DNA EXTRACTION LAB

ACTIVITY AT A GLANCE

Goal:
To introduce students 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, students will:

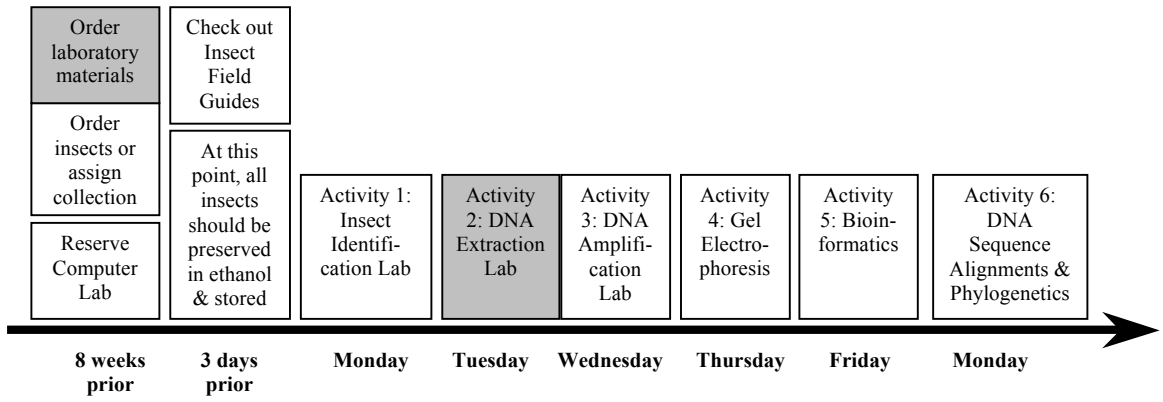
- Isolate total genomic DNA from morphospecies identified in the Insect Identification Lab.
- Develop pipetting skills to accurately aliquot small volumes.

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

In this activity, students will extract total genomic DNA from each of their 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, students will also prepare positive and negative controls using *Nasonia vitripennis* wasps that are infected and uninfected with *Wolbachia pipientis*, respectively. The *Nasonia* controls may be obtained by contacting Dr. Seth Bordenstein at The Marine Biological Laboratory (sbordenstein@mbl.edu) at least three weeks prior to beginning the lab series.

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) and then macerating them in 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 and hydrolytic enzymes in the insect tissue that may degrade the DNA. 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

- Heat block @ 70°C*
- Vortexer (optional)
- Student morphospecies
- + and – *Nasonia* controls*
- Microtube pestles (one per insect sample)* (USA Scientific 1415-5390)
- Qiagen DNeasy Kit*
- P200 & P1000 pipets*
- P200 & P1000 pipet tips*
- Small glass beakers (for EtOH)
- Waste cups for tips, etc.
- Microcentrifuge*
- Gloves
- 1X Phosphate Buffer Saline* (10X PBS from Fisher BP399-500 and dilute to 1X)
- Sharpies
- Tweezers
- Kimwipes or paper towels
- Ethanol (95 - 100%)
- Tube racks* (USA Scientific 2396-5048)
- 1.5ml microcentrifuge tubes (USA Scientific 1415-9199)*

***provided with CIBT kit**

TEACHER PREPARATION

This lab requires attention to detail, but it's worth it. Aliquot all of your reagents ahead of time into labeled sets of tubes so that YOU don't get confused. Use the P1000 and P200 pipettes to aliquot the solutions into individual tubes for your students. Each student will be preparing two DNA extracts, one from their collected insect, and one from either a positive or negative control *Nasonia*. Each group of two students will therefore be doing four DNA extractions (two unknown DNA samples, one + *Nasonia* control, and one – *Nasonia* control). Students will use sterile transfer pipettes to add the aliquotted solutions to their DNA extractions. Make sure you hand out 4 differently colored microtube pestles per student group to minimize the risk of contamination between samples. You should also hand out small glass beakers (one per group of 4 students) with ethanol for the students to rinse their tweezers in between different samples.

Reagents	# of tubes needed for 16 students	# of tubes needed for each team of 2 students	Amount needed per tube	Pipette used to aliquot
PBS	32	4	180 μl	P200
Proteinase K	32	4	20 μl	P200
Buffer AL	32	4	200 μl	P200
Ethanol (95 - 100%)	32	4	200 μl	P200
Buffer AW1	32	4	500 μl	P1000
Buffer AW2	32	4	500 μl	P1000
Buffer AE	32	4	100 μl	P200

ACTIVITY PROCEDURE

Review the activity flow-chart (page 10) with your class and instruct them to revisit their hypothesis from the Insect Identification Lab Mini-Report. Students will work with their same partners from Lab 1 and follow the protocol outlined on the student sheet. They are encouraged to read through the procedure prior to beginning the activity in order to identify and understand the purpose of each reagent. Answers are shown below:

- 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 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

Student Activity Sheet Name:_____

DNA Extraction Lab

Hypothesis: Based on your sets of morphospecies from the Insect Identification Lab, formulate a hypothesis about the presence of *Wolbachia pipientis* endosymbionts in your specimens.

