Lesson 2: Is it Genetically Modified?

**Explain**

1) Present the question on slide 23 of the provided PowerPoint to students: “How could you detect the presence of genetically modified organisms in your food?” Ask students to discuss briefly with their neighbor and then ask for suggestions from the class.

2) Use slide 24 to introduce PCR, and then have students watch the DNA Learning Center’s 3-D PCR animation (http://www.dnalc.org/resources/3d/19-polymerase-chain-reaction.html). Review what students have just seen using slide 25 and ask students the following questions in a think-pair-share format.

   a. Why do scientists need to make copies of a region of DNA? Why can’t they just use isolated DNA from your cells (which contains the region of interest) without amplifying it?
     Genomic DNA from a single diploid cell has only two copies of the DNA region of interest.
     PCR allows you to generate billions of copies of the DNA you’re interested in without extracting genomic DNA from billions of cells. PCR also allows you to increase the concentration of the DNA you are interested in relative to the rest of the genomic DNA that you are not interested in.

   b. Think about the structure of DNA. When the temperature in a PCR reaction is raised in order to denature or separate the strands, what interactions are disrupted?
      The hydrogen bonds between paired bases on each strand are disrupted.

   c. Think about the point in PCR when primers become important. Primers are usually used at a high concentration relative to the template DNA. Why?
      Primers become important after the template DNA has been denatured and the temperature is being cooled. Theoretically, the two template DNA strands could re-anneal to each other during this step. However, the high concentration of primers ensures that primer molecules are much more prevalent and therefore more likely to anneal to the template strands rather than the template strands re-annealing to each other.

   d. When primers bind or anneal, what interactions are they forming with the template DNA strand? What about this annealing step in the cycle allows these interactions to form?
      When primers anneal to the template DNA, they are forming hydrogen bonds between complementary base pairs. The cooler temperature during the annealing step allows these bonds to form.

   e. Why are primers needed in PCR? (Hint: Think about DNA replication.)
      DNA polymerases require a 3’ end to extend from; they cannot synthesize a complementary strand spontaneously.

3) Have students complete the first part of the exercise in Appendix I. Students should compare results with a partner before starting the second part. Students may work on the second part on their own or with their partners.

   Students may struggle with the 2D representation of DNA. Explain that the given sequence is
one continuous strand of a section of DNA. Scientists use this shorthand instead of drawing out the structure of a section of DNA.


4) Draw a gel with wells on a piece of chart paper. Along the side, draw an arrow indicating the direction of movement of DNA fragments. In groups of 3-4, ask students to determine which ends of the gel correspond to the positive and negative electrodes. Ask for a volunteer to share his/her group’s answer and explanation.

5) Tell the groups that a DNA sample with the following fragments of known size was run in the first lane: fragment A - 150 bp, fragment B - 250 bp, fragment C – 150 bp and fragment D - 500 bp. Two PCR reactions designed to amplify the same 200 bp DNA fragment were run in lanes two and three. The reaction in lane two worked correctly, however the reaction in lane three failed. Ask students to determine what the resulting gel would look like. Ask for a volunteer to share his/her group’s answer and explanation.

6) Finally, ask students to think about how PCR coupled with agarose gel electrophoresis could be used to detect genetically modified organisms. What region of DNA would they amplify? If you know the sequence of the DNA that has been added to an organism, you can design primers to PCR amplify a section of it.

Elaborate

1) This activity will use Bio-Rad’s GMO Investigator Kit Plus Small DNA Electrophoresis Reagent Pack (http://www.bio-rad.com/prd/en/US/LSE/PDP/1128f1a0-662c-4450-ad12-1b3634f4f18b/GMO-Investigator-Kit). The instruction manual is available free, but you must order a paper copy through an online account. Each kit contains enough materials for eight groups of four students to perform the experiment.

2) Organize students into lab groups of four and provide each student with a copy of the lab report rubric (Appendix II). Provide each group with the list of GMO crops the FDA or USDA’s APHIS unit have evaluated (http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=bioListing or http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml#not_reg), as well as the USDA’s consumer information on certified organic products (http://www.ams.usda.gov/AMSv1.0/nop). Ask each group to identify the food they would like to test for GMOs and form a hypothesis (supported by reasoned explanation) as to whether they think the food will contain GMOs or not. Students may either seek to discover if conventional products contain GMOs or to confirm that organic products do not contain GMOs. Students should be asked to bring their test food in to the next class period.

3) For homework, students should read the Quick Guide protocol (pgs. 30-32), write down any questions they have, and complete the questions in Appendix III. Do not give students the Student Manual, as it explains information that students will be asked to discover on their own.

