Lab Report: GMO detection VIA PCR in an unknown food sample

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Bio 210A

San Diego Mesa College

Wednesday, May 13, 2015

Abstract:

Most crops of today contain some kind of GMO DNA in them. Our sample plant food contains corn, one of the common GMO crops. Our test food was Corn Bread, and due to the ingredients a GMO positive result is expected. To determine the presence of GMO in the sample, the DNA of interest was first isolated. Then PCR was be used with specialized primers. The DNA was then be run through gel electrophoresis to determine the presence of DNA of known base pair lengths. The results of this experiment detected a weak amount of plant DNA in our sample and no GMO DNA. However, due to the weak plant DNA result, the experiment was unable to conclusively determine the presence of GMO DNA. The hypothesis was not supported or disproven via the experiment as a conclusive result was not achieved.

Introduction:

GMOs, or Genetically Modified Organisms are organisms that have had their DNA and genetic properties directly manipulated by humans for more favorable traits (King 2003). By directly modifying an organism's genome, it is possible to create organisms with favorable characteristics in a fraction of the time that selective breeding requires. Genetic modification begins with the selection of a desirable gene. Once that gene is selected, PCR is used to amplify said gene and isolate it using gel electrophoresis (Kaufman 1996). Once isolated, the host DNA

is denatured, and nucleases are used like microscopic scissors to replace, remove, or insert the gene. Commonly modified traits are related to crop yield or virus/parasite resistance to maximize the amount of viable crops. GMOs can also be used in agriculture to create plants that last longer than their unmodified counterparts, making the plants more durable in general. Such is the case with the recently approved modified potato, designed to resist bruising and to fry with less byproducts by the addition of 13 genes (Pollack 2014).

In this lab, we will be determining the presence of GMO foods using PCR. We will be searching genomic DNA for certain genes that are unique to genetically modified organisms. There are multiple methods that can be used for identifying GMO foods. The first is the ELISA method. This method uses an optical sensor to determine the presence and amount of antigens and protein (Davidson College 2002). We, however, will use PCR, as the ELISA method is unable to directly find modified genomic DNA that is not expressed as a change in proteins. Using PCR, the DNA that has the genes that are from GMO foods will be isolated and amplified. *Tag* DNA Polymerase will be used to maximize the efficiency of the PCR, as *Tag* DNA Pol. does not denature at the high temperatures used to denature the DNA. PCR uses a very specific step by step process to achieve the amplification desired. The first step is to take the source DNA and heat it up. This breaks the hydrogen bonds between the double helix DNA strands, allowing complementary base pairing with free nucleotides. A primer must then be added to promote DNA Polymerase at two exact points, one going forward from 5' -> 3' at the start of the gene, and one going in reverse from 5' -> 3' at the a end of the gene. *Tag* DNA polymerase will then be added to ensure that the reaction takes place; additional polymerase is not necessary after each heating cycle. The solution will be annealed for about a minute, allowing the primers to bind onto the sites of the gene. Finally, the DNA will be reheated to 72 degrees, which is the optimal

temperature for *tag* DNA Polymerase to work at. Each time this process is repeated, the DNA is duplicated by a factor of two. This process creates extraneous strands of DNA, but due to the exponential nature of PCR DNA duplication, the extraneous strands are negligible after amplification is complete (Schneider 2007). Then, agarose gel will be set, and electrophoresis will take place. Electrophoresis categorizes molecules by size. Using a molecular weight ruler as a reference, the size and identities of molecules can be determined. Because of the UV interaction between agarose gel and DNA, the movement of the DNA can be seen under a UV light. Ethidium bromide is used to create this UV illumination with the DNA (Snyder).

Hypothesis: I believe our experimental food will contain the genes related to GMO foods due to the prevalence of GMO based products in inexpensive convenience food.

