PCR LAB

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 three morphospecies and three controls using Polymerase Chain Reaction (PCR).
2. *Understand* the basic principles of PCR.

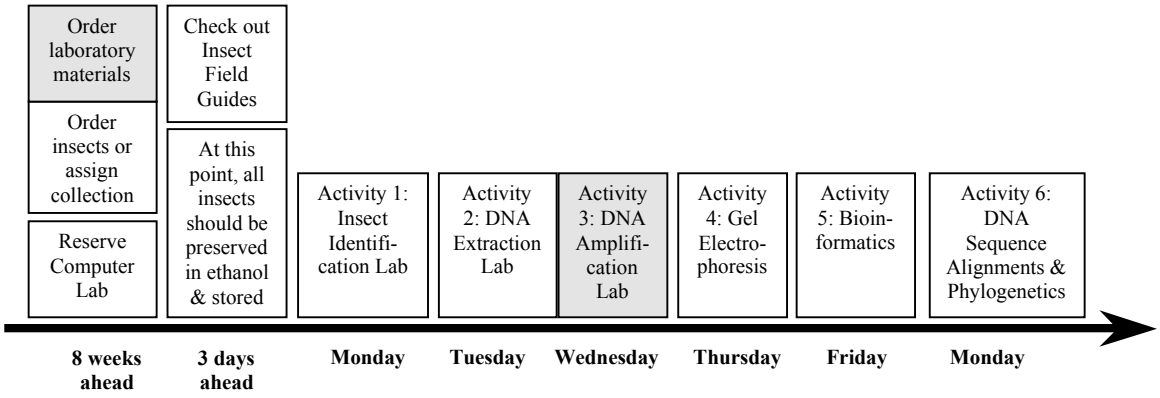
Prerequisite Skills:

- Prior practice with micropipettors.
- Familiarity with the roles and responsibilities of group work.

Teaching Time:

60 minutes

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



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 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. Contact Dr. Seth Bordenstein at The Marine Biological Laboratory for – and + *Nasonia* insect controls and + control DNA samples. As in the previous lab, students 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') are also provided by Dr. Bordenstein (sbordenstein@mbl.edu).

MATERIALS (per group of two students)

- Thermalcycler
- 3 DNA Samples from Morphospecies
- 2 DNA Samples from + and – *Nasonia* controls
- + DNA control
- Sharpie
- 6 PCR Ready Bead Tubes
- 1 box of P200 pipet tips
- 1 box of P20 pipet tips
- P200 and P20 pipettes
- Gloves, two pair
- 1 rack for holding PCR tubes (USA Scientific 2396-5048)
- 1 tube of Wspec-F primer (5 micromolar, 20μl)
- 1 tube of Wspec-R primer (5 micromolar, 20μl)
- 1 tube of d2H20 (200 μl)
- 1 waste cup for tips, tubes, etc.
- Saftey goggles

TEACHER PREPARTION

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://troi.cc.rochester.edu/~wolb/FIBR/workshops.html. As a group, program the themalcycler to the settings listed on the Student Sheet. Stress the importance of proper lab procedure in obtaining accurate results. Students will work with their same partners and follow the protocol outlined on the student sheet.

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.

_____
_____
_____
_____

MATERIALS

- 3 DNA Samples from Morphospecies
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- + DNA control
- Sharpie
- 6 PCR Ready Tubes
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- 1 waste cup for tips, tubes
- Saftey goggles

INTRODUCTION

In this activity, you will:

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

PREPARATION

The thermalcycler should be programmed for the optimum settings:

<u>1 cycle</u>
2 min @ 95 C

<u>38 Cycles</u>
30 sec. @ 94 C
45 sec @ 55 C
90 sec @ 72 C

<u>1 cycle</u>
10 min. @72 C

PROCEDURE:

1. Collect 6 PCR Ready tubes. Each of these already contains a preformulated, pellet of "master mix". This material contains Taq polymerase, $MgCl_2$, Buffer, and dNTPs.

> Note that you will use 6 tubes because a previously purified sample of *Wolbachia* DNA has been included as a procedural control.

Tube #	Tube Contents (Voucher #)
1	
2	
3	
4	- control
5	+ control
6	*Wolbachia* DNA

2. In 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. Place your six tubes with labels (initials and number) into the thermalcycler. Once everyone has prepared their samples, the thermalcycler can be turned on.

4. Clean up your lab station.