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Enzyme Lab

Introduction

When it comes to molecular biology, DNA and RNA are two important molecules that encompass all living organisms. A person's genome is the whole set of nucleic acids of an organism. An organism's genome is specific and unique. One person's genome will forever be unique to that person. In eukaryotes the genome is stored into the nucleus. For this lab the intracellular membrane must be dissolved in order to reach the DNA in the plant cell. PCR and Gel electrophoresis is used.

DNA molecules are the building block for an organism. One important characteristic of DNA is that it can synthesize protein through a cellular process called gene expression. Gene expression has two stages which are transcription and translation. Transcription is the first step in gene expression. The idea of transcription is to copy a gene DNA sequence to make an RNA molecule. In the initiation phase of transcription, RNA polymerase binds to the DNA molecule at the promoter. The function of RNA polymerase is to transcribe the genetic information in DNA and convert the information into an RNA molecule. One RNA polymerase binds to the gene, RNA polymerase separates the DNA strands (Areda & Boyles, 2017). The next phase of transcription is elongation. The template strand of DNA is used by RNA polymerase to build an RNA molecule out the complementary nucleotides. It is important to note that the RNA

transcript carries the same information as the strand of DNA but converts thymine to uracil. Termination is the last phase of transcription. The transcript is cut off at the stop codon and this releases the messenger RNA. Translation is the next stage of gene expression. This stage utilizes ribosomes to convert the mRNA genetic sequences to polypeptide sequences (Areda & Boyles, 2017).

To study DNA efficiently many labs use a polymerase chain reaction to produce millions of copies of a specific segment of DNA. The scientist who discovered PCR is named Kary B. Mullis. PCR utilizes DNA polymerase (also known as Tap DNA) to synthesize new strands of DNA. PCR starts breaking apart the hydrogen bonds turning the double helical structure of DNA into a single strand. This process denatures the strand of DNA. The mixture is placed in a lower temperature environment in order to anneal it (Areda & Boyles, 2017). After the annealing process, the mixture is placed in a high temperature environment because it is the optimal temperature of Tap DNA polymerase. The extension of the DNA by Taq DNA polymerase is called the extension phase. After a couple of cycles of this process, the result is a large amount of DNA that we are able to experiment with (Areda & Boyles, 2017).

Gel electrophoresis is another technique used in this lab. Electrophoresis is used to separate charged molecules like DNA according to the size of the molecules. Charged particles move through the gel due to an electric field being generated. Shorter strands of DNA move quicker than longer strands of DNA, so when an electric current is applied to the gel, the shooter strands of DNA will move towards the positively charged electrode quicker (Areda & Boyles, 2017). The difference in length is what sorts the DNA during gel electrophoresis. In the experiment a agarose gel is used and the use of dyes is used which enables the DNA to be seen

after the separation. The purpose of this lab is to isolate the plant DNA through PCR and gel electrophoresis. The end goal is to detect the presence of GMO's.

Methodology

DNA isolation is the first objective of this lab. Gather materials like a microtrfuge tube and the food sample. After placing the food sample in the microcentrifuge with the correct amount, turn on the centrifuge and turn it on. After the centrifuge, add 250 microliters of water to the sample. This is dilute the sample. Now, place the sample of the microtrifuge block heater for 5 minutes. After letting the sample cool off on the ice, mashing the selected food down with a pestle is needed to prevent overspill and reduce the amount of chunks in the sample. To make sure the mixture is consistent, liquefied, and broken down, the mixture will be placed in the microcentrifuge once more. Furthermore, using a pipette, extracting the liquid portion of the sample is needed to get rid of the chunks. Chelex is then mixed with the sample and placed on the microcentrifuge block heater for five minutes. Transfer the supernatant into a new tube and now tbe DNA should be isolated.

For the PCR reactions, take the sample tube with the isolated DNA sample and transfer 2 microleters of the food dNA to the first and second tube. Put in the primer and GMOs to the corresponding tube . After this mixture is made placing the tubes in the mircocentrigue will make sure the mixture is fully blended. In order to see the DNA sample in the gel, a dye must be mixed into the PCR sample. Loading the gen is fairly straightforward but requires the user to precisely place the sample into the gel at the right orientation. A pipette is used to load the gel. The next step of this lab is electrophoresis. Terminals are connected to the cover which will generate an electric field of 125 volts. After 45 minutes, the gel is completed and observations should be made.

