DNA EXTRACTION LAB

ACTIVITY AT A GLANCE

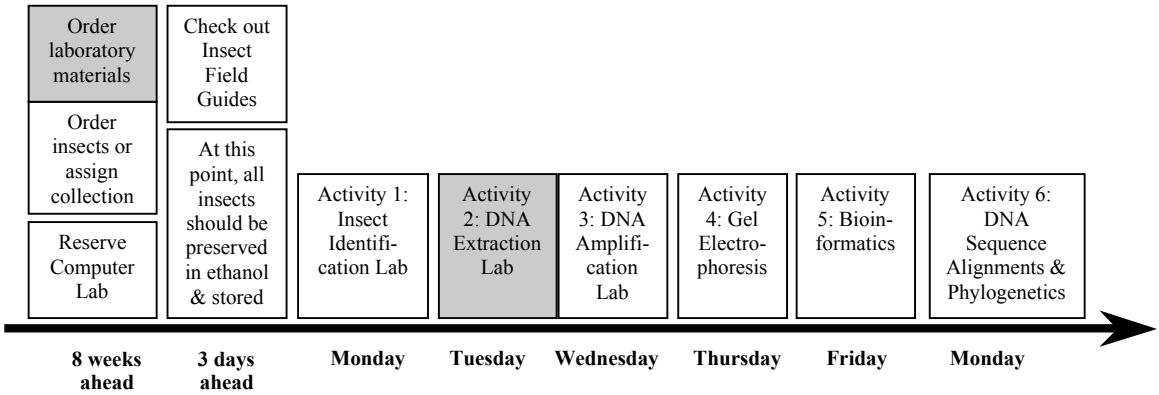
Goal:
To introduce students to DNA extraction techniques and to isolate genomic DNA from insects and *Wolbachia,* the endosymbiotic bacteria that live within the cells of over 20% of insect species.

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*



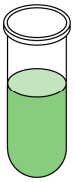
OVERVIEW

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 in the insect tissue. 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

- Incubator or water bath set @ 70˚ C
- Vortexer
- Student morphospecies
- + and – *Nasonia* controls
- Microtube Pestles (USA Scientific 1415-5390)
- Qiagen DNeasy Kit (#69504)
- P200 & P1000 pipets
- P200 & P1000 pipet tips
- Float racks for water bath
- Waste cups for tips, etc.
- Gloves
- 1X Phosphate Buffer Saline (10X PBS from Fisher BP399-500 and dilute to 1X)
- Sharpies
- Tweezers
- Kimwipes
- Ethanol (95-100%)
- Tube Racks (USA Scientific 2396-5048)
- 1.5ml microcentrifuge tubes (USA Scientific 1415-9199)

TEACHER PREPARTION

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. The Qiagen DNeasy Kit contains spin columns, collection tubes, Proteinase K, and buffers AL, AW1, AW2, and AE. Note that some of these solutions require 95-100% Ethanol added to them prior to the start and that the 1X PBS is not included in Qiagen Kit. You will also need to order the additional items in the list above. Set up each activity station with its own set of materials as reflected on the student sheet.

ACTIVITY PROCEDURE

Review the activity flow-chart (page 9) 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 and

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 refridgerator 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 group of two students)

- ❑ 3 different morphospecies
- ❑ + and – Nasonia controls
- ❑ Gloves
- ❑ Sharpie
- ❑ Tweezers
- ❑ 1 Box Kimwipes
- ❑ 1 box of P200 pipet tips
- ❑ 1 box of P1000 pipet tips
- ❑ P200 and P1000 pipets
- ❑ 1 waste cup for tips & tubes
- ❑ 5 Microtube pestles
- ❑ 1 tube of PBS Buffer (1ml)
- ❑ 1 tube of Proteinase K (110ul)
- ❑ 1 tube of Buffer AL (1ml)
- ❑ 1 tube of Buffer AW1 (2.5ml)
- ❑ 1 tube of Buffer AW2 (2.5ml)
- ❑ 1 tube of Buffer AE (500ul)
- ❑ 1 tube of ethanol (95-100% - 1ml)
- ❑ 5 spin columns
- ❑ 10 empty 1.5ml microcentrifuge tubes
- ❑ 5 empty 2.0ml collection tube
- ❑ 1 tube rack
- ❑ Safety goggles

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 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 *Wolbachia*, 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*, respectively. Review the activity flow-chart (page 9) and work with the same partners from Lab 1. Read through the procedure prior to beginning and 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 will put in each tube.

