PCR LAB

ACTIVITY AT A GLANCE

Goal:
Students are introduced to the Polymerase Chain Reaction (PCR) and its use as an essential laboratory procedure. They will learn about the role of PCR in this lab series and why it is necessary for the identification of *Wolbachia.* The method of PCR will be reviewed in detail. The steps of denaturation, annealing and extension performed by the PCR machine will be explained. The essentials of good pipetting skills will be stressed. This lab will present PCR as an application to identify *Wolbachia* in arthropods.

Learning Objectives:
Upon completion of this activity, students will use and understand one of the most useful biotechnology tools in the life sciences, 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. Students will *amplify* DNA extracted from two 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 bacterial ribosomal RNA. We will refer to this piece as 16S rDNA. The piece of DNA used for identifying the arthropod is the region that codes for the mitochondrial protein, cytochrome c oxidase I. We will refer to this piece as CO1.

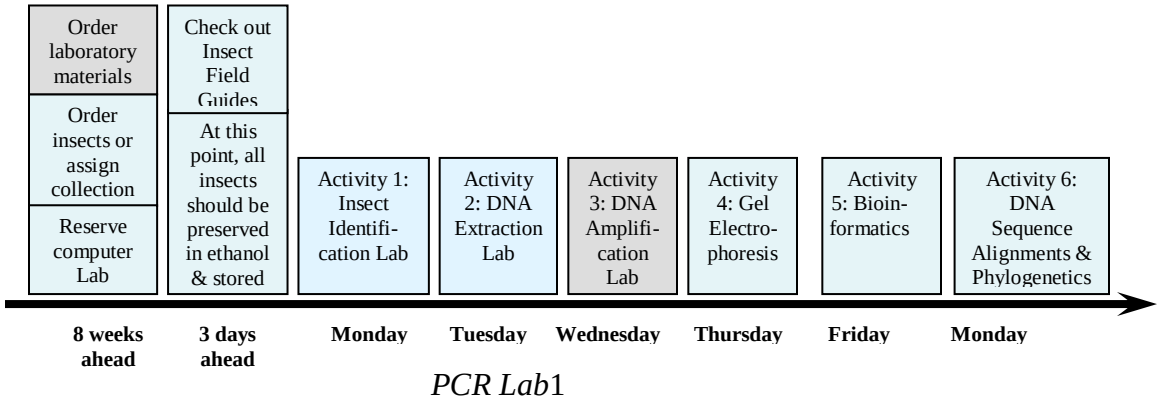
Prerequisite Skills: Prior practice with micropipettors.

Assessed Outcomes:
Assess the student's understanding of PCR and the role it has in the identification of *Wolbachia* in arthropods.
Assess the student's ability to stay organized and successfully complete the lab.

Teaching Time: 50 minutes

National Science Education Standards Addressed: *Unifying Concepts and Processes in Science, Science as Inquiry, Science and Technology, Life Science, Science in Personal and Social Perspectives, History and Nature of Science*

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 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 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 The *Wolbachia* Project (wolbachiaproject@vanderbilt.edu) at Vanderbilt University for positive and negative *Drosophila* insect controls and/or positive control DNA samples. As in the previous lab, students should work in groups of two.

Goals

In this activity, we will not only seek to amplify the possible *Wolbachia* DNA but we will also be amplifying a portion of Eukaryotic DNA. This second amplification is, in effect, a procedural control. Students can *hope* for a *Wolbachia* band, but will be guaranteed an insect (eukaryote) band. Therefore, they can be certain that their DNA isolation and amplification was done correctly as well as having a concrete band to read on their gels.

PCR Primers

Primers to specifically amplify a 438 bp fragment of the 16S ribosomal RNA gene (ubiquitous in all *Wolbachia*) are
Wspec-F (`5′-CAT ACC TAT TCG AAG GGA TAG-3′`) and
Wspec-R (`5′-AGC TTC GAG TGA AAC CAA TTC-3′`).
Primers to amplify a 708 bp fragment of the CO1 cytochrome oxidase gene (ubiquitous in arthropod mitochondria) are
LCO1490 (`5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′`) and
HCO2198 (`5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′`).

If you teach a high school class, these primers can be provided by the Bordenstein Lab at Vanderbilt University (wolbachiaproject@vanderbilt.edu). Further, we may be able to offer a free thermal cycler loaner program, so contact us several weeks in advance to coordinate shipping (high schools only).

