ 1) Add 180 µL of PBS and specimen to each *labeled* microtubes. MACERATE. *Using sealed microtip, crush abdomen and release white hemolymph.*

_____ 2) ADD 20 µL of Proteinase K and quickly go to #3.

_____ 3) QUICKLY add 200 µL of AL buffer.

_____ 4) Vortex for 10 seconds or invert 25 times.

_____ 5) Incubate @ 70°C for 10 minutes.

_____ 6) Add 200 µL of Ethanol and vortex.

_____ 7) If you are out of time ......Store in frig @ 4°C until tomorrow or KEEP GOING!

Protocol Day 2 (Purification & Elution):  ADD REAGENTS in THIS order: Ants, Nas -, and Nas +

_____ 1) Label Spin Columns to match microtubes.

_____ 2) Pipet liquid, avoiding exoskeleton, from specimen tubes into DNeasy Mini spin columns. Make sure labels match during transfer.

_____ 3) Centrifuge: 1 minute @ 8,000 rpm. *DNA is caught in the filter.*

_____ 4) Discard waste into waste beaker and reuse collection tube.

_____ 5) Add 500 µL of AW1. *(Washes the DNA.)*

_____ 6) Centrifuge: 1 minute @ 8,000 rpm.

_____ 7) Discard waste into waste beaker and reuse collection tube.

_____ 8) Add 500 µL of AW2.

_____ 9) Centrifuge: 3 minutes @ 13,000 rpm. *(This helps remove ethanol. You may want to let sample air dry for 5 minutes after centrifugation if you can’t reach 13,000 rpm.)*

_____ 10) Throw away collection tube and replace with a clean 1.5 mL eppendorf tube.

DNA Elution

_____ 11) Pipet 100 µL of AE DIRECTLY onto filter membrane.

_____ 12) Set at room temperature for 1 minute.

_____ 13) Centrifuge 1 minute @ 8,000rpm.

_____ 14) Discard spin column and KEEP 1.5 ml microtube containing the purified DNA.

_____ 15) MAKE SURE IT IS LABELED!!! *If you are out of time...Store in fridge @ 4°C until PCR.
Lab-3: PCR Lab

**ACTIVITY AT A GLANCE**

**Goal:**

To screen for *Wolbachia* symbiont DNA in the extracted DNA from insects using one of the most widely used biotechnology techniques in biological research, the **Polymerase Chain Reaction** (PCR). PCR amplifies DNA millions of times in just a few hours, so that the DNA becomes easy to detect and study in any fashion.

**Learning Objectives:**

Upon completion of this activity, you will use and understand one of the most useful biotechnology tools in the life sciences (PCR), understand DNA as the hereditary basis of life, utilize DNA as a diagnostic tool to discover microbes, and seamlessly transition their discovery-based science from organisms to molecules during this lab. You will also **amplify** DNA extracted from two ants and three controls using Polymerase Chain Reaction (PCR). The piece of DNA used for identifying *Wolbachia* is the region that codes for a **small subunit** of the **ribosomal RNA**. We will refer to this piece as **16S rDNA**. The piece of DNA used for identifying the insect is the region that codes for the mitochondrial protein, cytochrome **c oxidase** I (we will refer to it as CO1).

**Teaching Time:** 1 class period (50 minutes); the thermal cycler will have to run for 3 hours.

**OVERVIEW**

Most DNA analysis situations require fairly large amounts of DNA. Usually the amount in a few cells is not enough to fully analyze. A method called the **polymerase chain reaction** (PCR) has been developed to make many copies of DNA in a sample. PCR is essentially the microscope of the 21st century as it allows biologists to study the DNA of microorganisms that we cannot see by either eye or culture. It is revolutionizing research in microbial diversity, genetic disease diagnosis, forensic medicine, and evolution. In this portion of the lab series, you will use your samples from the DNA Extraction Lab to determine if your ants are infected. Your work could be new to science and potentially lead to new discoveries on the presence and absence of *Wolbachia* in insects. Contact Michele Bahr (mbahr@mbl.edu) at the Marine Biological Laboratory for – and + *Nasonia* insect controls and/or + control DNA samples. **Primers** to specifically amplify a **438bp fragment of the 16S** ribosomal RNA gene (ubiquitous in all *Wolbachia*) are WSPEC-F (5’-CATACCTATTGCGAGGATAG-3’) and WSPEC-R (5’-AGCTCGAGGTGAAACCATTCT-3’). **Primers** to amplify a **658bp fragment of the CO1** cytochrome oxidase gene (*ubiquitous in arthropod mitochondria*) are LCO1490 (5’-GGTCAACAATCATAAAGATATTGG-3’) and HCO2198 (5’-TAAACTTCAAGGGTACCAAAATCTA-3’). If you teach a high school class, these primers can be provided by Michele Bahr. **Further, we offer a free thermal cycler loaner program, so contact us several weeks in advance to coordinate shipping (high schools only).**
Two separate PCR reactions are necessary if you plan to check for Wolbachia and Mono/Polygyne because the annealing temperatures for the primers are different. The band produced by Wolbachia (region coding for the small subunit of the ribosomal RNA; the 16S rDNA is 438 bp amplicon) is very close to the shorter band produced by polygyne (423 bp amplicon).

