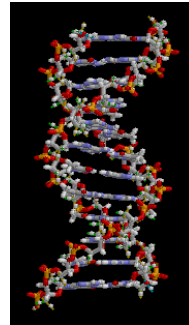
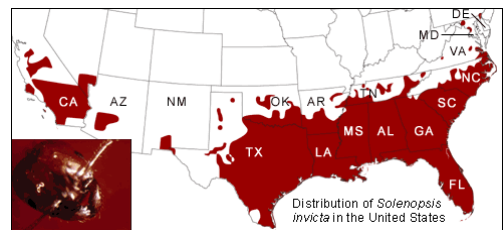


# Explorations in Fire Ant Molecular Biology: Colony Form and *Wolbachia* Endosymbiosis

*Real-World Biotechnology.*



*Real-World Science.*



*Real-World Importance.*



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## Background

**Real-world biotechnology. Real-world science. Real-world importance.** This series of laboratory explorations gives you all three. In it, you will conduct experiments that are designed to answer questions about a medically important organism, the red imported fire ant (RIFA) *Solenopsis invicta*, and a medically and agriculturally important bacterium, *Wolbachia*, that is an endosymbiont of the RIFA.

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

*Why medically important?* OUCH is the easiest reason. Those of us who have lived in the southeastern United States understand the “ouch”; those who do not, should be grateful they do not understand. A subset of those of us who have felt the sting of the RIFA have developed allergies to the sting; and some of us have the potential for severe, life-threatening allergic reactions, even from a single sting. So the RIFA isn’t just an annoying pest that can inflict pain on the unlucky person who steps onto its bed, or the weakened small animal that falls near its nest. It is an organism of true medical significance. *Wolbachia*, a bacterium that inhabits the abdomens of numerous insects and which can affect these populations, is also medically relevant. It turns out that it also inhabits filarial nematode parasites, which cause lymphatic elephantiasis and river blindness, as well as many other diseases; but in this case it is required for the parasites to survive in the host. Kill the *Wolbachia*, and you kill the host.

*Why biotechnology?* 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. The applications are far too numerous to begin to list. 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. In a bioinformatics module, you will be introduced to how the information obtained using these and other techniques can be combined with data from investigators across the globe and used to gain understandings not previously possible.

*Why real-world science?* The data you generate in these explorations will be entered into a world-wide database to help understand the distribution of *Wolbachia* in insects across the world. Put simply, the world is too large for a small subset of scientists to do this alone. The help of students and teachers across the globe is needed to have a true global understanding of where *Wolbachia* is, what its frequency in various insects is, if there are differences in the *Wolbachia* found in RIFA and those found in filarial nematodes; the list of questions is unending. These are not simply exercises to teach you techniques, although you will learn molecular techniques; these are real experiments with real-world importance and applications. 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*.

*The nitty-gritty details:* Put simply, 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 ([discover.mbl.edu](http://discover.mbl.edu)).

*Cooperative Investigations with Discover the Microbes Within! The Wolbachia Project,*  
A Howard Hughes Medical Institute Precollege Science Education Initiative

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 four core goals of this initiative are:

- (1) Engage high school students in nature and real-world research
- (2) Encourage nationwide participation in the collection and of new scientific data on bacterial endosymbionts (*Wolbachia*)
- (3) Enhance student interest in science through an integrative lab series spanning biodiversity to molecular biology

(4) Show students what it is like to be a scientist.

As part of the HHMI Precollege Science Initiative, *A Muse of Fire*, the projects developed here were generated in collaboration with Discover the Microbes Within: The *Wolbachia* Project. These projects are both part of and a specific expansion of Discover the Microbes Within: The *Wolbachia* Project, aimed at examining *Wolbachia* occurrence in the RIFA.

### **Objectives**

While not all high school students will enter fields that involve biotechnology, all will encounter (and do encounter) biotechnology in their daily lives. The most basic objective of this project is to improve student understanding of biotechnology by engaging students in active, application-oriented research. In short, in real research. Beyond learning techniques, students will learn applications of these techniques. While the specific system is the RIFA, DNA is, after all, DNA. It doesn't matter if it comes from a RIFA, its endosymbiont *Wolbachia*, a human (such as a high school student), a plant, a fungus, or the remains of a woolly mammoth: it all behaves the same. The techniques used here to study RIFA and *Wolbachia* are the same used in research labs and medical testing labs across the world. Another objective is to help students understand experimental design: how different molecular techniques are used in succession to arrive at an answer to a question—to test a hypothesis. A single technique alone is rarely useful; a well-executed series of experiments provides great information. Finally, a higher-level objective is to engage students in real scientific research that helps them improve their problem-solving skills—to think, to analyze, to interpret. Some students may wish to develop projects of their own based on what they have learned in these modules; they may wish to expand their work, develop hypotheses, ask more questions. Because the techniques are universal, and insects plentiful, the variety of questions which can be pursued are significant.

## DNA EXTRACTION LAB

### ACTIVITY AT A GLANCE



#### Goal:

To introduce you to DNA extraction techniques and to isolate genomic DNA from insects and *Wolbachia*, the endosymbiotic bacteria that live within the cells of over 20% of insect species.

#### Learning Objectives:

Upon completion of this activity, you will transition from fieldwork and morphological classification (Lab 1) to molecular biology (Lab 2) and biotechnology (Lab 3). You will also learn about DNA as a diagnostic tool to discover unseen microbes, increase abilities in biotechnology, and understand the process of inquiry and discovery-based research. In addition, you will also *isolate* total genomic DNA from morphospecies identified in the Insect Identification Lab.

#### Prerequisite Skills:

- Prior practice with micropipettors.

### OVERVIEW



In this activity, you will extract total genomic DNA from each of your three morphospecies using Qiagen's DNeasy Tissue Culture Kit. Total genomic DNA includes DNA of the insect host as well as any symbiotic bacteria, if present. In addition to the 3 unknown morphospecies, you will also prepare positive and negative controls using *Nasonia vitripennis* wasps that are infected and uninfected with *Wolbachia pipientis*, respectively.

