Are deer ticks carrying Lyme disease?

Contributors

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Intended Audience

<table>
<thead>
<tr>
<th>Grade</th>
<th>Status</th>
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<tbody>
<tr>
<td>K-4</td>
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<td>5-8</td>
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Intended Audience

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

Description

Students will compare extracted DNA by comparing DNA bands using agarose gel electrophoresis.

Abstract

Students will go through a computer-based polymerase chain reaction (PCR) followed by a gel-electrophoresis computer simulation. Students will load practice gels in order to gain micropipetting skills. Students will view the results of a gel electrophoresis DNA from DNA they extracted in a previous lab (Tick DNA Extraction), or an example photo of a gel will work (example is included in the powerpoint). Students will determine which DNA bands were genetically similar based on the gel photo.

Core Themes Addressed

<table>
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<tr>
<th>Microbial Cell Biology</th>
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<tr>
<td>Microbial Genetics</td>
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<td>Microorganisms and Humans</td>
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<td>Microorganisms and the Environment</td>
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<td>Microbial Evolution and Diversity</td>
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<td>Other –Comparing genetics</td>
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Keywords

Deoxyribonucleic acid, gel electrophoresis

Learning Objectives

At completion of this activity, learner will

1. List the steps of PCR along with their significance
2. Load gels correctly
3. Draw conclusions based on DNA bands as to which DNA bands were genetically similar

National Science Education Standards Addressed

Standard C: Life Science
– DNA/genetic comparison.
Are deer ticks carrying Lyme disease?

Student Prior Knowledge

- DNA extraction (see Tick DNA extraction lab)
- Be able to properly use micropipetters
- Be able to use the computer simulation programs

Teacher Background Information

Students will go through a computer-based polymerase chain reaction (PCR) followed by a gel-electrophoresis computer simulation. Students will load practice gels and an actual gel electrophoresis. After students become familiar with the techniques, they will look at a photo of a gel and determine which DNA bands of the ticks are positive for Lyme disease based on band brightness/presence. It is important to be able to accurately operate a micropipette and understand how gel electrophoresis works.

Class Time

This activity will require a minimum of one 50-minute class period

Teacher Preparation Time

This lesson will require approximately 10 minutes of preparation time.

1.) Fill eight 1.5 microcentrifuge vials with colored water (water + 1 drop food coloring)
2.) Place eight practice gels around the lab to create stations.
3.) Make 4 agarose electrophoresis gels by adding .75 grams of agarose to 75 ml of buffer and heating in the microwave for each gel. (Gel electrophoresis boxes will be placed between groups so they can be shared between 2 groups.)

Safety Precautions

All students should wear the appropriate safety attire (minimally aprons and goggles, and gloves if available).
Materials and Equipment

Each group of 4 will have:

- Computer with internet access
- 1 practice gel
- 1 1.5 ml microcentrifuge vial filled with colored water
- 1 5-50 microliter pipette
- 1 box pipette tips
- 1 beaker (for used tips)

Methods

1. Set computer to PCR simulation (website under references).
2. Open a new internet tab and pull up the gel electrophoresis simulation (website under references).
3. Have students go through each simulation along with you in front of the classroom.
4. Break students into groups of 4 and instruct them to go to a station.
5. Each person will get to load 2 practice wells in the practice gel.
6. Everyone will get a chance to load one well in the gel electrophoresis.
7. Call groups to the front and let them load DNA.
8. After each group is completed, place the lid on the gel box, oriented from black to red and start the gel.
9. Show students a photo of a sample gel and let them tell you which DNA is similar based on the bands shown.

Tips/Suggestions

- Be sure that all students have completed a practice gel. You can prepare an actual gel electrophoresis if gel boxes and gel ingredients are available, and allow each student to fill one well. It would be beneficial for students to see this step so they can link all the steps of gel electrophoresis together.
  - To prep gel add ~15 minutes to class prep times and complete the following steps:

- To make gel:
  - Gel should be made in a 200ml gel.
  - Mix .75 grams agarose with 75ml TBE buffer in a 200ml flask
  - Microwave mixture in short bursts (~20 seconds each) until agarose powder is dissolved into the buffer
  - Set-up gel box with combs for wells in place
  - Pour in buffer/agarose mixture
  - Allow gel 15-20 minutes to Harden
References

Original lab by Derek Tucker (on MBI website)

Extension/Additional Resources

learn.genetics.utah.edu/content/labs/pcr/ (The computer-based polymerase chain reaction (PCR) simulation) (Last accessed 2/26/2012)

learn.genetics.utah.edu/content/labs/gel/(The computer-based gel-electrophoresis computer simulation) (Last accessed 2/26/2012)

Answers to Student Handouts

Gel Electrophoresis Questions
1. What is the purpose of agarose gel electrophoresis?
We use gel electrophoresis in order to compare DNA sequences.