The second PCR reaction will amplify the Gp-9 alleles, the region of DNA associated with one queen colonies (Monogyne) and multi-queen colonies (Polygyne). There are two different alleles, Gp-9<sup>+</sup> and Gp-9<sup>−</sup>, which are different lengths. Monogyne colonies produce one band (517 bp amplicon). Polygyne colonies produce two bands (517 bp and 423 bp amplicon).

There are three steps in one PCR cycle:
1) Denature – hydrogen bonds are broken so DNA helix unzips; the highest temperature in the cycle.
2) Anneal – primers attach to target DNA; the coolest temperature of cycle.
3) Extension – Taq polymerase initiates extension of complement strand.

**MATERIALS**

- 2 DNA Samples from ants
- 2 DNA Samples from + and – *Nasonia* controls (from Woods Hole)
- + DNA *Wolbachia* control (from Woods Hole)
- Sharpie
- 6 PCR Ready Tubes
- 1 box of P200 pipet tips
- 1 box of P20 pipet tips
- P200 and P20 pipettes
- Gloves, 2 pair
- 1 rack for holding PCR tubes
- 1 tube of Wspec-F primer (5 micromolar, 20 µl)
- 1 tube of Wspec-R primer (5 micromolar, 20µl)
- 1 tube of CO1-F primer (5 micromolar, 20 µl)
- 1 tube of CO1-R primer (5 micromolar, 20 µl)
- 1 tube of d2H20 (200 µl)
- 1 waste cup for tips, tubes
- Saftey goggles
- If you teach a high school class, the primers, + and – Nasonia controls and + DNA control can be provided by Michele Bahr at the Marine Biological Laboratory (mbahr@mbl.edu).

**TEACHER PREP**

It is highly recommended to combine the proper amounts of primer for the class in one tube before lab to reduce the amount of pipetting with very small quantities.

The PCR protocol for Wol/Co1 and Mono/Polygyne are both included but you must do the Wol/Co1 protocol first to verify that your students were successful in their DNA extraction. If the extraction was not successful, do NOT use their DNA samples for further testing. It will waste the PCR Ready Tubes. Refer to the egel picture on the cover sheet. Notice that lanes 5 and 6 do not have any bands, indicating unsuccessful DNA extraction.
**1st PCR RXN - PREPARATION FOR WOLBACHIA ENDOSYMBIOT PRESENCE DETERMINATION:**
The thermal cycler should be programmed for the optimum settings below.

**1 cycle**
- 1 min. @ 94 C

**38 Cycles of PCR**
- 1 min. @ 94 C
- 1.5 min. @ 45 C
- 1 min. @ 72 C

**1 cycle**
- 5 min. @72 C
- **Store at 4°C**

---

**2nd PCR RXN - PREPARATION FOR MONOGYNE VS POLYGYNE DETERMINATION:**
For determining monogyn vs. polygyne colonies (Gp-9 amplification) the thermal cycler should be programmed for the optimum settings below.

**1 cycle**
- 2 min @ 94 C

**35 Cycles of PCR**
- 15 sec. @ 94 C
- 15 sec @ 55 C
- 30 sec @ 72 C

**1 cycle**
- 5 min. @72 C
- **Store at 4°C**
Lab-3: PCR Procedure – Wolbachia/CO1

Student Checklist

Student: ____________________________________________ Date: ____________________

1) Obtain a 5-strip PCR Ready tubes (contains preformulated, pellet of Taq polymerase, MgCl₂, Buffer, and dNTPs. Write your group number on tube.

2) Make sure you load tubes in the following order so you won’t have to label specimens: (HINGE POINTED AWAY FROM YOU).