The extraction of total genomic DNA involves three distinct steps:

1. *Cell Lysis:*



2. *Elimination of Cellular Debris:*

This will be followed by two washes steps with two buffers (AW1 and AW2)



3. *DNA Elution:*



## MATERIALS

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- |   |   |
|---|---|
| <ul style="list-style-type: none"><li><input type="checkbox"/> Incubator, heat block, or water bath set @ 70° C</li><li><input type="checkbox"/> Vortexer</li><li><input type="checkbox"/> Centrifuge</li><li><input type="checkbox"/> Student morphospecies</li><li><input type="checkbox"/> + and – <i>Nasonia</i> controls</li><li><input type="checkbox"/> Microtube Pestles (USA Scientific 1415-5390)</li><li><input type="checkbox"/> Qiagen DNeasy Kit (#69504)</li><li><input type="checkbox"/> P200 pipets</li><li><input type="checkbox"/> P200 pipet tips</li><li><input type="checkbox"/> Float racks for water bath</li></ul> | <ul style="list-style-type: none"><li><input type="checkbox"/> Waste cups for tips, etc.</li><li><input type="checkbox"/> Gloves</li><li><input type="checkbox"/> 1X Phosphate Buffer Saline (10X PBS from Fisher BP399-500 and dilute to 1X)</li><li><input type="checkbox"/> Sharpies</li><li><input type="checkbox"/> Tweezers</li><li><input type="checkbox"/> Kimwipes</li><li><input type="checkbox"/> Ethanol (95-100%)</li><li><input type="checkbox"/> Tube Racks (USA Scientific 2396-5048)</li><li><input type="checkbox"/> 1.5ml microcentrifuge tubes (USA Scientific 1415-9199)</li></ul> |
|---|---|

## ACTIVITY PROCEDURE

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Review the activity flow-chart (page 9) to familiarize yourself with the procedures before the lab is to take place. Also, revisit your hypothesis to have a better understanding of what it is you are looking for. You will work with your same partners from Lab 1 and follow the protocol outlined on the following sheet. Before you begin, take a moment to become familiar with each of the reagents involved in this experiment (this will come in handy during the procedures).

- Phosphate Buffered Saline (PBS): a salty solution of constant pH to keep tissues, cells, and proteins intact during maceration
- Proteinase K: an enzyme that catalyzes the breakdown of cellular proteins (including DNase) by splitting them into smaller peptides and amino acids
- Buffer AL: a cell lysis solution that breaks open cell and nuclear membranes
- Ethanol: used to precipitate DNA from the extracted material
- Buffer AW1 and AW2: solutions that wash the DNA attached in the column membrane of contaminants
- Buffer AE: a solution that elutes the DNA from the membrane and allows stable storage of DNA for years in the refrigerator or freezer

## DNA Extraction Lab

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

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### VOCABULARY

|                         |  |
|-------------------------|--|
| Cell Lysis              |  |
| DNA Purification        |  |
| Macerating              |  |
| DNA Elution             |  |
| Hydrolytic Enzymes      |  |
| Monogyne                |  |
| Polygyne                |  |
| Morphospecies           |  |
| Nasonia Controls        |  |
| Pestles                 |  |
| Wolbachia Endosymbionts |  |

### PRE LAB QUESTIONS

1. What is the purpose of a positive and negative control?

---

2. What is the percentage of insect species that have *Wolbachia*?

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3. What is the difference between monogyne and polygyne colonies?

---

4. What are the cellular structures and elements that serve as a barrier to getting your DNA out of the cell and intact?

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5. Research scientists who are trying to determine the identification of a new flu virus would perform a DNA extraction from human tissues. Will the method used to isolate DNA be similar or different from the method you used to isolate insect and *Wolbachia* DNA?

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**PROCEDURE**

*Preparation*

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

| Tube # | Contents (Voucher #)     |
|--------|--------------------------|
| 1      |                          |
| 2      |                          |
| 3      | - <i>Nasonia</i> control |
| 4      | + <i>Nasonia</i> control |

2. Collect five 1.5 ml microcentrifuge tubes.

3. Using a Sharpie marker, number them 1-5 along with your initials

**DNA Isolation Flow Chart**



↓  
Add 180  $\mu$ L of PBS and Ant Specimen

↓  
Macerate

↓  
**\*Do this step quickly**

↓  
Add 20  $\mu$ L of Proteinase K and 200  $\mu$ L of AL buffer

↓  
Vortex and Incubate at 70 °C for 10 minutes

↓  
Add 200  $\mu$ L of Ethanol and vortex

Transfer contents from tube to the DNeasy spin columns



Centrifuge for 1 minute at 8,000 rpm



Discard the waste into a waste container



Add 500  $\mu$ L of AW1 to each tube



Centrifuge for 1 minute at 8,000 rpm



Discard the waste into a waste container



Add 500  $\mu$ L of AW2 to each tube



Centrifuge for 3 minutes at 13,000 rpm



Discard the waste into a waste container



Transfer spin column into a 1.5 mL tube



Add 100  $\mu$ L of AE Buffer to each tube



Let incubate at room temperature for 1 minute



Centrifuge for 1 minute at 8,000 rpm



Discard spin column and KEEP the tube



Explorations in Fire Ant Molecular Biology: Colony Form and *Wolbachia* Endosymbiosis

Store in freezer until you are ready to do PCR

## PCR LAB

### ACTIVITY AT A GLANCE

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**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 three morphospecies 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.

**Prerequisite Skills:** Prior practice with micropipettors.

### OVERVIEW

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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 21<sup>st</sup> 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 decipher if *Wolbachia* symbionts are present within your morphospecies.

Your work could be new to science and potentially lead to new discoveries on the presence and absence of *Wolbachia* in insects. As in the previous lab, you should work in groups of two. Primers to specifically amplify a 438bp fragment of the 16S ribosomal RNA gene (ubiquitous in all *Wolbachia*) are WSPEC-F (5'-CATACCTATTCGAAGGGATAG-3') and WSPEC-R (5'-AGCTTCGAGTGAAACCAATTC-3'). Review the basic principles of PCR before class and remember to revisit your hypothesis from the DNA Extraction Lab to know what you are looking for.

## PCR Lab



*Hypothesis:* Based on extracted DNA from your sets of morphospecies and the estimated global frequency of *Wolbachia pipientis* endosymbionts (20%), formulate a hypothesis for your own specimens.

