**Testing if Foods are Genetically Modified by Analyzing DNA**

**Introduction**

Genetically Modified Organisms (GMOs), are organisms that “have had their DNA modified to include a region of DNA from a distantly related organism.” (Simms, 2017). This process, put very simply, is done by genetic engineering which is, “a technique that allows to detect, isolate, multiply and transplant specific genes in another living organism.” (Arcieri, 2017). With genetic engineering, organisms, such as crops, are able to have more desirable traits. This includes, but is not limited to, “improving crop yield, reducing the use of agrochemicals and adding nutritional properties to staple crops.” (Giraldo, 2008). Though there seems to be many more advantages some there are some disadvantages and controversies. Some of the concerns and controversies include, “The uncontrolled combinations of genes and the release of hazardous GMO viruses are real, frightening risks (Bergmans et al., 2008). And, gene therapy, for instance, engenders ethical dilemmas and to some, fears of designer children and the unlimited extension of human life spans” (Goldbas, A., 2014).

During this lab, in order to determine whether the food samples were genetically modified or not, a process called polymerase chain reaction (PCR) was used. This process is used to make many copies of the original material, which is necessary to identify if a specific gene is present. There are 4 different components of PCR: 10x buffer solution, dNTPs, two complementary primers, and DNA polymerase enzyme. The 10x buffer solution makes a suitable chemical environment for the enzyme to function properly. The primers define the region that will be amplified. Lastly, enzyme functions to add bases in the DNA. Also used in order to detect if the food sample was genetically modified was a technique called Agarose Gel Electrophoresis. This technique is used to separate DNA fragments based on their size and their charge. The bigger the fragments are, the harder it is for them to travel through the pores in the agarose material, but no matter what size, the DNA fragments cannot move through the pores if there is no electrical field. The electrical field helps the negatively charged DNA fragments move through the gel from the cathode (negative electrode) to the anode (positive electrode).

The purpose of this experiment was to see if different food samples were genetically modified. This was done by first extracting the DNA from the food sample by using the InstaGene Matrix. The InstaGene Matrix removes any Mg2+ (positively charged) present. After extracting the DNA, the process mentioned earlier in the paper, PCR, is used. This process has 3 separate steps: denaturation, annealing, and extension. During denaturation, the DNA is heated to allow the separation of the two strands in the DNA. In annealing, the temperature is lowered to allow the primers to bind to the DNA. Lastly, during extension, the temperature is moved to optimal temperature for the DNA polymerase to work and make copies of the original DNA. Once the PCR process is finished, the Agarose Gel Electrophoresis technique is used which, as previously stated, is used to separate the DNA fragments by their charge and size. The bigger the fragments are, the harder it is for them to travel through the pores in the agarose material, but no matter what size, the DNA fragments cannot move through the pores if there is no electrical field. The electrical field helps the negatively charged DNA fragments move through the gel from the cathode (negative electrode) to the anode (positive electrode). A stain called GelGreen, which binds the base pairs, is used to stain the DNA. The DNA segments need to be stained in order to be seen properly. Then, using the DNA ladder, the DNA fragment’s size is determined. Next, the instructor prepares the gel so that the gel can be loaded followed by the instructor running the gel. After the gel is run, the DNA fragments can be seen using an LED light. Lastly in the GMO experiment, the gel must be analyzed by measuring the distance the fragments traveled in the Hi Lo DNA ladder in order to see if the promoter cauliflower mosaic virus is present. I hypothesized that the apple and celery are genetically modified because they have the promoter sequence cauliflower mosaic virus as shown by 190 base pairs in the electrophoresis gel. If the apple and celery are genetically modified, then they will contain cauliflower mosaic virus promoter.

**Materials and Methods**

For the first part of this lab DNA was extracted from the food samples. In order to do this, 2 grams of each food was weighed out. Food item number 1 was placed in the mortar and then grinded in the mortar for 1 minute. Then, using a glass pipette, 10 milliliters of distilled water was added to the mortar and grinded together for two minutes in order to break down the cell walls. After this, another 10 milliliters was added and the mixture was grinded until it was smooth enough to pipette. Next, using a micropipettor with a clean tip, 50 microliters was removed from the mortar and added to a 1.5 milliliter tube containing InstaGene Matrix. Then, a sharpie was used to label the tube as “food item #1, item name, section and group number.” After, the tube was parafilmed and shaken well. Lastly, the mortar and pestle were washed using a 10% bleach solution and dried with a clean paper towel. Then, the process starting from placing the food in the mortar and ending with cleaning the mortar and pestle, was repeated with food item number 2 and 3. Once the steps were all done with food item number 3, all 3 of the 1.5 milliliter tubes were put into a 95° C water bath for 5 minutes in order to destroy proteins and enzymes in the DNA that could degrade it. After 5 minutes, the tubes were removed from the water bath and dried off using a paper towel. Next, the instructor centrifuged the tubes at maximum speed for 5 minutes. Lastly, the tube was carefully removed by the instructor from the centrifuge.