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<tbody>
<tr>
<td>1) Ants</td>
<td>2) Ants</td>
<td>3) Nas -</td>
<td>4) Nas +</td>
<td>5) + Wol Control – purified sample of Wol DNA (procedural control)</td>
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</tbody>
</table>

Wolbachia & COI Primer Sets - Directions/group (Recommended for teacher to make total for class and aliquot total needed for each group.)

3) Each tube should have a total volume of 25 µL. Please check off each upon completion. VORTEX Primer Mix & DNA Template before obtaining the following volumes.

_____a) 23 µL Primer Mix/Group:

* 15 µL deionized water
* 2 µL Wspec-Forward
* 2 µL Wspec-Reverse
* 2 µL COI-F
* 2 µL COI-R

_____b) 2 µL of your specimen DNA template (THIS DNA MUST BE SAVED FOR FUTURE USE IN MONO/POLYGYNE PCR!)

*Cap and gently tap the bottom of each tube to mix the components. Place in thermal cycler. BIORAD 8 x 12 = 96

*Document the location of each group in thermal cycler diagram below.
Lab-4: Agarose Gel Electrophoresis Lab

ACTIVITY AT A GLANCE

Goal:

To determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product.

Learning Objectives:

Upon completion of this activity, you will have integrated scientific discovery, inquiry and biotechnology. You will also understand that DNA contains hereditary information in the form of genes, how DNA samples separate based upon different sizes, learn how to stain and visualize DNA samples. We will be using agarose gel electrophoresis to determine the presence and size of Wolbachia 16S rDNA amplified by our PCR.

Materials Needed / Group:

- Your 5 PCR products & rack
- P200 & P20 pipets & tips
- Gloves
- Sharpie
- 6x Loading Buffer
- DNA Ladder (if available)
- Agarose Powder
- Gel casting tray and combs
- QUIKView DNA stain (Ward’s 38V 9014)
- Staining trays
- Balance
- Weighing paper
- Spatula
- 500 ml flask
- 100 ml graduated cylinder
- Hotplate
- Oven mitt
- Gloves
OVERVIEW

Introduction:

Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is pulled through the pores of the gel by the electrical current. DNA has a negative charge because of the phosphates composing its backbone. Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose, and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

Goal:

To determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product.

Learning Objectives:

In this activity you will learn how DNA samples separate based upon different sizes and learn how to stain and visualize DNA samples. We will be using agarose gel electrophoresis to determine the presence and size of *Solenopsis Gp-9* alleles and, separately, *Wolbachia 16S rDNA* amplified by our PCR.
Lab-4: Agarose Gel Procedure – Wol/Co1

Student: ____________________________  Date: ________________

Preparing the gel

1. Measure 1.25 g Agarose powder and add it to a 500 ml flask.
2. Add 125 ml TAE Buffer to the flask. (1% solution; note the total gel volume well vary depending on the size of the casting tray)
3. Melt the agarose in a microwave or hot water bath until the solution becomes clear.
   (i) if using a microwave, heat the solution for several short intervals - 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.
6. Place the combs in the gel casting tray.
7. Pour the melted agarose solution into the casting tray and let cool until it is solid (it turns a light blue as it solidifies).
8. Carefully pull out the combs and remove the tape.

   Place the gel in the electrophoresis chamber.

Add enough TAE Buffer so that there is about 2-3 mm of buffer over the gel.

Loading the gel

1. Add 5 µl of 6X Loading Buffer to each 25 µl PCR reaction
2. Record the order each sample will be loaded on the gel, including who prepared the sample, the DNA template - what organism the DNA came from, controls and ladder.

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<th>DNA Template</th>
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3. Carefully pipette 20 μl of each sample/Sample Loading Buffer mixture into separate wells in the gel.
4. Pipette 10 μl of the DNA ladder standard (if available) into at least one well of each row on the gel.

**Running the gel**
1. Place the lid on the gel box, connecting the electrodes appropriately (positive (red) and negative (black))
2. Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber.
3. Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
4. Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes.
5. Let the power run until the blue dye approaches the end of the gel, then turn off the power, disconnect the electrodes, remove the lid and the gel using gloves.