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### MATERIALS

- 2 DNA Samples from RIFA
- 2 DNA Samples from + and –  
*Nasonia* controls
- + DNA control for Mono/Polygyne  
Determination
- + DNA control for *Wolbachia*
- Sharpie
- 11 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 master mix for allele 1 GP-  
3 (mono/polygyne amplification) (5  
uM of each primer + purified water,  
25ul total)
- 1 tube of master mix for allele 2 GP-  
3 (mono/polygyne amplification) (5  
uM of each allele + purified water,  
25ul total)
- 1 tube of master mix for *Wolbachia*  
DNA amplification (5 uM of each  
allele + purified water, 25ul total)
- 1 waste cup for tips, tubes
- Safety goggles

**INTRODUCTION**

In this activity, you will learn what Polymerase Chain Reaction (PCR) does, how it works, and why it is useful to research in the biological sciences. You will use PCR to make many copies of *Wolbachia* DNA (if present) from the extracted DNA of the three morphospecies and controls. The piece of DNA used for identifying *Wolbachia* is a region that codes for a small subunit of the ribosomal RNA (16S rRNA) that is unique to *Wolbachia*.

**VOCABULARY**

|                   |  |
|-------------------|--|
| PCR               |  |
| DNA Amplification |  |
| Microbes          |  |
| Morphospecies     |  |
| Ribosomal RNA     |  |
| Primers           |  |
| Thermacycler      |  |
| Denature          |  |
| Taq polymerase    |  |
| MgCl <sub>2</sub> |  |
| Buffer            |  |
| dNTPs             |  |

**PRE LAB QUESTIONS**

1. What is the purpose of heating up the DNA?

---

2. What is the purpose of cooling down the DNA?

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3. What is the role of the primers?

---

**PREPARATION**

1. The thermalcycler should be programmed for the optimum settings below.

1 cycle  
2 min @ 95 C



38 Cycles  
30 sec. @ 94 C  
45 sec @ 55 C  
90 sec @ 72 C



1 cycle  
10 min. @ 72 C

1 cycle  
Hold @ 4 C

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

2. Collect 11 PCR Ready tubes. Each of these already contains a preformulated, pellet of “master mix”. This material contains **Taq polymerase, MgCl<sub>2</sub>, Buffer, and dNTPs.**

3. Label each tube with the appropriate number and your initials.

| Tube # | Tube Contents     |
|--------|-------------------|
| 1a     | Ant DNA 1         |
| 2a     | Ant DNA 2         |
| C1a    | + control ant DNA |

4. Add 23 ul master mix for GP-3 allele 1 to each tube.

5. Add 2 ul of appropriate DNA to each tube (be sure to change tips each time!)

6. Repeat with another set of 3 PCR Ready tubes, labeled as follows:

| Tube # | Tube Contents     |
|--------|-------------------|
| 1b     | Ant DNA 1         |
| 2b     | Ant DNA 2         |
| C1b    | + control ant DNA |

7. Add 23 ul master mix for GP-3 allele 1 to each tube.
8. Add 2 ul of appropriate DNA to each tube (be sure to change tips each time!)
9. With the remaining 5 PCR Ready tubes, label as follows:

| Tube # | Tube Contents        |
|--------|----------------------|
| 1      | Ant DNA 1            |
| 2      | Ant DNA 2            |
| 3      | - control            |
| 4      | + control            |
| 5      | <i>Wolbachia</i> DNA |

10. Add 23  $\mu$ L master mix for GP-3 allele 1 to each tube.
11. Add 2  $\mu$ L of appropriate DNA to each tube (be sure to change tips each time!)
12. Place your six tubes with labels (initials and number) into the thermalcycler.  
**\*Once everyone has prepared their samples, the thermalcycler can be turned on.**
13. When the reaction is complete, store PCR reactions in the freezer (-20C).

## PCR Flow Chart



1

2

3

4

5



Add 23  $\mu$ L of master mix to each tube



Add 2  $\mu$ L of DNA template from each sample to its correlating tube.

**\*Be sure to change the pipette tips for each DNA template!**



Cap and gently tap the bottom of each tube to mix the components.



Place your six tubes with labels (initials and number) into the thermalcycler.

**\*Once everyone has prepared their samples, the thermalcycler can be turned on.**



Clean up your lab station.



When the thermalcycler is done (~3hrs), store each sample in the  $-20^{\circ}\text{C}$  freezer

# Agarose Gel Electrophoresis Lab



## ACTIVITY AT A GLANCE

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### 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 (per group of two students)

- ❑ Your 11 PCR products
- ❑ 4 Gloves
- ❑ 1 sharpie
- ❑ 1 box of P200 pipet tips
- ❑ 1 box of P20 pipet tips
- ❑ P200 and P20 pipets
- ❑ 1 rack for holding PCR tubes
- ❑ 6X Loading Buffer (Fisher TAK-9156)
- ❑ DNA ladder (Fisher PR-G3161)
- ❑ Safety goggles
- ❑ 1 e-gel electrophoresis apparatus
- ❑ 1 SYBR safe e-gel
- ❑ 1 power supply
- ❑ 1 transilluminator
- ❑ 1 gel imaging hood with camera



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

### **VOCABULARY**

|                 |  |
|-----------------|--|
| Agarose         |  |
| Agarose Gel     |  |
| Electrophoresis |  |
| Electrode       |  |

### **PRE LAB QUESTIONS**

1. What type of charge does DNA have?

---

2. Which will move faster through the agarose gel: small or large DNA?

---

3. How will you view the DNA on the gel?

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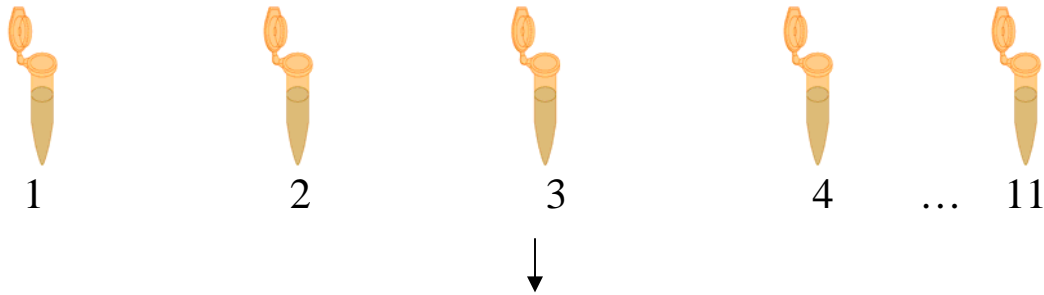
4. What is the purpose of the Loading Dye?

