Species Diversity:  
Part 1

Contributors

Kelly Dabney  
Graduate Student  
Georgia Southern University, GA

Donna Hanson  
Partner Teacher  
Bradwell Institute, GA

Intended Audience

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-12</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Activity Characteristics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Classroom Setting</td>
<td>X</td>
</tr>
<tr>
<td>Requires special equipment</td>
<td>X</td>
</tr>
<tr>
<td>Uses hands-on manipulatives</td>
<td>X</td>
</tr>
<tr>
<td>Requires mathematical skills</td>
<td></td>
</tr>
<tr>
<td>Can be performed individually</td>
<td></td>
</tr>
<tr>
<td>Requires group work</td>
<td>X</td>
</tr>
<tr>
<td>Requires more than one (45 min class)</td>
<td>X</td>
</tr>
<tr>
<td>period</td>
<td></td>
</tr>
<tr>
<td>Appropriate for special needs student</td>
<td>X</td>
</tr>
</tbody>
</table>
Introduction

Description

Students use mosquito diversity to evaluate the presence or absence of pesticides. In part one, they students complete virtual labs and practice micropipetting.

Abstract

Students use mosquitos, a pollinator species, as an indicator of unintentional pesticide run-off. Students compare DNA from a known site of pesticide spraying (a farm), a site which commonly receives run-off (a river located near the farm), a site where no spraying occurs (near a well), and an unknown (near a school or other area that is not supposed to receive spraying). They examine the DNA banding patterns produced by each sample and use those to determine whether pesticide spraying unintentionally impacts the unknown site. In this portion of the activity, students complete two virtual labs, which gives them a deeper understanding of how DNA samples are processed prior to analysis. Students also practice micropipetting, an essential skill when performing DNA gel electrophoresis.

Core Themes Addressed

<table>
<thead>
<tr>
<th>Microbial Cell Biology</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial Genetics</td>
<td></td>
</tr>
<tr>
<td>Microorganisms and Humans</td>
<td></td>
</tr>
<tr>
<td>Microorganisms and the Environment</td>
<td>X</td>
</tr>
<tr>
<td>Microbial Evolution and Diversity</td>
<td></td>
</tr>
<tr>
<td>Other – Ecology, species diversity</td>
<td>X</td>
</tr>
</tbody>
</table>

Keywords

Gel electrophoresis, DNA analysis, indicator species, ecology

Learning Objectives

At completion of this activity, learner will:

1. Describe the process of gel electrophoresis in own words, explain how and why it works.
2. Identify 3 potential uses of DNA analysis.
3. Explain why scientists use PCR on DNA samples.
4. Read the resultant gel and articulate the results of the DNA comparison.
National Science Education Standards Addressed

**Standard A**: Science as inquiry
- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

**Standard C**: Life science
- Interdependence of organisms
- Behavior of organisms

**Standard E**: Science and technology
- Abilities of technological design
- Understandings about science and technology

**Standard F**: Science in personal and social perspectives
- Personal and community health
- Natural resources
- Environmental quality
- Natural and human induced hazards
- Science and technology in local, national, and global challenges

**Standard G**: History and nature of science
- Science as a human endeavor
- Nature of scientific knowledge
Student Prior Knowledge

Students should know what DNA is, how it replicates, and what human impact means. Use Powerpoint 1 to provide students with background.

Teacher Background Information

Micropipettes are standard laboratory equipment in micro and molecular biology laboratories. They are used to measure and transfer small volumes of liquids.

Disposable plastic tips are placed on the end of the micropipettor for use in measuring liquids.

The plunger on top of the micropipette can be twisted to set the desired volume of liquid to be measured. It also has two different stopping points when depressed. The first stopping point is used to measure out the desired volume of liquid. The second stop is only used for the complete discharging of liquid from the tips.

Micropipettes are expensive and certain rules should be followed. Never adjust the volume beyond the specific micropipette’s range. Always keep pipettes upright when liquid is in the plastic tip.

Class Time

This activity will require a minimum of one 90 minute class period.

1. Introductory PowerPoint – gel scenario explanation. (~15 minutes)
2. Practice micropipetting. (~30 minutes)
3. Virtual DNA extraction and PCR labs. (~45 minutes)

Teacher Preparation Time

This lesson will require approximately 10 minutes of preparation time:

Set up 8 trays with 1 15ml tube with plain water, 1 tube with colored water, and 6 empty 1.5 microcentrifuge tubes.