Materials and Methods:

DNA Isolation from food samples:

Materials:

- Screwcap tubes with 500ul Instagene (x2)
- DNase and RNase free water
- Sample
- Non-GMO certified control
- Sterile knife
- Scale + Weighing boat/paper
- 2 DPTP
- Mortar and pestle (Grinder)

- Waterbath at 95-100C
- MicroCentrifuge

Methods:

Two screw caps were labeled “Non-GMO” and “Test” to ensure the separation of DNA samples. 0.5 grams of certified Non-GMO sample was weighed and placed in a mortar. This was the non-GMO control. Using a DPTP, 5ml of DNase and RNase free water was added for every gram of food. In this case, 2.5 mL of water was added as the sample was only .5g. The water assisted in the grinding and breaking of the cells. The sample was then ground for two minutes. An additional 2.5mL of water was added. Grinding was continued until the sample was consistently ground. 50ul of the ground DNA solution was added to the screw cap labeled ‘Non-GMO’. This screw-cap contained the Instagene matrix. This ensured the removal of Mg2+ from the solution, which is a cofactor for DNase. Removal of Mg2+ inhibits DNase from degrading the DNA sample. The screw cap was then recapped and shaken. This same procedure was followed for the test food sample. Once both samples were isolated, the two screw caps were placed in a water bath at the 95C setting for 4 minutes. This served to denature the DNase, inactivating it. The sample was then stored on ice to prevent degradation until PCR amplification was ready to take place.

PCR Amplification:

Materials:

- Microcentrifuge
- Ice Bath

- GMO Master Mix (Red)
- Plant Master Mix (Green)
- GMO+ DNA Control
- Test Food DNA
- Non-GMO food Control
- PCR Tubes x 6
- Pen
- 2-20ul Micropipette
- 2-20ul pipet tips
- PCR Machine

Methods:

First, the DNA samples were centrifuged at a high RPM setting to separate the DNA from the ECM and the Instagene beads. Six PCR tubes were then numbered to insure correct pairing of DNA and Master Mix. Refer to Table I for the correct pairings.

Table I: PCR DNA MM Combinations

Tube	DNA	Master Mix
1	20ul Non-GMO Control	20ul Plant (Green)
2	20ul Non-GMO Control	20ul GMO (Red)
3	20ul Test DNA (Corn Bread)	20ul Plant (Green)
4	20ul Test DNA (Corn Bread)	20ul GMO (Red)
5	20ul GMO+ Control DNA	20ul Plant (Green)
6	20ul GMO+ Control DNA	20ul GMO (Red)

The tubes remained chilled to ensure that the Master Mix and/or DNA did not degrade. 20ul of indicated Master Mix was added to the PCR tubes listed in Table I with their respective tube numbers. A clean pipette tip was used each time to ensure cross contamination did not occur. PCR is very sensitive and will amplify any contaminants. 20ul of indicated DNA was then added to each tube based on their respective tube numbers in Table I. Care was taken to insure that the InstaGene beads remained in the screw caps and were not transferred to the PCR tubes. The Instagene beads can interfere with the PCR Process. The PCR tubes were then placed in a PCR machine configured with the settings in Table II.

Table II: PCR Machine settings

Hot Start	94C	2 Minutes	1X
Denature	94C	1 Minute	Repeat Seq. 40X
Anneal	59C	1 Minute	Repeat Seq. 40X
Elongate	72C	2 Minutes	Repeat Seq. 40X
Final Extension	72C	10 Minutes	1X
Hold	4C	Indefinitely	1X

Gel Electrophoresis

Materials:

- Agaros Gel
- EtBr
- Gloves
- Food DNA samples
- Running buffer (1x TAE) (300ml - 350ml)

- Orange G dye
- PCR Molecular ruler
- 2-20ul pipet
- 1-20ul pipet tips
- Gel electrophoresis machine'
- UV Light
- UV Goggles
- Power Supply