---

**PREPARATION**

1. Retrieve your PCR samples that you stored overnight in the freezer
2. Let samples thaw before you begin the procedure
3. Once thawed, Pipet 3  $\mu$ l of 6X Loading Dye into your PCR reaction tubes
4. Then mix well
5. Follow the flowchart to execute the proper procedure

## Agarose Gel Electrophoresis Flow Chart

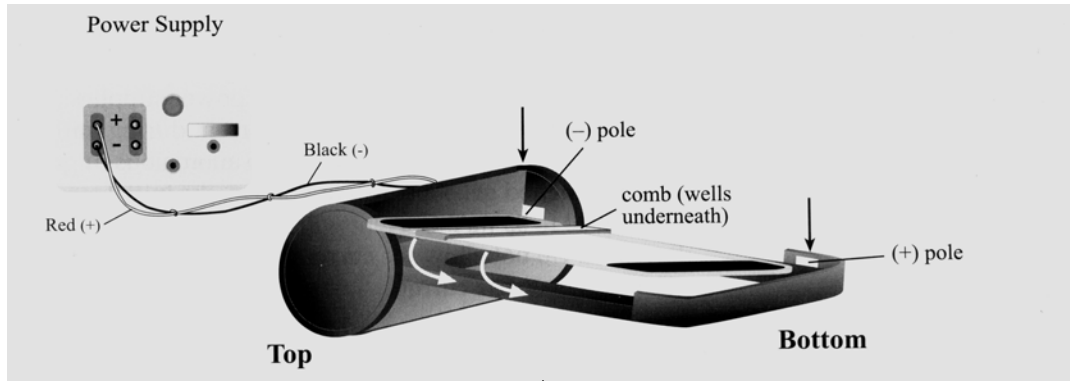


Record the order each sample will be loaded on the gel, including who prepared the sample, the DNA template - what organism the DNA came from, controls and ladder.

| <i>Lane #</i> | <i>Prepared by</i> | <i>DNA Template</i> | <i>Notes</i> |
|---------------|--------------------|---------------------|--------------|
| 1             |                    | DNA Ladder          |              |
| 2             |                    |                     |              |
| 3             |                    |                     |              |
| 4             |                    |                     |              |
| 5             |                    |                     |              |
| 6             |                    |                     |              |
| 7             |                    |                     |              |
| 8             |                    |                     |              |
| 9             |                    |                     |              |
| 10            |                    |                     |              |
| 11            |                    |                     |              |
| 12            |                    |                     |              |

Carefully open the e-gel packet (with scissors)

Insert the gel (with the comb in place) into the apparatus right edge first. Press firmly at the top and bottom to seat the gel in the base (you should hear a snap when it is in place). The Invitrogen logo should be located at the bottom of the base. See the diagram below.



Connect the power supply appropriately

Pre-run the gel (with the comb in place) for 1-2 minutes at 60-70V (or 40-50 mA). Do not exceed 2 minutes.

Turn off the power supply. Remove the comb by using both hands to gently lift the comb by rolling the comb slowly towards you.

Carefully pipette 20  $\mu$ l of each sample/Sample Loading Buffer mixture into separate wells, starting with lane #2 in the gel. You can store the remaining solution in the freezer.

Pipette 10  $\mu$ l of the DNA ladder standard into lane #1 on the gel.

Run the gel at 60 to 70 volts for approximately 30 – 40 minutes. Do not run longer than 60 minutes. Let the power run until the buzzer sounds, then turn off the power

Remove the gel and visualize on the transilluminator

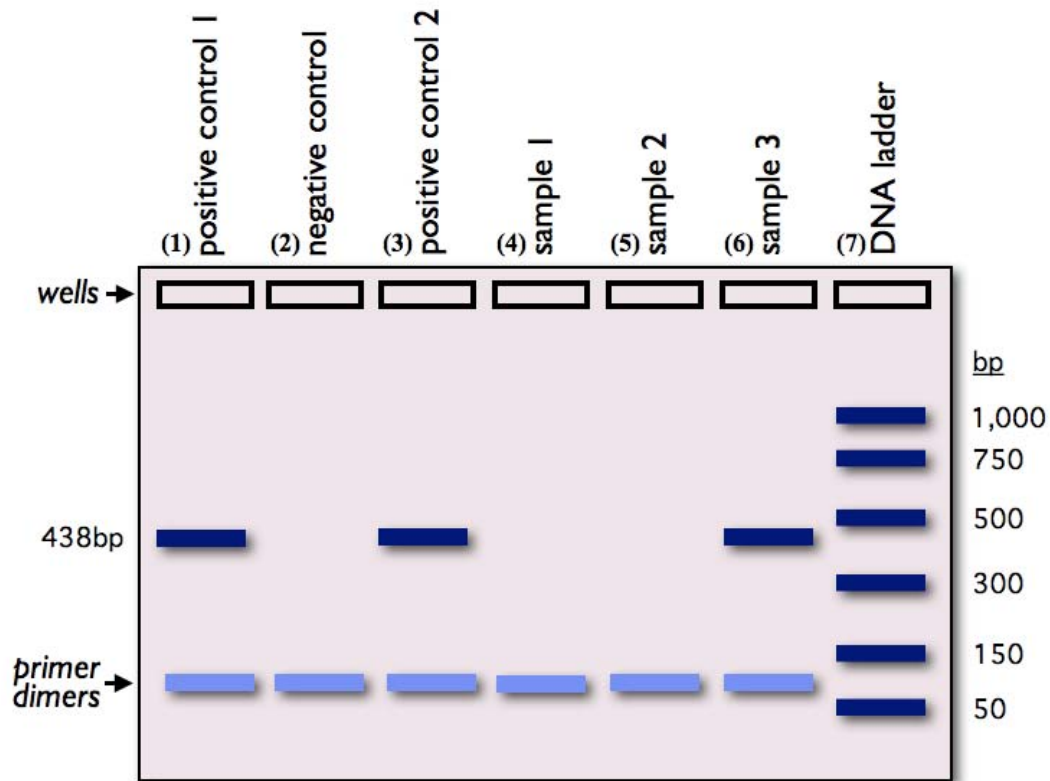
Take a photographic image of your gel for analysis

