ACTIVITY AT A GLANCE

**Goal:**
To screen for *Wolbachia pipientis* 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, students will:
1. Amplify DNA extracted from two morphospecies and three controls using Polymerase Chain Reaction (PCR).
2. Understand the basic principles of PCR.

**Prerequisite Skills:**
- Prior practice with micropipettors.
- Familiarity with the roles and responsibilities of group work.

**Teaching Time:**
60 minutes

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**Timeline for Teaching Discover the Microbes Within: The Wolbachia Project**

- **Activity 1: Insect Identification Lab**
- **Activity 2: DNA Extraction Lab**
- **Activity 3: DNA Amplification Lab**
- **Activity 4: Gel Electrophoresis**
- **Activity 5: Bioinformatics**
- **Activity 6: DNA Sequence Alignments & Phylogenetics**

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**PCR Lab-CIBT Version**
OVERVIEW

Most DNA analysis situations require fairly large amounts of DNA. Usually the amount in a few cells is not enough for a full analysis. A method called the Polymerase Chain Reaction (PCR) has been developed to amplify the amount 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 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, students should work in groups of two. The PCR primers provided in the CIBT kit specifically amplify a 438bp fragment of the 16S ribosomal RNA gene (ubiquitous in all *Wolbachia*). These are WSPEC-F (5’-CATACCTATTCGAAGGGATAG-3’) and WSPEC-R (5’-AGCTTCGAGTGAACCAATTC-3’).

MATERIALS (per team of two students)

- Thermalcycler (one per class)*
- 2 DNA samples from morphospecies
- 2 DNA samples from + and – *Nasonia* controls*
- + Wolbachia DNA control*
- Sharpie (extra fine point)
- 5 PCR Ready Bead Tubes*
- 1 box of P20 pipet tips*
- Gloves, two pairs
- 1 rack for holding PCR tubes (USA Scientific 2396-5048)*
- 1 tube of Wspec-F primer (5 micromolar, 20 µl)*
- 1 tube of Wspec-R primer (5 micromolar, 20 µl)*
- 1 tube of sterile water (150 µl)*
- 1 waste cup for tips, tubes, etc.
- 1 microcentrifuge with rotor for PCR tubes (one per class)*
- Safety goggles (optional)

*provided with CIBT kit

TEACHER PREPARATION

Set up each activity station with its own set of materials as reflected above.

ACTIVITY PROCEDURE

Review the basic principles of PCR with your class and instruct them to revisit their hypothesis from the DNA Extraction Lab. Download the lecture material on DNA-based technologies and PCR Basics. This lecture describes how techniques such as PCR are changing the landscape of biological research and where PCR has even been mentioned in contemporary movies and TV shows today. This lecture as well as others can be downloaded for free at http://jbpc.mbl.edu/~sbordenstein/workshop.html.
As a group, program the thermalcycler to the settings listed on the Student Activity Sheet. Most likely your thermalcycler has a ready-to-go program named ‘1wolbach’ (or similar) already listed in its protocol library. This protocol has an additional step preprogrammed: after the last 10-min cycle @ 72°C, a final cycle of unspecified duration @ 4°C follows – this allows you to run the PCR reaction overnight while keeping the samples at refrigerator temperature after the reaction until used for the next lab (Agarose Gel Electrophoresis).

Stress the importance of labeling and proper lab procedure in obtaining accurate results. Students will work with their same partners and follow the protocol outlined on the Student Activity Sheet.

Experience tells us that labeling the PCR tubes on the flat hinge part of the rounded lid provides the best chance of staying through the thermocycling. Instruct your students to place the tube number in that flat spot while their initials can be placed on the rounded lid (and re-done later if necessary).

Given the small amounts needed and available, it is not practical to aliquot out a few µl of Wolbachia control DNA for each student team. Instead you may want to go around to each group and add the 2 µl to their prepared PCR Tube #5 yourself.
Discover the Microbes Within: The *Wolbachia* Project

Student Activity Sheet  Name:__________

**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 (per team of 2 students)**

- [ ] 2 DNA samples from morphospecies
- [ ] 2 DNA samples from + and – *Nasonia* controls
- [ ] + DNA control
- [ ] Sharpie (extra fine point)
- [ ] 5 PCR Ready Bead Tubes
- [ ] 1 box of P20 pipet tips
- [ ] P20 pipette
- [ ] Gloves, 2 pairs
- [ ] 1 rack for holding PCR tubes
- [ ] 1 tube of Wspec-F primer (5 micromolar, 15 µl)
- [ ] 1 tube of Wspec-R primer (5 micromolar, 15 µl)
- [ ] 1 tube of sterile water (150 µl)
- [ ] 1 waste cup for tips, tubes
- [ ] Safety goggles (optional)

**INTRODUCTION**

In this activity, you will:

1. Amplify DNA extracted from two morphospecies and three controls using a procedure called Polymerase Chain Reaction (PCR).
2. Understand the basic principles of PCR.

**PREPARATION**

The thermocycler should be programmed for the optimum settings. If using a CIBT thermocycler, this program will be called ’1wolbach’ or similar.

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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
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**PROCEDURE**

1. Collect 5 PCR Ready tubes. Each of these already contains a preformulated, pellet of “Master Mix”. This material contains Taq polymerase, MgCl$_2$, Buffer, and dNTPs. Label each tube (number and initials) according to which DNA sample they will contain.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Tube Contents (Voucher #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>- control</td>
</tr>
<tr>
<td>4</td>
<td>+ control</td>
</tr>
<tr>
<td>5</td>
<td><em>Wolbachia</em> DNA</td>
</tr>
</tbody>
</table>

   Note that you will use 5 tubes because a previously purified sample of *Wolbachia* DNA has been included as a procedural control.

2. To each tube, add the materials in the sequence below (total volume of 25 µl). A new pipette tip should be used for each step:
   a. Add 19 µl of sterile distilled water to each tube
   b. Add 2 µl of Primer W-spec forward to each tube
   c. Add 2 µl of Primer W-spec reverse to each tube
   d. Add 2 µl of DNA template from each sample to its correlating tube. Be sure to change the pipette tips for each DNA template!

3. Cap and gently tap the bottom of each tube to mix the components. Make sure the labeling (number and initials) is still in place and re-label the tubes if necessary. Place your five tubes into the microcentrifuge and briefly spin them for a few seconds to make sure all the solutions collect at the bottom of the PCR tubes (tweezers may be helpful for this task). The rotor can hold 16 PCR tubes, which means 3 teams can spin their samples at the same time (add a dummy PCR tube to hole 16 to balance the centrifuge).

4. Using tweezers, place your five tubes into the thermocycler’s green tube holder in the logical order from left to right: tube #1, #2, #3, #4, and #5. The first group of students places their tubes in the upper left corner of the tube holder, the next group skips a hole in the same row and adds their tubes to the right. The third group starts a new row in the tube holder, etc. Keeping such a system and order is essential as the labeling on those tiny PCR tubes may come off during the approximately 3 hrs. of thermocycler action with varying temperature ranges. This way you can be confident to be able to re-label the PCR tubes if necessary.
5. Once everyone has prepared their samples, the thermocycler can be turned on and the appropriate protocol (1wolbach) started.

6. Clean up your lab station.