Methods

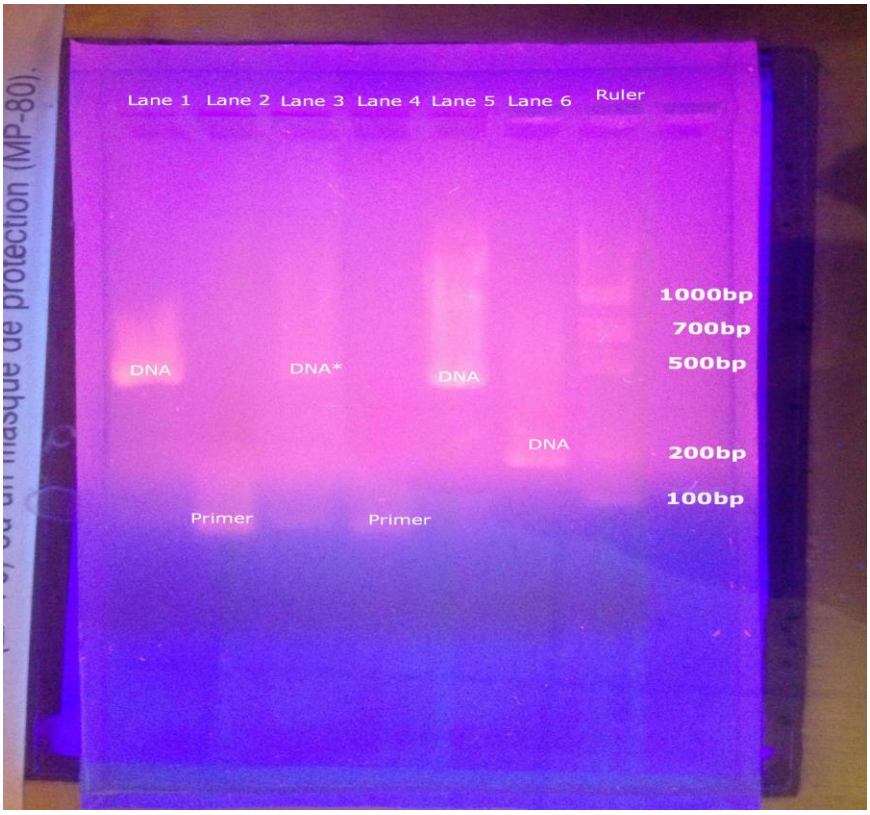
Using gloves, a casting tray was set by pouring Agarose gel with 1.5ul of EtBr into the casting tray mold. The EtBr was used to make the DNA illuminate under UV light. The casting tray was then left for 30 minutes and was allowed to solidify. Once the tray was solid, the well comb was removed and the gel cartridge was moved to the electrophoresis machine. The Electrophoresis chamber was thrn filled with the TAE buffer. This allowed electricity to flow through the gel, which moved the DNA molecules. 10ul of Orange G was then added to each sample tube. This assisted in placing the DNA molecules into the wells. It also insured that the DNA did not run off the edge of the gel. The six samples were then placed into the wells in order from left to right in wells 1-6, with the well number corresponding to the indicated tube number. The molecular ruler was placed in the 7^{th} well. The Agarose gel was then run at 100V for 20 minutes. Care was taken to ensure the orange dye did not go off the edge of the cartridge, as this could mean the DNA had also gone over the edge. Upon completion of electrophoresis, the gel was moved to the UV box and slid off the cartage onto the UV light. The UV light illuminated the DNA bands, showing us the location in relation to the molecular rule

Results:

PCR Lane combinations:

Lane	DNA	Master Mix
1	20ul Non-GMO Control	20ul Plant (Green)
2	20ul Non-GMO Control	20ul GMO (Red)
3	20ul Test DNA	20ul Plant (Green)
4	20ul Test DNA	20ul GMO (Red)
5	20ul GMO+ Control DNA	20ul Plant (Green)
6	20ul GMO+ Control DNA	20ul GMO (Red)
7	20ul Ruler (1000,700,500,200,100bp)	N/A

Figure 2A: Raw Gel DNA visualization



*Inconclusive due to the low intensity of the result.

Discussion:

The GMO test used cornbread bought from the Mesa cafe as the test DNA. This cornbread was first ground up and liquefied, while a non-GMO control was also prepared in the same manner separately. They were then put into two separate screw caps, both containing Instagene mix. The screw caps were then submerged in a 95C water bath for 5 minutes. They were then removed, cooled, and centrifuged to ensure Instagene matrix separation before being mixed with our master solutions. PCR was done at the GMO-BioLab setting on the terminal cycler, completing 40 cycles, which amplified the DNA with the respective primers in the master solutions. After amplification, 6 test tubes were put into 6 wells, along with a molecular ruler in well 7. These six samples contained 5 controls and 1 test. The controls consisted of a certified Non-GMO sample with the plant mix as a positive plant master mix control, a Non-GMO sample with the GMO master mix as a GMO master mix negative control, the test sample with the plant master mix as a control to see if DNA is present in the sample at all, a GMO Positive sample with the plant master mix as a negative plant master mix control, and finally a GMO positive sample with the GMO master mix as a GMO master mix positive control.