Safety Precautions

Students should use safety glasses when working with liquids. Food coloring has the capacity to stain clothing, so warn students and/or provide them with aprons.
Materials and Equipment

1. Access to a computer lab or laptop cart with internet access
2. Micropipetting practice, supplies per group of 3-4 students:
   a. 6 empty 1.5ml microcentrifuge tubes
   b. Food coloring
   c. Micropipette
   d. 1.5ml microcentrifuge tube rack
   e. 20 pipette tips
   f. Plain water
   g. 1 waste container (Tupperware, cup, or any other small container)
   h. Safety glasses
3. Micropipetting practice, supplies per class:
   a. Plain water
   b. 2 practice gels
   c. 2 micropipettes
   d. Rectangular dish (to hold practice gel)
   e. 4ml loading dye
   f. 2 boxes of pipette tips

Methods

1. Show students how to operate a micropipette prior to distributing any micropipetting supplies. Define first stop, second stop, and plunger.
2. Distribute micropipetting handout. Have students fill in the correct order of the micropipetting procedure. Check the handout, and distribute supplies to groups that have correctly completed the procedure.
3. Students will pipette different amounts of water and dye to familiarize them with the process.
4. After students finish practicing, have them come up to the front bench. Guide students in the loading of a practice gel. The practice DNA gels are quite durable, so remind students that real gels are much more fragile.
5. After successful completion of the gel loading, give students the handout for the virtual labs and allow them to complete that part of the activity.
References

- This activity was modified from a lab created by Michelle Carlson of the Molecular Biology Initiative (MBI). Original content can be found on the MBI website, at: http://cost.georgiasouthern.edu/biology/mbi/ (Last accessed: 2/29/12)
- The virtual PCR and DNA extraction activities use the wonderful virtual laboratories created by the University of Utah. Labs can be accessed at: http://learn.genetics.utah.edu/ (Last accessed: 2/29/12)

Answers to Student Handouts

Practice Micropipetting

What is the correct order when preparing for pipetting?

Push down on the plunger to the second stop  __9__
Push down on the plunger to the first stop  __4,8__
Put on pipette tip  __2__
Release pipette plunger  __10__
Prepare all liquids  __1__
Place pipette in liquid  __5__
Slowly release the pipette plunger  __6__
Place pipette over new container  __7__
Push down on the plunger to the first stop  __4,8__
Set your pipette to the correct volume  __2__

Virtual Labs

Follow along the DNA extraction virtual lab (http://learn.genetics.utah.edu/content/labs/extraction/) and answer these questions in complete sentences.

1) What are three ways DNA can be used?

   1. Test for genetic disease.
   2. Analyze forensic evidence
   3. Study a gene involved in cancer.

2) Where is DNA located? How much DNA can be found in one cell?

   DNA is located in the nucleus of each cell. Around two meters of DNA is in each nucleus.
3) What kind of cells are we using to isolate DNA?

We are using epithelial cells from inside the cheek.

4) What are the four steps to purify DNA?

1. Collect cheek cells
2. Burst cells open to release DNA
3. Separate DNA from proteins and debris
4. Isolate concentrated DNA

5) What are the 10 materials used in the DNA purification?

1) Warm water bath, 2) Buccal swab, 3) Micropipettes, 4) Lysis solution, 5) Concentrated salt solution, 6) Sample tubes, 7) Resuspension buffer, 8) Ethanol, 9) Isopropyl alcohol, 10) Centrifuge

6) What is the purpose of the lysis solution?

The purpose of the lysis solution is to degrade the cell membranes and release DNA.

7) What is the purpose of the salt solution?

The salt solution causes proteins and cellular debris to clump together.

8) How does a centrifuge work?

A centrifuge spins at a very high speed and causes objects in solution to separate based on mass (in this example heavy proteins and debris accumulate at the bottom of the tube while DNA stays in solution).

9) What can you do with DNA once it is extracted from a cell?

DNA can be frozen or used for various analyses once it is extracted.

Follow the PCR virtual lab (http://learn.genetics.utah.edu/content/labs/extraction/) and answer these questions in complete sentences.

10) What does PCR do?

PCR takes a specific length of DNA and copies it many times.

11) How many copies of DNA can PCR create?