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

2. Collect five 1.5 ml microcentrifuge tubes. Using a Sharpie marker, number them 1-5 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 (ul) of PBS buffer into each tube to macerate the insects in.
2. Place the small insect or abodmen of a larger insect into the buffer (no larger than $2mm^2$) 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 ul of Proteinase K (destroys Dnases that break down DNA), and 200 ul of buffer AL (lysis

buffer to break open cells). Mix by vortexing for 10sec 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 four samples. Be sure to use a different pestle and pipet tips for each tube.
5. Incubate for at least 10 minutes at 70ºC.
6. Add 200 ul of Ethanol (96-100%) to each tube. This will precipitate DNA from the extracted material
7. Vortex.

Cellular Debris Removal

1. Collect five DNeasy spin columns fitted in five 2.0 ml collection tubes and label the lids of the spin columns 1-5 with your initials.
2. Pipet the liquid from tube 1 of the above steps (with or without exoskeleton) into the DNeasy Mini spin column #1. Using a new pipet tip for each transfer, repeat this process with the four other tubes. Make sure to keep tube numbers consistent.
3. Centrifuge for 1 minute at 6000g or 8,000 rpm. The DNA is now caught in the filter of the spin column. Discard the flow through waste into the 2.0ml collection tubes in the waste bucket.
4. Place the spin column containing the DNA from tube 1 in the same emptied 2.0ml collection tube.
5. Repeat for your other 4 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 at 6000g or 8000rpm.
8. Again, discard the flow through waste in the 2.0ml collection tubes in the waste bucket and place the DNeasy Mini spin column from tube 1 into the same emptied 2 ml collection tube labeled "1"; repeat for your other 4 tubes.
9. Add 500 µl of Buffer AW2 (a second wash buffer) to each of your 5 tubes and centrifuge for 3 minutes at 20,000 g or

13,000 rpm (or max speed if your centrifuge doesn't go that high)*. Discard flow-through and collection tubes. This step is also removing the ethanol.

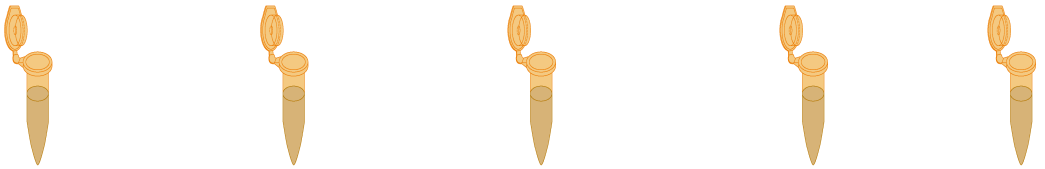
10. Place your spin columns into lidded 1.5 ml microcentrifuge tubes. Again, be sure to label the lids of each tube 1-5 and include your initials this time. These will contain your purified DNA samples.

***Note-if the centrifuge you are using cannot attain this speed, you can allow the tube to air dry for 5 minutes. This will evaporate the ethanol.**

DNA Elution and Dilution

1. Pipet 100ul of Buffer AE directly onto the membrane. This is an elution buffer that rinses the DNA off the spin column filter and into the 1.5ml tube.
2. Incubate at room temperature for 1 minute.
3. Centrifuge (6000g or 8,000 rpm) for 1 minute to elute.
4. Discard the spin column and KEEP the labeled 1.5ml tube. Store in 4ºC fridge until PCR.

DNA Isolation Flow Chart



1 2 3 4 5

↓

Add PBS and Macerate

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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