_____
_____
_____

MATERIALS (per team of two students)

- ❑ 2 different morphospecies
- ❑ + and – Nasonia controls
- ❑ Gloves
- ❑ Sharpie
- ❑ Tweezers
- ❑ Small glass beaker w/ethanol to rinse tweezers
- ❑ 1 Box Kimwipes or paper towels
- ❑ P200 & P1000 pipettors and tips
- ❑ 1 waste cup for tips & tubes
- ❑ 4 microtube pestles (4 different colors)
- ❑ 4 tubes of PBS Buffer (180 µl each)
- ❑ 4 tubes of Proteinase K (20 µl each)
- ❑ 4 tubes of Buffer AL (200 µl each)
- ❑ 4 tubes of Buffer AW1 (500 µl each)
- ❑ 4 tubes of Buffer AW2 (500 µl each)
- ❑ 4 tubes of Buffer AE (500 µl)
- ❑ 4 tubes of ethanol (95 - 100%, 200 µl each)
- ❑ 4 spin columns
- ❑ 8 empty 1.5 ml microcentrifuge tubes
- ❑ 4 empty 2.0 ml collection tubes
- ❑ 1 tube rack
- ❑ Safety goggles (optional)

INTRODUCTION

In this activity, you will:

- Isolate total genomic DNA from morphospecies identified in the Insect Identification Lab.
- Develop pipetting skills to accurately aliquot small volumes of reagents.

In this activity, you will extract total genomic DNA from each of the two morphospecies using Qiagen's DNeasy 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 morphospecies, 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 10) and work with the same partners from Lab 1. Read through the procedure prior to beginning the activity in order to identify and understand the purpose of each reagent.

BEFORE YOU BEGIN

After the teacher reviews the entire procedure, note the purpose of each reagent:

- Phosphate Buffered Saline (PBS): _____
- Proteinase K: _____
- Buffer AL: _____
- Ethanol: _____
- Buffer AW1: _____
- Buffer AW2: _____
- Buffer AE: _____

PROCEDURE

Preparation

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

Tube #	Contents (Voucher #)
1	
2	
3	- control
4	+ control

2. Collect four 1.5 ml microcentrifuge tubes. Using a Sharpie marker, number them 1 - 4 along with your initials.

Cell Lysis

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

1. Place 180 microliters (µl) of PBS buffer into each tube to macerate the insects in.
2. Place the small insect or abdomen of a larger insect into the buffer (no larger than 2 mm^2) of Tube 1 with tweezers. If the insect is preserved in ethanol, briefly blot it dry on a Kimwipe. Blot the ethanol away of your + and – *Nasonia* controls as well.
3. Take Tube 1 and macerate **THOROUGHLY** using a microtube pestle. **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 three samples. Be sure to use a different pestle and pipette tip for each tube. Also rinse your tweezers with ethanol in between different samples.
5. Incubate in the heat block for at least 10 minutes at 70ºC.
6. Add 200 µl of ethanol (95 - 100%) to each tube. This will precipitate DNA from the extracted material
7. Vortex or invert the tubes 25 times.

***STOPPING POINT IF NEEDED. 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.
2. Pipette the liquid from Tube 1 of the above steps (with or without exoskeleton) into the DNeasy spin column #1. Using a new pipette tip for each transfer, repeat this process with the three other tubes. Make sure to keep tube numbers consistent.
3. Centrifuge for 1 minute. The DNA is now caught in the filter of the spin column. Discard the flow-through waste from the 2.0 ml collection tubes into the waste bucket.
4. Place the spin column containing the DNA from Tube 1 into the same emptied 2.0 ml collection tube.
5. Repeat for your other three 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.
8. Again, discard the flow-through waste in the 2.0 ml collection tubes into the waste bucket and place the DNeasy spin column from Tube 1 into the same emptied 2.0 ml collection tube labeled "1"; repeat for your other 3 tubes.
9. Add 500 µl of Buffer AW2 (a second wash buffer) to each of your 4 tubes and centrifuge for **3** minutes. Discard flow-through and collection tubes. This step is also removing the ethanol.
10. Place your spin columns into lidded 1.5 ml microcentrifuge tubes. Again, be sure to label the lids of each tube 1 - 4 and include your initials this time. These will contain your purified DNA samples.
11. Allow your tubes to air dry for 5 minutes. This will evaporate any excess 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 for 1 minute to elute. Make sure that the open lids of the 1.5 ml tubes don't interfere with the centrifugation.
4. Discard the spin column and KEEP the labeled 1.5 ml tube. Store in refrigerator at 4ºC until used in the PCR lab.

DNA Isolation Flow Chart

1 2 3 4

↓

Add PBS and Macerate

↓

Add Proteinase K and AL

↓

Vortex and Incubate

↓

Add Ethanol

↓

Spin Through a Filter and Discard Flow-through

↓

Wash with Buffer AW1

↓

Spin and Discard Flow-through

↓

Wash with Buffer AW2

↓

Spin and Discard Flow-through

↓

Add Elution Buffer, Spin, and **Keep Flow-through**