PCR can copy DNA segments billions of times.
12) List at least four types of cells that you can extract DNA from.

DNA can be extracted from blood, saliva, skin cells and hair follicles.

13) What is the first step of PCR? Why do you perform that step?

The first step of PCR is the addition of primers. They are added so that only a specific DNA sequence is copied – otherwise the entire length of DNA would be copied.

14) What are primers? What is the purpose of adding primers?

Primers are short sequences of DNA that match each end of the DNA segment that is meant to be copied. They attach to each side of the sequence and direct DNA polymerase to begin transcribing DNA at that location.

15) What is the purpose of adding nucleotides to the PCR tube? Can PCR work without nucleotides?

Nucleotides are the building blocks of DNA. They are needed in order for new lengths of DNA to be generated. No, PCR will not be able to create new DNA lengths without available nucleotides.

16) What is DNA polymerase? How does it function?

DNA polymerase is a complex of proteins that copies a cell’s DNA during cell division.

17) What temperature does the thermocycler reach first? Why does it get so hot?

The first temperature reached by the thermocycler is 95°C which causes the DNA double helix to separate and form two strands of single stranded DNA.

18) There are always far more primers than DNA in the PCR tube. Why?

The overabundance of primers makes it probable that a primer will attach to each single strand of DNA before they have a chance to reattach.

19) How many cycles have to pass before you start getting your desired fragments?

After the third cycle desired fragments of DNA are obtained.

20) How many fragments are produced after 5 cycles? After 30?

After 5 cycles 32 fragments are present. After 30 cycles over one billion fragments are present.
Introduction

Humans continuously shape the environments they live in, intentionally or unintentionally. To examine the unanticipated repercussions of pesticide spraying, we will conduct a simulated genetic analysis of the mosquito species present in an area that is not supposed to receive pesticide spraying. To prepare for our genetic analysis, you will first practice micropipetting, then conduct 2 virtual labs. The virtual labs lead you through the process of DNA extraction, the means by which we isolate DNA, and polymerase chain reaction (PCR), an essential process that can rapidly produces millions of copies of a DNA sample.

Student Background Knowledge

Students should know what DNA is, how it replicates, and what human impact means.

Vocabulary

Restriction enzyme: an enzyme used to cut DNA; recognizes a specific sequence of bases and cuts the DNA strand at that exact location

Gel electrophoresis: a means of separating DNA fragments based on size, allowing for comparison of multiple DNA samples

DNA extraction: a method for isolating usable genomic DNA from cells

Polymerase chain reaction (PCR): a method of making multiple copies of a DNA sequence, involving repeated reactions with a polymerase

Species diversity: the number of species in a particular community or habitat

Indicator species: an animal or plant species that can be used to assess the ecological health or condition of a particular habitat

Safety Considerations

Have students wear safety goggles when dealing with liquids. Since food coloring can stain clothes, give warn students and give them the option to wear aprons.
Materials Checklist

<table>
<thead>
<tr>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5ml tube rack</td>
</tr>
<tr>
<td>Empty 1.5ml microcentrifuge tubes (6)</td>
</tr>
<tr>
<td>15ml tube with clear water</td>
</tr>
<tr>
<td>15ml tube with colored water</td>
</tr>
<tr>
<td>5-50μl micropipette</td>
</tr>
<tr>
<td>Box of micropipette tips</td>
</tr>
<tr>
<td>Safety glasses (1/student)</td>
</tr>
<tr>
<td>Waste container</td>
</tr>
</tbody>
</table>

Procedure

Exercise 1: Micropipetting practice

1) Place 6 of the 1.5 mL tubes into the tube rack
2) Label tubes 1-6
3) Pipet the correct amount of water into each of your tubes (see table)
4) Pipet the correct amount of food coloring into each of your tubes (see table)

Table 1: The amount of liquid to be placed in each tube.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Amount of water</th>
<th>Amount of food coloring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>45</td>
</tr>
</tbody>
</table>

5) Check each tube for pipetting accuracy
   a. Set pipet to 50 uL
   b. Pipet out all liquid from tube 1
   c. Check for empty space at the bottom of your pipet tip or excess liquid in tube
   d. Place liquid back in tube 1
   e. Repeat for tubes 2-6

Exercise 2: Gel loading practice

Using the techniques you just learned each student should practice pipetting into the practice gel. There are 2 major rules for pipetting into gels:
- Never place the pipet tip into the gel well (you could puncture the gel).
- Never push the plunger to the 2nd stop (introduces air bubbles into the well).