Gel Electrophoresis was used to measure the size of the DNA. The gel was examined under a UV light and compared to the ruler in lane 7. Our results were inconclusive. Lane 1 was Non-GMO control with Plant primers as a control. Lane 1 showed a bright line below 500bp. For reference, the plant primer product bp is 455. Therefore Lane 1 is positive for plant DNA. This was an expected result for one of the control lanes. Lane 2 contained a mix of the Non-GMO DNA with the GMO Master Mix. Under UV light, lane 2 contained a mark at 100BP. This is

likely the amplified primers for the GMO Master Mix and not a product of amplified plant DNA. Thus this result is negative, as expected for the negative control lane. Lane 3 contained a mix of Test DNA and the Plant Master mix. Well 3 shows a faint line under 500bp, which is likely the result of PCR amplified plant DNA. Well 3 also shows a faint line at 100bp which is likely the Plant Master Mix primers. This is an expected results that shows that despite the heavy processing of the test sample, there was enough DNA intact for PCR to amplify. However, possibly due to the heavy processing, the line is very faint. Lane 4 was a mix of the Test DNA and the GMO Master Mix; this lane is effectively the unknown test. Well 4 showed a faint line at 100bp, likely the GMO Master mix primers. There was no PCR amplified DNA detected. However, due to the faint line in Lane 3, a negative result here cannot be concluded as the DNA may have been too weak to replicate due to processing. Lane 5 contained GMO-Positive DNA and the Plant Master mix. Lane 5 showed a bright line at just under 500bp. This is a positive result for plant DNA. This was an expected result as GMO foods also contain plant DNA. The final lane, Lane 6, contained our GMO positive DNA and the GMO Master Mix. Lane 6 showed a bright line at exactly 200bp, indicating a positive result for GMO DNA. This was expected, as it was our GMO positive control test. Lane 7 was our molecular ruler, showing 5 bright lines at known DNA intervals. Due to the consistency of between our control lines and the ruler lines, it can be concluded that the ruler worked as intended, showing bp marks at 100,200,500,700, and 1000bp.

The weak line in Lane 4 could be due to a number of factors. The most likely cause is the processing that goes into milled products. Such an intense chemical process likely denatures proteins and DNA to the point that they cannot be amplified. Such is the case with cornbread, whose flour is milled to remove impurities. Other factors, however unlikely, may have also

contributed to the partial result. The separation of the Instagene matrix and the DNA is crucial to the PCR amplification process. Any contamination of the final sample with the Instagene matrix could have interfered with the amplification. This is because the Instagene matrix contains the cofactors needed for DNase to break down the DNA. If these cofactors (Mg^{2+}) are present, the DNase may degrade the DNA. Failure of the PCR Thermal Cycler can be ruled out as a possibility due to the control DNA giving the expected results. The last obvious point of failure is the incorrect addition of the DNA into the wells in the electrophoresis gel. The pipette may have punctured the gel, or not delivered enough of a sample due to operator error.

Conclusion: The experiment was unable to determine the presence of GMO in the test sample. The positive test control line in well 3 was very faint, because of this, it is impossible to determine if the negative result in well 4 is accurate. The hypothesis was not supported or unsupported as the test itself was inconclusive. Further experiments will take ingredients that make up before processing to ensure enough undamaged DNA is present.

I believe the use of GMO foods is generally beneficial to the wellbeing of millions of people. However, the copyright laws surrounding GMO foods allow large scale patent trolling by companies such as Monsanto in cases of accidental cross-pollination. This patent trolling is unacceptable and there need to be safeguards put in place to prevent Monsanto from pursuing legal action against small family farms due to accidental cross pollination. Additionally, safeguards need to be in place to prevent genetic stagnation, as this increases the likelihood of a superbug wiping out the whole crop.

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