2. Why does the DNA migrate from negative to positive?
DNA migrates from negative to positive because DNA is negatively charged (opposites attract).

3. Do small or large DNA fragments migrate further down the gel? Why?
Smaller DNA fragments will migrate further down the gel because they will pass more easily through the gel matrix than larger pieces of DNA.

4. How are we able to see the DNA bands?
We are able to view DNA bands through UV lighting and staining.

5. What is the purpose of PCR (Polymerase Chain Reaction)?
PCR is a tool used to amplify DNA.

6. Where is the DNA located?
DNA is located in the nucleus of the cell.

7. What does a restriction enzyme do?
Restriction enzymes cleave DNA at specific sites so that the desired fragment of DNA is cut out.

Results
8. Were any of the ticks carrying Lyme disease? Was your prediction correct?
Yes, the first two ticks were carriers of Lyme disease.
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Introduction

Gel electrophoresis is useful when comparing the likeness of DNA. This tool is used often in paternity testing and testing for genetically inherited diseases. Gel electrophoresis works by pulling various strands of DNA negative to positive regions through an electrically charged gel matrix. Larger pieces of DNA will not travel as far through the gel as smaller pieces of DNA, because they will get caught in the gel. Use agarose gel electrophoresis (DNA fingerprinting) to find out if the ticks we extracted DNA from last week were carriers of Lyme disease.

Student Background Knowledge

- Be able to properly use micropipettes
- Have a basic understanding of PCR and gel electrophoresis

Vocabulary

Deoxyribonucleic acid: a double-stranded nucleic acid that contains the genetic information for cell growth, division, and function

Gel electrophoresis: a molecular technique used to separate and compare lengths of strands of DNA

Safety Considerations

Be sure you are extremely careful when plugging in gel electrophoresis (gels should run from negative to positive or black to red).

Materials Checklist

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Procedure

- Watch and follow along with PCR simulation.
- Break into groups of 4 and go to a station.
- Each person will get to load 2 practice wells in the practice gel.
- Everyone will get a chance to load one well in the gel electrophoresis. Wait until your group is called, then come to the front to load DNA.
- The instructor will place the lid on the gel box, oriented from black to red and start the gel.
- We will view the results of last week’s DNA extraction.
Are ticks carrying Lyme disease?

Name:
Date:
Period:

Are ticks in Southeast Georgia carrying Lyme Disease?

Objective: Use agarose gel electrophoresis (DNA fingerprinting) to see if any ticks we extracted DNA from last week were carrying Lyme disease.

Hypothesis: Make a prediction concerning the percentage of ticks carrying Lyme disease.

FYI: Agarose gel electrophoresis is a method used to separate molecules based on size using an electric field. The gel is placed in a box and covered with a buffer solution. When hooked up to a source of electricity, the DNA will migrate from the negative electrode (black) to the positive electrode (red). It does this because DNA is a negatively charged molecule. DNA fragments migrating through the gel are separated based on size. Small fragments meet less resistance and thus travel further through the gel. DNA itself is not visible within the gel so we add a dye that attaches to the DNA.

Draw in the bands you see in the resulting gel:
Procedure:

1. Watch and follow along with PCR simulation.
2. A person from each group will get a chance to load one well in a gel. Wait until your group is called, then come to the front to load DNA.
3. We will view the results of last week’s DNA extraction.

Gel Electrophoresis Questions
1. What is the purpose of agarose gel electrophoresis?

2. Why does the DNA migrate from negative to positive?

3. Do small or large DNA fragments migrate further down the gel? Why?

4. How are we able to see the DNA bands?

5. What is the purpose of PCR (Polymerase Chain Reaction)?

6. Where is the DNA located?

7. What does a restriction enzyme do?

Lyme Disease

8. Were any of the ticks carrying Lyme disease? Was your prediction correct?