Determine if your ant DNA is polygyne or mongyne and if *Wolbachia* is present

Record this on the chart below

Dispose of the gel in the garbage and clean up your lab station

| <i>Lane #</i> | <i>DNA Template</i> | <i>Polygyne/Monogyne (P or M)</i> | <i>Wolbachia (+ or -)</i> |
|---------------|---------------------|-----------------------------------|---------------------------|
| 1             | DNA Ladder          | n/a                               | n/a                       |
| 2             |                     |                                   |                           |
| 3             |                     |                                   |                           |
| 4             |                     |                                   |                           |
| 5             |                     |                                   |                           |
| 6             |                     |                                   |                           |
| 7             |                     |                                   |                           |
| 8             |                     |                                   |                           |
| 9             |                     |                                   |                           |
| 10            |                     |                                   |                           |
| 11            |                     |                                   |                           |
| 12            |                     |                                   |                           |

**Sample Gel**

**Please answer the following questions about the sample gel:**

- What is the purpose of the positive and negative controls (lanes 1-3) in this experiment?
- What is the function of the DNA ladder?
- What does the band in lane #6 indicate?
- What are primer dimers? and what do they say about the presence or absence of *Wolbachia* in a sample?

## Bioinformatics Lab

*This lab activity is taken without changes from discover.mbl.edu, the Discover the Microbes Within! The Wolbachia Project website.*

### ACTIVITY AT A GLANCE

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*"Understanding nature's mute but elegant language of living cells is the quest of modern molecular biology. From an alphabet of only four letters representing the chemical subunits of DNA, emerges a syntax of life processes whose most complex expression is man....The challenge is in finding new approaches to deal with the volume and complexity of data, and in providing researchers with better access to analysis and computing tools in order to advance understanding of our genetic legacy and its role in health and disease."*

From the National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>

#### Goal:

- Module 1: To show the ways in which the NCBI online database classifies and organizes information on DNA sequences, evolutionary relationships, and scientific publications.
- Module 2: To identify an unknown nucleotide sequence from an insect endosymbiont by using the NCBI search tool BLAST

#### Teaching Time:

45 minutes

#### Introduction:

This exercise represents two interrelated modules designed to introduce the student to modern biological techniques in the area of Bioinformatics. Bioinformatics is the application of computer technology to the management of biological information. The need for Bioinformatics has arisen from the recent explosion of publicly available genomic information, such as that resulting from the Human Genome Project. To address this, the [National Center for Biotechnology Information \(NCBI\)](http://www.ncbi.nlm.nih.gov/) was established in 1988 as a national resource for molecular biology information. The NCBI creates public-access databases, develops software tools for analyzing genome data, and disseminates biomedical information - all for the better understanding of molecular processes affecting human health and disease. The NCBI is a virtual goldmine both in terms of available resources, and treasures yet to be discovered. We will investigate the GenBank DNA sequence database, which is responsible for organizing millions of nucleotide sequence records.

**Online Resources:** There are a number of online, educational resources devoted to learning bioinformatics. For details that summarize what we will cover in this exercise and more, see:

- BLAST for beginners (Helps the learner with a slide show; we will use this one!):  
<http://www.geospiza.com/outreach/BLAST/index.html>
- Similarity search (Summarizes the basic concepts and vocabulary of BLAST)  
<http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/similarity.html>
- NCBI Education (Provides educational tutorials, software, and mini-courses):  
<http://www.ncbi.nlm.nih.gov/Education/index.html>

**Significance and Supplies Needed:** By completing this project, you will be exposed to the tools and databases currently used by researchers in molecular and evolutionary biology, and you will gain a better understanding of gene analysis, taxonomy, and evolution. While no computer programming skills are necessary to complete the modules in this work, prior exposure to personal computers and the Internet will be assumed. The main program that you will need is an Internet browser, such as Netscape Navigator or Internet Explorer.

Student Activity Sheet Name: \_\_\_\_\_

**Bioinformatics Lab****MODULE 1: Sequence Taxonomy**

**Objective:** The goal of this module is to introduce you to the number and diversity of nucleotide sequences in the NCBI database.

Begin by linking to the NCBI homepage ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). If you ever get lost, always return to this page as a starting point. Select '**TaxBrowser**' at the top right. The NCBI Taxonomy database contains the names of those organisms whose sequences have been deposited. Only a small fraction of the millions of species estimated to exist on earth is represented! Select the option '**Taxonomy Statistics**' in the middle of the left-side navigation bar.

1. For the 'all dates' column, how many Bacterial Species were in the sequence database? \_\_\_\_\_
2. For the year 1999, how many new Bacterial Species were added to the sequence database? \_\_\_\_\_ Wow, what a difference a few years makes!

Interestingly, the sequence data from extinct organisms are even listed in the GenBank database. Let's look for a gene sequence from a 120 Mya old insect preserved in amber! From your last website,

- Select the '**Taxonomy**' option in the right of the top menu bar
- Select '**Taxonomy home**' in the left-hand navigation menu
- Select '**Extinct organisms**' in the bottom of the left-hand navigation menu to see the organism list
- Scroll down to Insects on the main page and select '**Libanorhinus succinus (a beetle from Lebanese amber 120-135 Mya)**'.
- This page gives you very specific information about the ancestry of this organism. Select the option '**Arthropoda**'.

3. What are some other organisms that belong to this phylum of animals? \_\_\_\_\_

Can you think of any body traits that these organisms have in common? \_\_\_\_\_

4. Go back one page. How many 'Nucleotide' sequences have been deposited into the Entrez Records from this organism? \_\_\_\_\_

5. What is the name of the gene that was sequenced for this organism (to find out, click on the number 1 next to nucleotide)?\_\_\_\_\_
6. How many nucleotide base pairs does this DNA entry contain? (the answer is in the first line of the flatfile after you select the Identification link)\_\_\_\_\_

Scroll through the complete reference report on this sequence. A lot of information may seem confusing, but it is all there to provide scientists with as much information as possible about this sequence. At the bottom of the screen, you will find the nucleotide sequence (all of the A,T,G,C base pairs) of this gene. Click on the **PUBMED** '8505978' to directly link to the title, authors, and abstract of the published paper! Amazing, now you can read the research article that discovered this nucleotide sequence.

7. Select the '**NCBI**' link in the top left corner of the screen (next to the DNA symbol) to return to the NCBI home page. Great! That's where we started with Module 1.