TEACHER PREPARATION

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

MATERIALS (per group of two students)

- ❑ Thermal cycler for the class
- ❑ 2 DNA samples from Morphospecies (number of samples can vary)
- ❑ 2 DNA samples from positive and negative *Drosphila* controls
- ❑ Positive DNA control
- ❑ Sharpie, fine tip
- ❑ Thermo Scientific DreamTaq Green PCR Master Mix 2x (K1081)
- ❑ 1 box of P200 pipet tips
- ❑ 1 box of P20 pipet tips
- ❑ P200 and P20 pipettes
- ❑ Ice bucket and Styrofoam coffee cups for ice
- ❑ Gloves, two pair
- ❑ 1 rack for holding PCR tubes
- ❑ 1 Primer Mix tube of primers containing
  12 µl Wspec-F primer (5 µM)
  12 µl Wspec-R primer (5 µM)
  12 µl CO1-F primer (5 µM)
  12 µl CO1-R primer (5 µM)
  15 µl sterile distilled H_2O, provided with DreamTaq Master Mix
- ❑ 1 waste cup for tips, tubes
- ❑ Safety goggles
- ❑ Squeeze bottle of 70% ethanol
- ❑ 0.2 ml PCR tubes

Note: Students can also pipette each primer and the water individually for practice.

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 https://my.vanderbilt.edu/discover/lab-series/. As a group, program the thermal cycler to the settings listed on the Student Sheet. If you borrow the thermal cycler from Vanderbilt University, the settings are preloaded under the program "Wolbachia". 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

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- ❑ 2 DNA samples from positive and negative *Drosophila* controls
- ❑ Positive DNA control
- ❑ Sharpie, fine tip
- ❑ Thermo Scientific DreamTaq Green PCR Master Mix 2x
- ❑ 1 box of P200 pipet tips
- ❑ 1 box of P20 pipet tips
- ❑ P200 and P20 pipettes
- ❑ Gloves, 2 pair
- ❑ Styrofoam coffee cup with ice
- ❑ 1 rack for holding PCR tubes
- ❑ 1 Primer Mix tube of primers with
  12 µl Wspec-F primer (5 µM)
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- ❑ 1 waste cup for tips, tubes
- ❑ Safety goggles
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- ❑ 0.2 ml PCR tubes

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) and arthropod DNA from the extracted DNA of the two morphospecies and control insects. As well as a previously extracted DNA sample which is positive for *Wolbachia.* This positive control DNA will be provided by Vanderbilt University. 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*. The piece of DNA used for identifying arthropod DNA is a region that codes for the cytochrome oxidase I protein in animal mitochondria (CO1).

PREPARATION

The thermal cycler should be programmed for the optimum settings below. If you are a high school teacher and borrowed the Bio-Rad MyCycler from Vanderbilt University, the settings are already programmed in as "Wolbachia".

1 cycle
2 min. @ 94° C

30 Cycles
30 sec. @ 94° C
45 sec. @ 49° C
1 min. @ 72° C

1 cycle
10 min. @72° C
Hold @4° C

PROCEDURE

1. Remove all unnecessary items at your lab station. Clean all surfaces, including equipment by wiping down with 70% ethanol.
2. Collect five 0.2 ml PCR tubes, number and label them with your initials.

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

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

3. To determine the recipe for your reaction use the number of samples to be tested plus 1 to make up for loss while pipetting. For the 5 samples above use a factor of 6 to determine the amounts:

Per reaction:

Wolbachia forward primer (Wspec-F)	2 µl
Wolbachia reverse primer (Wspec-R)	2 µl
Cytochrome Oxidase forward (Co1-F)	2 µl
Cytochrome Oxidase reverse (Co1-R)	2 µl
Deionized sterile water	2.5 µl
Taq Master Mix 2x	12.5 µl
Total	23 µl

2 µl of DNA template makes a total reaction volume of 25 µl.

Make your Primer Mix separately from the Taq Master Mix. For six reactions:

Wspec-F	12 µl	
Wspec-R	12 µl	
Col-F	12 µl	
Co1-R	12 µl	
dH_2O	15 µl	
Total	63 µl	63 µl / 6 = 10.5 µl per tube
Taq Master Mix	75 µl	
Total	138 µl	75 µl / 6 = 12.5 µl per tube

Check math: 138 µl / 6 = 23 µl per PCR reaction

3. Add the above primers and H_2O to a tube labeled "Primer Mix". Use your pipette to mix by pumping in and out, or vortex briefly.

4. Place the PCR tubes on ice. Add 10.5 µl of Primer Mix to each tube.

5. After adding Primer Mix to each of your PCR tubes, add 12.5 µl of Taq Master Mix to each tube. Keep tubes on ice to keep polymerase from activating once you add the Taq mix.

6. Add 2 µl of DNA template from each sample to its correlating tube. ***Be sure to change the pipette tips for each DNA template!***

7. Cap and gently tap the bottom of each tube to mix the components. If drops are still on the side of the tube, spin very gently in a centrifuge for a couple of seconds at a time, until all liquids are collected in the bottom. Place your five tubes with labels (initials and number) into the thermal cycler. Once everyone has prepared their samples, the thermal cycler can be turned on.

8. Clean up your lab station, and wipe surfaces with ethanol.

9. When the thermal cycler is done (~2 hours), store the samples in the 4° C fridge.

10. Proceed to the Gel Electrophoresis Lab.