1. ____ See your instructor to practice pipetting 10 µl of loading dye into the practice gel.

2. ____ Write down the 2 major rules for pipetting into gels in your lab book.

**Exercise 3: Virtual Labs**

1. Follow along the DNA extraction virtual lab ([http://learn.genetics.utah.edu/content/labs/extraction/](http://learn.genetics.utah.edu/content/labs/extraction/)) and answer the questions on the worksheet in complete sentences.

2. Follow the PCR virtual lab ([http://learn.genetics.utah.edu/content/labs/pcr/](http://learn.genetics.utah.edu/content/labs/pcr/)) and answer the questions on the worksheet in complete sentences.
Student Worksheet
Practice Micropipetting

Name: ______________________________________
Block:__________

(Adapted from Michelle Carlson)

Objective: This lab will be used to practice correct pipetting techniques

Warm-up Question:

1) What is the correct order when preparing for pipetting?
   - Push down on the plunger to the second stop ___
   - Push down on the plunger to the first stop ___
   - Put on pipette tip ___
   - Release pipette plunger ___
   - Prepare all liquids ___
   - Place pipette in liquid ___
   - Slowly release the pipette plunger ___
   - Place pipette over new container ___
   - Push down on the plunger to the first stop ___
   - Set your pipette to the correct volume ___

Materials: (groups of 3)

<table>
<thead>
<tr>
<th>1.5 mL tubes (6)</th>
<th>Food coloring (1)</th>
<th>Pipet (1)</th>
<th>1.5 mL tube rack (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipet tips (20)</td>
<td>Practice gel (1)</td>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

Exercise 1: Learning to Pipet

1) ____Place 6 of the 1.5 mL tubes into the tube rack
2) ____Label tubes 1-6
3) ____Pipet the correct amount of water into each of your tubes (see table)
4) ____Pipet the correct amount of food coloring into each of your tubes (see table)

Table 1: The amount of liquid to be placed in each tube.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Amount of water</th>
<th>Amount of food coloring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>
5) Check each tube for pipetting accuracy
   a. ___ Set pipet to 50 uL
   b. ___ Pipet out all liquid from tube 1
   c. ___ Check for empty space at the bottom of your pipet tip or excess liquid in tube
   d. ___ Place liquid back in tube 1
   e. ___ Repeat for tubes 2-6

Question:
How accurate was your group at pipetting? How do you know how accurate you were?
_____________________________________________________________________________
_____________________________________________________________________________

Exercise 2: Gel loading practice

Using the techniques you just learned each student should practice pipetting into the practice gel. There are 2 major rules for pipetting into gels:

- Never place the pipet tip into the gel well (you could puncture the gel).
- Never push the plunger to the 2nd stop (introduces air bubbles into the well).

1. ___ See your instructor to practice pipetting 10 µl of loading dye into the practice gel.
2. ___ Write down the 2 major rules for pipetting into gels in your lab book.
Follow along the DNA extraction virtual lab (http://learn.genetics.utah.edu/content/labs/extraction/) and answer these questions in complete sentences.

1) What are three ways DNA can be used?

2) Where is DNA located? How much DNA can be found in one cell?

3) What kind of cells are we using to isolate DNA?

4) What are the four steps to purify DNA?

5) What are the 10 materials used in the DNA purification?

6) What is the purpose of the lysis solution?

7) What is the purpose of the salt solution?
8) How does a centrifuge work?

9) What can you do with DNA once it is extracted from a cell?

Follow the PCR virtual lab (http://learn.genetics.utah.edu/content/labs/pcr/) and answer these questions in complete sentences.

10) What does PCR do?

11) How many copies of DNA can PCR create?

12) List at least four types of cells that you can extract DNA from.

13) What is the first step of PCR? Why do you perform that step?

14) What are primers? What is the purpose of adding primers?

15) What is the purpose of adding nucleotides to the PCR tube? Can PCR work without nucleotides?
16) What is DNA polymerase? How does it function?

17) What temperature does the thermocycler reach first? Why does it get so hot?

18) There are always far more primers than DNA in the PCR tube. Why?

19) How many cycles have to pass before you start getting your desired fragments?

20) How many fragments are produced after 5 cycles? After 30?