## Bioinformatics Lab

### MODULE 2: Sequence Searching and BLAST

**Objective:** The goal of this module is to retrieve genetic sequence data from the NCBI database that identifies the '*Wolbachia* sequence' you generated. The Basic Local Alignment Search Tool (BLAST) is an essential tool for comparing a DNA or protein sequence to other sequences in various organisms. Two of the most common uses are to a) determine the identity of a particular sequence and b) identify closely related organisms that also contain this particular DNA sequence.

**A slide show introduction (optional):** Begin by linking to a BLAST for beginners slide show that is simple and easy to follow

(<http://www.geospiza.com/outreach/BLAST/index.html>). Let the slide show guide your learning by clicking on the bright green arrow to proceed through the pages. Note that this slideshow is not updated and based on the old BLAST format. It is meant to give a general feel for using BLAST and it is not necessary to complete the whole slide show.

**Using BLAST to identify a fake sequence and your '*Wolbachia* Sequence':** Begin by linking to the NCBI homepage ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Select '**BLAST**' in top menu bar. With your new knowledge of Sequence Searching and BLAST, let's begin with a sequence you make up and then your *Wolbachia* sequence.

- Select '**nucleotide BLAST**' under the Basic BLAST category
- **Input your own nucleotides (A,T,G,C) that fill one complete line** into the Query Sequence Box. This is referred to as the query sequence.
- **VERY IMPORTANT - Click on the circle** for 'Others (nr etc.)' under Choose Search Set
- Select '**BLAST!**' at end of page. A new window appears.
- Wait for the results page to automatically launch. The wait time depends on the type of search you are doing and how many other researchers are using the NCBI website at the same time you are!

1. Did your sequence produce a significant match (also known as 'hit' to another sequence in the genetic database (a significant hit is an E value below E-10)\_\_\_\_\_ If yes, how many\_\_\_\_\_
2. How many sequences did it search in the database?\_\_\_\_\_
3. How many nucleotide letters did it search in the database?\_\_\_\_\_

- Select **Home** at the top of the BLAST page.
- Select '**nucleotide BLAST**' under the Basic BLAST category
- **Enter your *Wolbachia* sequence below into the Search box.** (At this point in the lab, if students generated their own *Wolbachia* sequences, they

could BLAST their own sequence. Here everyone will BLAST the same sequence provided to you below)

>Your *Wolbachia* Sequence (either use the new *Wolbachia* sequences from the insects that the class discovered infections in, provided by the Bordenstein Lab, or use the one provided online (shown below) so you can copy and paste it in the box. <http://serc.carleton.edu/microbelife/k12/bioinformatics/module2.html>

```
GTTGCAGCAATGGTAGACTCAACGGTAGCAATAACTGCAGGACCTAG
AGGAAAAACAGTAGGGATTAATAAGCCCTATGGAGCACCAGAAATTA
CAAAAGATGGTTATAAGGTGATGAAGGGTATCAAGCCTGAAAAACCA
TTAAACGCTGCGATAGCAAGCATCTTTGCACAGAGTTGTTCTCAATGT
AACGATAAAGTTGGTGTGTTACAACAACGTGCTCAATACTAAGTAGC
AACATGATAATGGAAGCTTCAAAATCAATTGCTGCTGGAAACGATCGT
GTTGGTATTA AAAACGGAATACAGAAGGCAAAGATGTAATATTA AA
GGAAATTGCGTCAATGTCTCGTACAATTTCTCTAGAGAAAATAGACGA
AGTGGCACAAGTTGCAATAATCTCTGCAAATGGTGATAAGGATATAGG
TAACAGTATCGCTGATTCCGTGAAAAAAGTTGGAAAAGAGGGGTGTA
TAACTGTTGAAGAGAGTAAAGGTTCAAAGAGTTAGAAGTTGAGCTG
ACTACTGGCATGCAATTTGATCGCGGTTATCTCTCTCCGTATTTTATTA
CAAATAATGAAAAAATGATCGTGGAGCTTGATAATCCTTATCTATTAA
TTACAGAGAAAAAATTAATATTATTCAACCTTACTTCCTATTCTTGA
AGCTATTGTTAAATCTGGTAAACCTTTGGTTATTATTGCAGAGGATATC
GAAGGTGAAGCATTAAAGCACTTTAGTTATCAATAAATTGCGTGGTGGT
TAAAAGTTGCTGCAGTAAAAGCTCCAGGTTTTGGTGACAGAAGAAAG
GAGATGCTCGAAGACATAGCAACTTAACTGGTGCTAAGTACGTCATA
AAAGATGAACTT
```

- **Select 'BLAST!'** A new window appears
4. How long (query length) is the *Wolbachia* sequence that you used to search the database? \_\_\_\_\_
  5. What is the E-value and Max score of the best hit (in this case, the first matching sequence)? \_\_\_\_\_ and \_\_\_\_\_
  6. What is the most likely identity of this sequence? (click on the blue 'Accession' link to the left of the top hit)
 

\_\_\_\_\_

What is the title of the scientific publication that reported this sequence (click on the PUBMED 16267140 link)

\_\_\_\_\_
- **Go back twice** when you're done.

- **Select Distance tree of results**<sup>NEW</sup> just above the list of the sequences producing significant alignments. This will open a separate page with a phylogenetic tree that includes your sequences (highlighted in yellow with blue dot)
- **Print the phylogenetic tree** (if you can print) **and discuss** what the tree tells you about the evolutionary relatedness of your *Wolbachia* strain to other strains in the database. The class might want to create a portfolio of their trees along with a picture and general information on their insects

7. What does a phylogenetic tree show? For instance, what does the length and order of the branches tell you about evolutionary relatedness?

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8. What is your strain most closely related to in the phylogenetic tree?

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- **Return** to the page with your BLAST results
- **Select Home** at the top of the BLAST page.
- **Select ‘nucleotide BLAST’** under the Basic BLAST category
- **Now enter only the first 25 base pairs of your *Wolbachia* sequence below into the Search box.**

>Your *Wolbachia* Sequence  
GTTGCAGCAATGGTAGACTCAACGG

- As you did before, **select ‘BLAST!’** A new window appears

9. What is the E-value and Max score of the best hit (the first matching sequence)? \_\_\_\_\_ and \_\_\_\_\_. Is the E-value more or less significant than you BLASTED the longer *Wolbachia* sequence in step 3?