**Gel Staining**
1. Place into the staining dish.
2. Add warmed (50-55°C) staining mix.
3. Allow gel to stain for at least 25-30 minutes (the entire gel will become dark blue).
4. Pour off the stain (the stain can be saved for future use).
5. Rinse the gel and staining tray with water to remove stain.
6. Fill the tray with warm tap water (50-55°C). Change the water several times as it turns blue. Gradually the gel will become lighter, leaving only dark blue DNA bands. Destain completely overnight for best results.
7. View the gel against a white light box or bright surface.
8. Record the data while the gel is fresh, very light bands may be difficult to see with time.
Lab-5: PCR Procedure – Mono/Polygyne

Student Checklist

(Solenopsis Gp-9 allele)

Student: __________________________________________ Date: ______________

1) Obtain a 2-strip PCR Ready tubes (contains preformulated, pellet of Taq polymerase, MgCl2, Buffer, and dNTPs. Write your group number on tube.

2) Make sure you load tubes in the following order so you won’t have to label specimens:
   (HINGE POINTED AWAY FROM YOU).

Mono/Polygyne Primer Sets - Directions/group (Recommended for teacher to make total for class and aliquot total needed for each group.)

3) Each tube should have a total volume of 25 µL. Please check off each upon completion. VORTEX Primer Mix & DNA Template before obtaining the following volumes.

   _____ a) 23 µL Primer Mix/Group:
       * 15 µL deionized water
       * 2 µL Primer 26BS
       * 2 µL Primer 16BAS
       * 2 µL Primer 24BS
       * 2 µL Primer 24bAS

   _____ b) 2 µL of your specimen DNA template

*Cap and gently tap the bottom of each tube to mix the components. Place in thermal cycler.

BIORAD 8 x 12 = 96

*Document the location of each group in thermal cycler diagram below.

PCR Protocol: Mono/Polygyne – 1 cycle(2min@94C)-35cycles(15sec@94C; 15sec@55C; 30sec@72C)-1cycle(5min@72C)-4°C for storage
Lab-6: Gel Procedure – Mono/Polygyne

Student Checklist

Name:__________________________________________________Date:________________

1) Follow the same procedure for making an agarose gel, loading and staining a gel as described in Lab 4.

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

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GLOSSARY of TERMS

Aliquot to dispense equal volumes of reagents
Amplicon target sequence of DNA to be amplified
Anneal second step of PCR cycle; primers attach to target DNA
Colony a group of organisms of the same kind living or growing in close association
Denature first step of PCR cycle; DNA helix unzips as hydrogen bonds are broken
dNTP (deoxynucleotide triphosphates) concerning DNA; guanine, cytosine, adenine, and thymine
Elongation third step in PCR cycle; Taq polymerase initiates formation of complement strand
Elute to remove substance absorbed to a surface using a solvent (removing DNA from spin column filter)
Endemic natural to or characteristic of a specific area or region; indigenous
Habitat the natural environment of an organism; place that is natural for the life and growth of an Organism
Hemolymph a circulatory fluid in certain invertebrates
Macerate breaking into pieces using a liquid
Monogyne a colony of ants with one queen
Negative Control specimen in experiment not infected; should not produce infected band on gel; purpose-to verify no contamination occurred during the experiment
Order the usual major subdivision of a class or subclass in the classification of organisms, consisting of several families
Pedicel the stem of the abdomen between the thorax and the gastra
Polygyne a colony of ants with many queens
Positive Control specimen in this experiment infected with Wolbachia; used to demonstrate correct band pattern on gel for infected insects
Primer short single strand of DNA (oligonucleotide) that will flank the DNA sequence of interest
Protocol the predefined written procedural method of an experiment
Species the major subdivision of a genus or subgenus, regarded as the basic category of biological classification, composed of related individuals that resemble one another, are able to breed among themselves, but are not able to breed with members of another species.
Terrestrial living on or in the ground; not aquatic, arboreal, or aerial
Un-Infested Area area not containing ants or ant mounds
1. Which is being used as a negative control in the Wolbachia experiment _____.
   A) Infected Wolbachia  B) Uninfected Wolbachia
   C) Infected Nasonia    D) Uninfected Nasonia (p.12)

2. Who won a Nobel Prize in 1993 for developing PCR _____.
   A) Kary Mullis (p. 5)  B) James Watson
   C) Seth Bordenstein    D) Rob Rockhold

3. Which part of DNA contributes to its charge? ____
   A) Nitrogen base   B) phosphates (p. 23)  C) sugar   D) uracil

4. Term for oligonucleotide used to amplify target segment of DNA? ____
   A) promoter         B) poly A tail
   C) primers (p. 5)   D) gene

