Appendix F
Teacher Answer Guide

Lesson 1  Extraction of DNA from food samples

1. How can you test a food to find out if it contains material derived from a genetically modified organism (GMO)?

   There are two methods to test for foods containing GMOs. The ELISA test is used to see if particular proteins are in a sample. PCR is used to amplify regions of GMO genomes.

2. In what organelles is plant DNA located?

   Plant DNA is not only found in the nucleus, it is also found in the mitochondria and chloroplasts. Plants and other autotrophic organisms are the only organisms with chloroplasts. Plant DNA is more difficult to obtain intact because the cell wall must be destroyed.

3. Many foods containing GM crops are highly processed. Can you suggest how DNA from whole plants may differ from that extracted from processed foods, e.g., corn chips, cornmeal, etc.?

   High temperatures or physical manipulation of the plant tissue during processing may destroy or fragment DNA.

4. What molecules are present in the cell that might interfere with DNA extraction?

   Enzymes, such as DNases, may degrade DNA. Metal ions act as cofactors and coenzymes for enzymes that degrade DNA. Cellulose plant cell walls may act as a barrier to DNA extraction.

5. Why do you also perform analysis on food that is known to be a non-GMO control?

   To make sure samples have not been contaminated. It is also used as a comparison to show how a non-GMO banding pattern should look.

6. Why was the non-GMO food control prepared prior to your test food sample?

   In the grinding process, airborne particles can travel through the air and contaminate samples of non-GMO foods. Also a mortar and pestle that is not properly washed can transfer minute sample. PCR only needs ONE molecule of DNA to make amplified product.

Lesson 2  Set Up PCR Reactions

1. What chemicals and molecules are needed for PCR, and what is the function of each component?

   - Taq DNA polymerase – a polymerase that is not sensitive to heat. It links the deoxynucleotide triphosphates to make a DNA strand that is complementary to the template
   - Deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP) – the basic units that are connected to make the complementary strand
   - Primers – short sequences of DNA that serve as beginnings of newly synthesized DNA.
   - Buffers and cofactors needed to make the reaction take place at an optimal rate
3. Examine the 150 base sequence below.

5'TAGAAAAGGA AGGTGGCTCC TACAAATGCC ATCATTGCGA TAAAGGAAAG
3'ATCTTTTCT TCACCAGGAGATGTTACCG ATCAAACGCT ATTTCCCTTTC
forward 5'AGGAAGGTGG3'

GCTATCATTC AAGATGCTTC TGCGACAGT GGTCGCAAAG ATGGACCCCC
CGATAGTAAG TTTCTACGGAGACGGCTGTCA CCAGGGTTTC TACCTGCGGG

3'CTTCT GCAAG5' Reverse primer
ACCCACGAGG AGC ATCGTGG AAAAGAAAGA CGTTCAAACC ACGTCTTCAA3'

TGGGTGCTCC TCGTAGCACC TTTTTCTTCT GCAAGGTGG TGCAGAAGTT5'

Write in the sequence of the complementary strand and mark the 3' and 5' ends of the complementary strand (see italics)

Remembering that DNA polymerases can only add nucleotides to the 3' end of DNA, design a forward primer and a reverse primer, each 10 bases long, to amplify a target sequence of the DNA that is at least 100 bp long. Write the sequence of the primers below, with their 3' and 5' ends indicated. Also indicate on the sequence above which strand they are complementary to (will anneal to).

These primers can be any sequence so long as their orientation and complementarity matches these examples in bold.
Forward primer 5'AGGAAGGTGG3'
Reverse primer 3'CTTCTGCAAG5'

If you are teaching about primer design in more depth, you may want to give more criteria for their primer design such as % GC content and primer dimer formation.

4. Why are you performing two PCR reactions on each DNA sample?

One reaction is a control to show we extracted plant DNA using primers to a universal plant DNA sequence. The second reaction is to identify the GMO target sequence.

5. What is the purpose of the GMO positive control DNA?

We want to make sure our PCR reaction worked; if the positive control produces a positive result but I do not get a band in my test sample, the test is most likely non-GMO. If I do not get the 200 base pair band in the positive control, I can assume the PCR reaction did not work.
Lesson 3  Electrophoresis of PCR products

1. Why did you resolve your PCR products by electrophoresis?

   Gel electrophoresis separates DNA molecules based on charge and size. After the bands are separated the gel is stained to visualize the band pattern. We can calculate the size of the DNA molecules, in base pairs, in each band.

2. Explain why DNA fragments separate according to size in an electrophoresis gel.

   DNA is negatively charged and is repelled by the negative electrode (cathode) and attracted by the positive electrode (anode) when an electric current is applied across the gel. It separates because different lengths of DNA move through the gel matrix at different rates. Longer fragments move more slowly than shorter fragments.

3. Why do you need a molecular mass ruler alongside your samples?

   We need a molecular mass ruler to calculate the size of each of our bands. We know exactly how many bands are in the ruler and the size of each of those bands. We can graph the size of the bands against the distance they moved in the gel to create a standard curve. We can then measure the distance our PCR product bands moved in the gel and use our standard curve to calculate the sizes of the product bands.

4. 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</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample 1: Non-GMO food control with plant primers</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Sample 2: Non-GMO food control with GMO primers</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Sample 3: Test food with plant primers</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Sample 4: Test food with GMO primers</td>
<td>Don't know</td>
</tr>
<tr>
<td>5</td>
<td>Sample 5: GMO positive control DNA with plant primers</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Sample 6: GMO positive control DNA with GMO primers</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Lesson 4  Drying Gels and Analysis of Results

1. What was your test food?

   Answer to be determined by instructor.

2. Did your test food generate a 200 bp band with GMO primer (lane 4)?

   Yes or no.

3. What does this tell you about the GMO status of your food?

   A band indicates that the food may be GMO-positive, the absence of a band indicates the food may be GMO-negative.

4. What other information do you need to confirm the GMO status of your sample?

   If there was a band in lane 4, we need to determine that there was not contamination of the samples to ensure the result is not a false positive.
   If there was no band in lane 4, we need to confirm that DNA was extracted from the sample and that the PCR reaction was functioning properly to ensure the result is not a false negative.

5. How do the results of your other five PCR reactions help support or undermine your result for your test food?

   Refer to the flow chart on next page.

6. If you were to repeat the procedure what laboratory practice might yield better results?

   Accept all reasonable answers.