4) Before beginning the lab, discuss any student questions arising from the Quick Guide protocol, especially those that concern technical details.

5) We suggest the following student roles at each step. Before beginning the experiment, have each group decide who will perform what role. Make sure that each student has at least one pipetting role.

a. DNA extraction
To Pearson, c. b. c. 

ii. Weighing/grinding – this person prepares the non-GMO and test slurries

iii. Pipetting – this person pipettes the slurries into the appropriate tubes

iv. Incubating/centrifuging – this person performs the water bath and centrifuging steps with the help of the timer

b. PCR

i. Labeling – this person labels the PCR tubes

ii. Reading – this person calls out the appropriate reagent to add to each tube to the pipetters

iii. Master mix pipetting – this person transfers the appropriate master mix to each PCR tube based on the reader’s instructions

iv. Sample pipetting – this person transfers the appropriate sample to each PCR tube based on the reader’s instructions

c. Electrophoresis

i. Gel chamber setup – this person sets up the gel chamber with buffer and the gel

ii. Sample prep – this person spins down the PCR tubes and adds loading dye

iii. Load and run the gel – this person loads the prepared samples and the ladder on the gel and starts the gel running

iv. Stain and photograph – this person stains the gel and photographs it for the group

6) To make sure students understand why they are performing each step, we propose some questions before each phase of the experiment. Students should discuss with each other as necessary, and/or as a class, and record their answers. This kit breaks down into the following chunks of lab activity:

a. Isolate DNA from food samples.

Before students begin, ask, What is the first step you need to complete to analyze your test food? What physical processes does this entail?

b. Amplify DNA by PCR.

i. Before students begin, ask, What is the purpose of this step? What ingredients and/or cycling steps ensure that you will amplify the piece of DNA you intend to amplify during a PCR reaction?

ii. Once the PCR reactions are running, this is a good time to introduce students who do not have experience with gel electrophoresis to the delicate task of loading a gel. Submerge a practice gel (does not need to contain EtBr) in buffer and allow students to take turns loading 5-10 µL of loading dye into the wells. (6X dye can be purchased cheaply from many laboratory supply companies.) Students should practice until they feel comfortable with their ability to load wells without piercing them or pipetting the sample into the buffer.

c. Analyze PCR products by gel electrophoresis.

i. Before students begin, ask, What is the purpose of this step? What is the purpose of the size markers?

ii. Check each group’s gel set up before they turn on the power supply.

iii. While the gels are running, students can work on their lab reports.

iv. If students photograph their stained gels, the photo can serve as their raw data and there is no need to dry the gels.

d. If your school does not have a thermocycler or gel electrophoresis equipment, consider contacting a local college/university or biotech company. Labs may have equipment they may be
willing to donate or lend.

(e.) Throughout the lab, students should take notes on their procedures they are doing, the goal of each step, and any questions they may have.

**Evaluate**

1) Students should complete their lab reports.

2) Have each group project the picture of its gel for the class and present its data and conclusions. Groups with controls that did not give expected results may be hesitant to present their data. Reassure these students that experiments do not always work, and that they should not be embarrassed as long as they analyze their results thoroughly and objectively and can give a reasonable explanation of what went wrong.
Appendix I—PCR Activity (adapted from Bio-Rad’s GMO Investigator Kit manual)

Examine the 150-base sequence (below) from the *Agrobacterium tumefaciens* nopaline synthase terminator.

1) Write in the sequence of the complementary strand on the line below the given sequence and mark the 3' and 5' ends of the complementary strand.

2) Design a forward PCR primer and a reverse PCR primer, each 16 bases long, to amplify a target sequence from the given DNA that is 100 bp long (Hints: With respect to 5’ and 3’ ends, how do DNA strands hybridize? What end does DNA polymerase extend from? Are the primers included in the final product’s length?). Write the sequence of the primers between the given sequence and the complementary sequence, with their 3’ and 5’ ends indicated. Also indicate which strand each one will anneal to.