5. Wolbachia is an example of a/an _____.
   A) virus           B) bacteria (p. 3)  C) protist    D) fungus

6. The DNA polymerase currently used in PCR was isolated from the bacterium _____.
   A) E. coli         B) Saccharomyces cerevisiae
   C) Thermus aquaticus (p. 5)      D) Drosophila melanogaster

7. Which step of PCR requires the highest temperature?
   A) denature (p. 5)  B) elongation  C) anneal  D) replication

8. Which one of the following destroys DNAses during DNA Extraction?
   A) PBS Buffer       B) ethanol  C) AW-2   D) Proteinase K (p. 9)

9. Which substance will cause DNA to precipitate?
   A) PBS Buffer       B) ethanol (p. 9)  C) water   D) Proteinase K

10. _____ colonies have several queens.
    A) Anodyne  B) Pseudogyne   C) Polygyne(p. 3)   D) Monogyne
Name _______________________________ Date ________________

Pre/Post Test - Wolbachia
Circle the correct choice.

1. **Which is being used as a negative control in the Wolbachia experiment _____**.
   A) Infected *Wolbachia*  B) Uninfected *Wolbachia*
   C) Infected *Nasonia*  D) Uninfected Nasonia

2. **Who won a Nobel Prize in 1993 for developing PCR _____**.
   A) Kary Mullis  B) James Watson
   C) Seth Bordenstein  D) Rob Rockhold

3. **Which part of DNA contributes to its charge? _____**
   A) Nitrogen base  B) phosphates  C) sugar  D) uracil

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   A) *E. coli*  B) *Saccharomyces cerevisiae*
   C) *Thermus aquaticus*  D) *Drosophila melanogaster*

7. **Which step of PCR requires the highest temperature?**
   A) denature  B) elongation  C) anneal  D) replication

8. **Which one of the following destroys DNAses during DNA Extraction?**
   A) PBS Buffer  B) ethanol  C) AW-2  D) Proteinase K

9. **Which substance will cause DNA to precipitate?**
   A) PBS Buffer  B) ethanol  C) water  D) Proteinase K

10. _____ colonies have several queens.
    A) Anodyne  B) Pseudogyne  C) Polygyne  D) Monogyne
Standards which are in Alignment with the Study

Alignment to the Standards

National Science Education Standards:
- Unifying Concepts and Processes in Science
- Systems, order and organization
- Evidence, models, and explanation
- Change, constancy and measurement
- Evolution and equilibrium
- Science as Inquiry
- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry
- Life Science
- Biological Evolution
- Interdependence of organisms
- Matter, energy and organization in living systems
- Science and Technology
- Understandings about science and technology
- Science in Personal and Social Perspectives
- Science and technology in local, national, global challenges
- History and Nature of Science
- Science as a human endeavor
- Nature of scientific knowledge

MS Biology II Science Framework Aligned with “A Muse of Fire: Wolbachia”

2010 Proposed Mississippi Science Framework for Biology II

1. Apply inquiry-based and problem-solving processes and skills to scientific investigations.
   a. Use current technologies such as CD-ROM, DVD, Internet, and on-line data search to explore current research related to a specific topic. (DOK 3)
   b. Clarify research questions and design laboratory investigations. (DOK 3)
   c. Demonstrate the use of scientific inquiry and methods to formulate, conduct, and evaluate laboratory investigations (e.g., hypotheses, experimental design, observations, data analyses, interpretations, theory development). (DOK 3)
   e. Evaluate procedures, data, and conclusions to critique the scientific validity of research. (DOK 3)
   f. Formulate and revise scientific explanations and models using logic and evidence (data analysis). (DOK 3)
g. Collect, analyze, and draw conclusions from data to create a formal presentation using available technology (e.g., computers, calculators, SmartBoard, CBL’s, etc.) (DOK 3)

2. **Describe and contrast the structures, functions, and chemical processes of the cell.**
   
   c. Analyze and describe the function of enzymes in biochemical reactions.
   
   - The impact of enzymatic reactions on biochemical processes
   - Factors that affect enzyme function (e.g., pH, concentration, temperature, etc.) (DOK 2)

3. **Investigate and discuss the molecular basis of heredity.**

   d. Assess the potential implications of DNA technology with respect to its impact on society. (DOK 3)
   
   - Modern DNA technologies (e.g., polymerase chain reaction (PCR), gene splicing, gel electrophoresis, transformation, recombinant DNA) in agriculture, medicine and forensics

   e. Develop a logical argument defending or refuting bioethical issues arising from applications of genetic technology (e.g., the human genome project, cloning, gene therapy, stem cell research). (DOK 3)