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10. Is the identity of the best hit different from when you used the complete nucleotide sequence? \_\_\_\_\_

11. From the two BLAST searches, what can you deduce about how the length of a query sequence affects your confidence in the sequence search?

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- 
- **Close all web windows.** This exercise is now complete. You successfully mastered one of the state-of-the-art tools used by most molecular and evolutionary biology researchers today. There is a lot of information on the NCBI website. Feel free to explore the website and you can find more tutorials at:  
<http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html>

Name \_\_\_\_\_ Date: \_\_\_\_\_

**True/False***Write true or false in the blank provided.*

- \_\_\_\_\_ 1) One fire ant can sting several times.
- \_\_\_\_\_ 2) An ant colony that contains several queens is called a monogyne colony.
- \_\_\_\_\_ 3) Most stinging insects, such as wasps, have venom with a larger proportion of alkaloids than protein.
- \_\_\_\_\_ 4) Symbiosis is the living together of dissimilar organisms.
- \_\_\_\_\_ 5) Endosymbiosis is when one organism lives but does NOT replicate inside another one.
- \_\_\_\_\_ 6) Smaller DNA molecules move through the agarose gel faster than larger molecules.
- \_\_\_\_\_ 7) DNA has a positive charge.
- \_\_\_\_\_ 8) The target segment of DNA to be amplified is also known as the "amplicon".

**Multiple Choice***Write the letter of the choice that best completes the statement or answers the questions.*

- \_\_\_\_\_ 9) In the name *Solenopsis invicta*, *Solenopsis* represents which taxon level?  
a) species      b) order      c) kingdom      d) genus      e) class
- \_\_\_\_\_ 10) Where was the red imported fire ant first introduced in the USA?  
a) Biloxi, MS      b) Mobile, AL      c) Miami, FL      d) New Orleans, LA
- \_\_\_\_\_ 11) How many polygyne colonies can be found per acre?  
a) 10      b) 100      c) 1,000      d) 10,000
- \_\_\_\_\_ 12) What percent of all animal species are insects?  
a) 85%      b) 65%      c) 50%      d) 45%      e) 25%
- \_\_\_\_\_ 13) What percent of ALL tested insect species harbor heritable symbionts called *Wolbachia*?  
a) 1%      b) 5%      c) 10%      d) 20%      e) 50%
- \_\_\_\_\_ 14) Which organelle is thought to have originated by microbial endosymbiosis in eukaryotic evolution?  
a) ribosome      b) mitochondria      c) lysosome      d) centriole      e) endoplasmic reticulum

- \_\_\_\_\_ 15) Who developed and won a Nobel Prize for PCR?  
a) Seth Bordenstein    b) Kary Mullis    c) James Watson    d) Francis Collins  
e) Robin Rockhold
- \_\_\_\_\_ 16) Which volume is the same as 2 mL?  
a) 2  $\mu$ L    b) 20  $\mu$ L    c) 200  $\mu$ L    d) 2,000  $\mu$ L
- \_\_\_\_\_ 17) Which of the following will allow you to amplify DNA?  
a) PCR    b) electrophoresis    c) vortexing    d) centrifugation
- \_\_\_\_\_ 18) Which part of DNA contributes to its charge?  
a) nitrogen bases    b) phosphates    c) deoxyribose sugar    d) uracil
- \_\_\_\_\_ 19) Term for oligonucleotide used to amplify target segment of DNA?  
a) promoters    b) poly A tail    c) primers    d) gene
- \_\_\_\_\_ 20) Which of the following is NOT found in the bead of a PCR Ready tube?  
a) magnesium chloride    b) Taq polymerase    c) primers    d) dNTP's
- \_\_\_\_\_ 21) *Wolbachia* is an example of a/an:  
a) virus    b) fungus    c) bacteria    d) protest
- \_\_\_\_\_ 22) How many subunits compose a ribosome?  
a) 1    b) 2    c) 3    d) 4    e) 5
- \_\_\_\_\_ 23) The DNA polymerase currently used in PCR was isolated from the bacterium  
a) *E. coli*    b) *Saccharomyces cerevisiae*    c) *Thermus aquaticus*    d) *Drosophila melanogaster*
- \_\_\_\_\_ 24) Which step of PCR requires the highest temperature?  
a) DNA synthesis    b) primers anneal    c) denature DNA    d) elongation
- \_\_\_\_\_ 25) The instrument used to conduct PCR is called a  
a) vortexer    b) thermal cycler    c) gel electrophoresis    d) microcentrifuge  
e) micropipettor
- \_\_\_\_\_ 26) Which one of the following destroys DNAses?  
a) Proteinase K    b) elution buffer    c) Taq polymerase    d) primers
- \_\_\_\_\_ 27) Gel electrophoresis can be used to analyze all of the following EXCEPT  
a) carbohydrates    b) nucleic acids    c) DNA    d) proteins
- \_\_\_\_\_ 28) Which segment of an ant contains the venom glands?  
a) head    b) mandibles    c) thorax    d) abdomen
- \_\_\_\_\_ 29) Which substance will cause DNA to precipitate?  
a) Proteinase K    b) water    c) ethanol    d) PBS buffer
- \_\_\_\_\_ 30) Which of the following would result from increasing the agarose concentration of a gel?  
a) increase migration speed and separation of smaller DNA molecules  
b) increase migration but decrease separation of smaller DNA molecules

- c) reduce migration speed but increase separation of smaller DNA molecules
- d) reduce migration and decrease separation of smaller DNA molecules

## Answer Key

- 1) T
- 2) F
- 3) F
- 4) T
- 5) F
- 6) T
- 7) F
- 8) T

- 9) D
- 10) B
- 11) C
- 12) A
- 13) D
- 14) B
- 15) B
- 16) D
- 17) A
- 18) B
- 19) C
- 20) C
- 21) C
- 22) B
- 23) C
- 24) C
- 25) B
- 26) A
- 27) A
- 28) D
- 29) C
- 30) C

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

### **2001 Mississippi Science Framework for Biology II**

- 1) Utilize critical thinking and scientific problem solving in designing and performing biological research and experimentation.
  - a. Demonstrate the proper use and care of scientific equipment used in biology.
  - b. Observe and practice safe procedure in the classroom and laboratory.
  - c. Apply the components of scientific processes and methods in the classroom and laboratory investigations.
  - d. Communicate results of scientific investigations in oral, written, and graphic form.
- 2) Investigate chemical processes of the cell that maintain life.
  - b. Investigate enzymatic reactions and identify factors that influence enzyme activity.
- 3) Explore the molecular basis of heredity.
  - c. Investigate modern DNA technologies (gene splicing, gel electrophoresis and recombinant DNA in agriculture, medicine, and forensics).
  - a. Review genetic principles for solving inheritance problems.
- 4) Investigate the role that natural selection plays in maintaining diversity.
  - c. Examine factors that affect populations, such as distribution, competition, migration, isolation, and disease.
- 5) Apply principles of classification to groups of organisms.
  - b. Compare classical (morphological) and modern (molecular) classification systems.