5’...TGCAGGCATGCAAGCGATCCCCGATCGTTCAACACATTTGGCAATAAAGTT
_______________________________________________________________
TCTTAAGATTGAATCCTGTGGCCGCTTTGCATGATTATCATATAATTTCTGT
_______________________________________________________________
TGAATTACGTAAAGCATGTAATAATTAACATGTAATGCATGACGT...3’
## Appendix I— Lab Report Rubric

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<thead>
<tr>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td><strong>Problem Definition</strong></td>
<td>Statement of the problem is limited or missing altogether.</td>
<td>Problem is poorly stated with major omissions.</td>
<td>Problem is adequately stated with minor omissions.</td>
<td>Problem is clearly and completely stated.</td>
</tr>
<tr>
<td><strong>Identification of Variables</strong></td>
<td>No identification of independent, dependent, and controlled variables.</td>
<td>Independent, dependent, and controlled variables are mentioned, but incorrectly identified.</td>
<td>Most variables are correctly identified as independent, dependent, or control, but at least one in incorrectly identified.</td>
<td>Independent, dependent, and controlled variables are all clearly and correctly identified.</td>
</tr>
<tr>
<td><strong>Hypothesis</strong></td>
<td>Hypothesis is limited or missing altogether.</td>
<td>Hypothesis poorly predicts the influence of the independent variable on the dependent variable, with a limited or no explanation.</td>
<td>Hypothesis somewhat predicts the influence of the independent variable on the dependent variable, with an adequate explanation.</td>
<td>Hypothesis clearly predicts the influence of the independent variable on the dependent variable, with a good explanation.</td>
</tr>
<tr>
<td><strong>Experimental Design</strong></td>
<td>Experimental design does not match the stated problem, is incomplete or missing. No attempt to control variables. Diagram of set-up is limited or missing altogether.</td>
<td>Experimental design matches stated problem to some extent. Little attempt to control variables. Procedures are incomplete. Major modifications or clarifications needed. Poor diagram of set-up.</td>
<td>Experimental design generally matches stated problem. Attempt at controlling variables made. Procedures are generally complete. Minor modifications or clarification needed. Good diagram of set-up.</td>
<td>Experimental design matches stated problem. Variables are controlled. Procedures are clear, complete, and replicable. A control is included when appropriate. Diagram of set-up is exceptionally clear.</td>
</tr>
<tr>
<td><strong>Data Presentation</strong></td>
<td>Data table and graph are very poorly organized, presented inappropriately, or missing altogether.</td>
<td>Data table and graph are poorly organized or presented inappropriately. Major omissions or errors present.</td>
<td>Data table and graph are presented appropriately. Minor errors or omissions present.</td>
<td>Data table and graph are very well organized and presented appropriately.</td>
</tr>
<tr>
<td><strong>Conclusions</strong></td>
<td>Conclusions are not related to stated problem, not supported by data or are missing.</td>
<td>Conclusions are related to stated problem and supported by limited data. Major errors in interpretation of results.</td>
<td>Conclusions are generally related to stated problem and supported by data. Minor errors in interpretation of results.</td>
<td>Conclusions are related to stated problem and fully supported by data.</td>
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Appendix III – Questions about the Protocol

1) In part one, you will be extracting DNA from two different samples. In living cells, where does DNA reside? What barrier(s) separate it from the cell’s outside environment? Given this information, why do you think it is necessary to extract DNA before doing anything else?

The Plant Master Mix contains primers designed to amplify 455 bps of a gene found in all plants, while the GMO Master Mix contains primers designed to amplify DNA sequence that is common only to plants that have been genetically modified (specifically, these primers amplify 200 bps of the most commonly used promoter and terminator sequences for GMO gene constructs).

2) Why is it important to set up a PCR reaction with your test food using the Plant Master Mix alongside the PCR reaction using the GMO Master Mix? What kind of control (i.e., positive or negative) is the Plant Master Mix PCR and what is it testing for?

3) Why are you also performing PCR with the Plant Master Mix and the GMO Master Mix on certified non-GMO food? What kind of control is the certified non-GMO food? If you are successful in extracting DNA from the certified non-GMO food and this sample does not become contaminated, what results do you expect in the Plant Master Mix PCR? What about the GMO Master Mix PCR?

4) Why are you also performing PCR with the Plant Master Mix and the GMO Master Mix on GMO positive control DNA? What results do you expect in the Plant Master Mix PCR? What about the GMO Master Mix PCR?
5) What results do you expect in each lane? Fill in the chart below.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Expect band (Yes, No, Don’t know)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample 1: Non-GMO food control with plant primers</td>
<td></td>
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<tr>
<td>2</td>
<td>Sample 2: Non-GMO food control with GMO primers</td>
<td></td>
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<tr>
<td>3</td>
<td>Sample 3: Test food with plant primers</td>
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<td>4</td>
<td>Sample 4: Test food with GMO primers</td>
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<tr>
<td>5</td>
<td>Sample 5: GMO positive control DNA with plant primers</td>
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<tr>
<td>6</td>
<td>Sample 6: GMO positive control DNA with GMO primers</td>
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