The next step in the process was the PCR process. First, the instructor removed 7 microliters of the supernatant from the 1.5 milliliter tube for food item number 1 using a micropipettor with a clean tip and put it into a .2 milliliter tube. Next, with a clean new tip, they used the micropipettor to remove 8 microliters of a solution containing 10X PCR buffer, the two primers targeting the cauliflower mosaic virus promotor region, the dNTPs and the Taq polymerase. After this was completed, the steps starting with removing 7 microliters and ending with removing 8 microliters of the solution, were repeated for food items number 2 and 3. Once all the tubes were finished, they were put in a thermocycler to run a PCR program.

The last steps in this lab were those in the agarose gel electrophoresis process. First the instructor prepared the gel by positioning the sample comb at one end of the gel tray in the casting apparatus. Then they used a weigh boat to measure 2 grams of agarose and added it to a 300-milliliter flask. Next, 100milliliters of 1X TAE buffer was added and the flask was covered. Once the flask was covered, the mixture was microwaved for 180 seconds during which it was removed every 60 seconds to be mixed thoroughly. After, a micropipettor was used to add 4 microliters of 10,000 X Gel Green and was allowed to cool for 1-2 minutes. Next, 100 milliliters of the 2% agarose solution was put into the gel tray. Lastly, they allowed the solution to solidify for 20-30 minutes before removing the comb from the gel and putting the gel tray into the electrophoresis chamber. The next step in the process was loading the gel. To do this a micropipettor was used to load 10 microliters of PCR product into a sample well and then 10 microliters of the DNA ladder was added to two center wells. Next was running the gel. After all the samples and ladders were loaded, a cover was placed on the chamber and the electrophoresis chamber was plugged in. Then the power supply was turned on and set to 120 millivolts. After, the DNA samples were left to run until the red band was situated at about 1/3 of the way from the bottom of the gel which should take about 60 minutes. Lastly was visualizing the DNA fragments and analyzing the gel. After electrophoresis was finished, the power supply was turned off and the gel was removed from the chamber. It was then observed with an LED light and safety glasses. Lastly, a ruler was used to measure the distance (in cm) that the fragments, in Hi Lo DNA ladder, traveled. The DNA ladder was used to show how far the control fragment traveled. This was done to observe if the food items contained 190 base pair sequence (CaMV promoter).

**Results**

After going through the entire procedure, the results of the food items tested are in wells 7 (apple), 8 (celery), and 9 (positive control, cornmeal) as seen in Figure 1. Looking at Figure 1, you can see that our food sample 1 (apple) and 2 (celery) did not have 190 base pairs sequence. The apple food sample does not have a DNA band at all according to Figure 1. Also, it should be noted that the positive control did not have a 190 base pair sequence.

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Sample 1

Sample 2

c

Positive

Negative

c

DNA Ladder

Negative

c

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

**Figure 1**

**Discussion**

The objective of this lab was to test different food items to see if they were genetically modified.My hypothesis was rejected because I got inconclusive results from both the apple and the celery. The test did not show a DNA band for the apple and the celery did not have a 190 base pair sequence, but this doesn’t mean that it is not genetically modified. Although my results could mean that both the apple and celery were not genetically modified, it could also mean that errors occurred causing skewed/incorrect results. One error that could have affected the results was when we were putting the food samples into the gel. The micropipettor with the apple sample in it was not emerged far enough into the gel which could’ve caused there not to be enough of the sample to read properly. The micropipettor with the celery sample was put in too deep in the well which caused a hole and could’ve skewed the results. Also, while making all of the samples, including the positive control, there could’ve been an error in mixing or the amount of a solution added which could’ve changed the results negatively. Similar to what we did in our lab was a test done in Lebanon. There they used PCR and gel electrophoresis in order to test different soybeans to see if they were genetically modified. They said, “At the screening stage, we tested twenty-three samples for the presence of p35S using conventional PCR and showed that all samples were positive” (Sakr, J. et al, 2014). In our lab we used the same technique, but we only did one test while the other experiment tested it multiple times. This could’ve caused the huge difference in our negative results vs their positive results. This also shows the differences between testing for GMO’s at a higher level vs. a lower level. In the future if I were to do this experiment again, I would run the test multiple times to avoid getting skewed results from small mistakes.

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