### **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)

- d. Organize data to construct graphs (e.g., plotting points, labeling x- and y-axis, creating appropriate titles and legends for circle, bar, and line graphs), draw conclusions, and make inferences. (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)

## **4. Demonstrate an understanding of the factors that contribute to evolutionary theory and natural selection.**

- c. Identify and provide supporting evidence for the evolutionary relationships among various organisms using phylogenetic trees and cladograms. (DOK 2)
- h. Develop a logical argument describing ways in which the influences of 20<sup>th</sup> century science have impacted the development of ideas about evolution (e.g., synthetic theory of evolution, molecular biology). (DOK 3)

- i. Analyze changes in an ecosystem resulting from natural causes (succession), changes in climate, human activity (pollution and recycling), or *introduction of non-native species*. (DOK 2)

**The *Wolbachia* Project: Discover the Microbes Within!**

| National Science Education Standards<br>(National Research Council 1996) | Activity 1: Insect<br>Identification | Activity 2: DNA<br>Extraction | Activity 3: DNA<br>Amplification | Activity 4: Gel<br>Electrophoresis | Activity 5:<br>Bioinformatics |
|--|--------------------------------------|-------------------------------|----------------------------------|------------------------------------|-------------------------------|
| <b>Unifying Concepts and Processes in Science</b>                        |                                      |                               |                                  |                                    |                               |
| Systems, order and organization  | ●                                    | ●                             |                                  |                                    | ●                             |
| Evidence, models and explanation   | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| Change, constancy and measurement  | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| Evolution and equilibrium  | ●                                    | ●                             | ●                                |                                    | ●                             |
| <b>Science as Inquiry</b>  |                                      |                               |                                  |                                    |                               |
| Abilities necessary to do scientific inquiry                             | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| Understandings about scientific inquiry                                  | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| <b>Life Science</b>  |                                      |                               |                                  |                                    |                               |
| The cell   |                                      | ●                             | ●                                |                                    | ●                             |
| Molecular basis of heredity  |                                      | ●                             | ●                                | ●                                  | ●                             |
| Biological evolution   | ●                                    | ●                             |                                  | ●                                  | ●                             |
| Interdependence of organisms   | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| Matter, energy and organization in living systems                        | ●                                    |                               |                                  |                                    |                               |
| <b>Science and Technology</b>  |                                      |                               |                                  |                                    |                               |
| Abilities of technological design  |                                      | ●                             | ●                                | ●                                  | ●                             |
| Understandings about science and technology                              | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| <b>Science in Personal and Social Perspectives</b>                       |                                      |                               |                                  |                                    |                               |
| Personal and community health  |                                      |                               |                                  |                                    | ●                             |
| Science and technology in local, national, global challenges             | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| <b>History and Nature of Science</b>                                     |                                      |                               |                                  |                                    |                               |
| Science as a human endeavor  | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| Nature of scientific knowledge   | ●                                    | ●                             | ●                                | ●                                  | ●                             |
| Historical perspectives  |                                      |                               |                                  |                                    | ●                             |

**VOCABULARY**

|                                |   |
|--------------------------------|---|
| Cell Lysis                     | the death of a cell by breaking the cellular membrane   |
| DNA Purification               | a solid phase extraction method to quickly purify nucleic acids   |
| Macerating                     | softening or breaking of a solid into pieces with a liquid  |
| DNA Elution                    | to obtain DNA from a column using a buffer  |
| Hydrolytic Enzymes             | an enzyme that catalyzes the hydrolysis of a chemical bond  |
| Monogyne                       | A colony with one queen   |
| Polygyne                       | a colony with multiple queens   |
| Morphospecies                  | a species typed by its morphology   |
| Nasonia Controls               | wasps that either have <i>Wolbachia</i> present (positive control) or not present (negative control)  |
| Pestles                        | a tool used to crush, grind, and mix substances   |
| <i>Wolbachia</i> Endosymbionts | a bacterium that inhabits the abdomens of numerous insects  |
| PCR                            | used to amplify a piece of DNA by <i>in vitro</i> enzymatic replication   |
| DNA Amplification              | the process of copying a double-stranded DNA molecule to form two double-stranded molecules   |
| Microbes                       | an organism that is microscopic (too small to be seen by the naked human eye)   |
| Ribosomal RNA                  | the function of the rRNA is to provide a mechanism for decoding mRNA into amino acids and to interact with the tRNAs during translation   |
| Primers                        | a strand of nucleic acid that serves as a starting point for DNA replication  |
| Thermocycler                   | a laboratory apparatus used to amplify segments of DNA via the polymerase chain reaction (PCR) process  |
| Denature                       | a process in which proteins or nucleic acids lose their 3D-structure (secondary structure) by application of some external stress or compound for example: heat   |
| Taq polymerase                 | a thermostable DNA polymerase named after the thermophilic bacterium <i>Thermus aquaticus</i> from which it was originally isolated, and is frequently used in polymerase chain reaction (PCR), methods for greatly amplifying short segments of DNA. |
| MgCl <sub>2</sub>              | Magnesium chloride salt; essential for replication  |
| Buffer                         | used for the purpose of lysing cells for use in   |

|                             |   |
|-----------------------------|---|
|                             | experiments that analyze the compounds of the cells   |
| dNTPs                       | a solution of all four deoxyribonucleotides   |
| Agarose                     | a gelatinous substance derived from seaweed   |
| Agarose Gel Electrophoresis | a method used in biochemistry and molecular biology to separate DNA, or RNA molecules by size |
| Electrode                   | an electrical conductor used to make contact with a nonmetallic part of a circuit             |