Data

Image 1

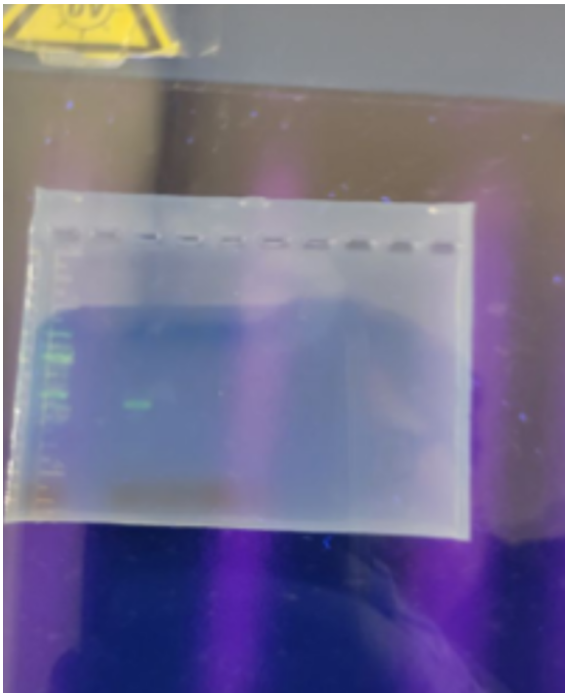


Image 2

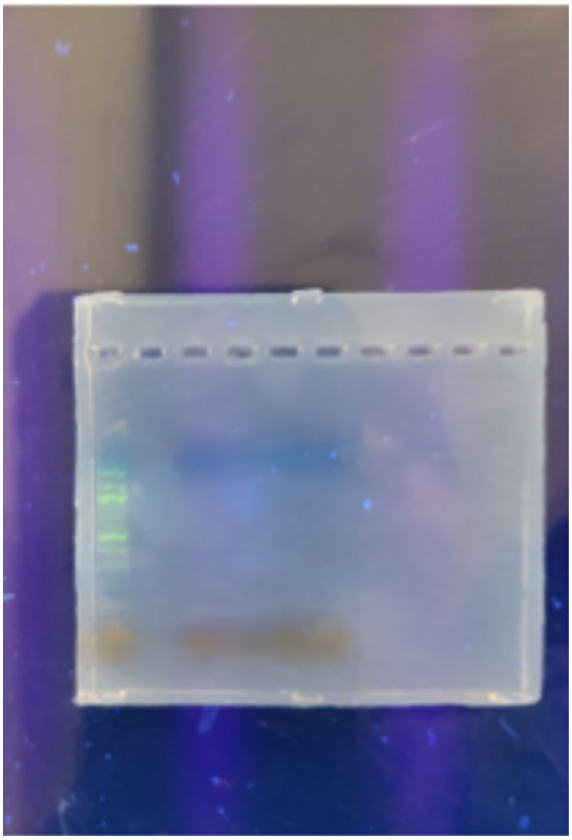
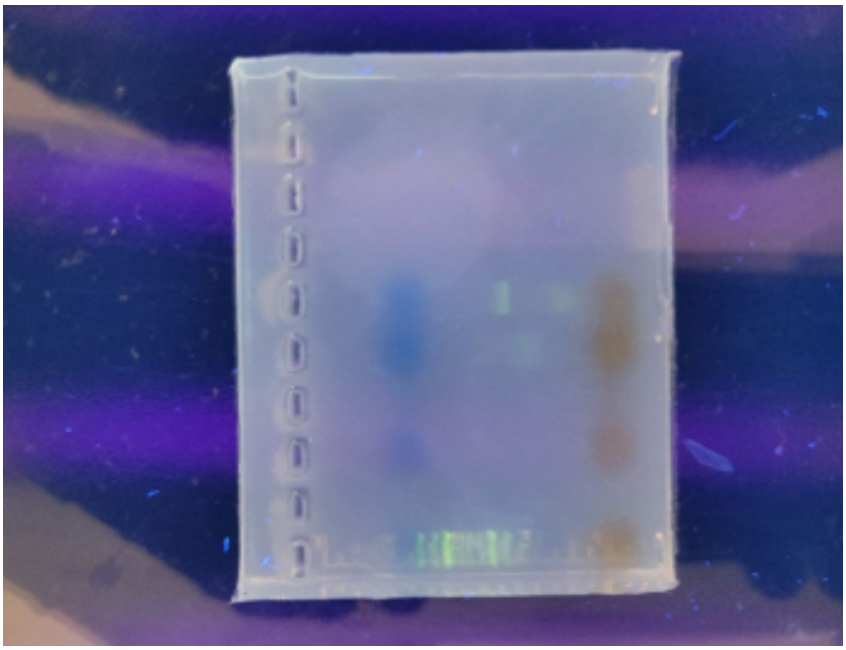
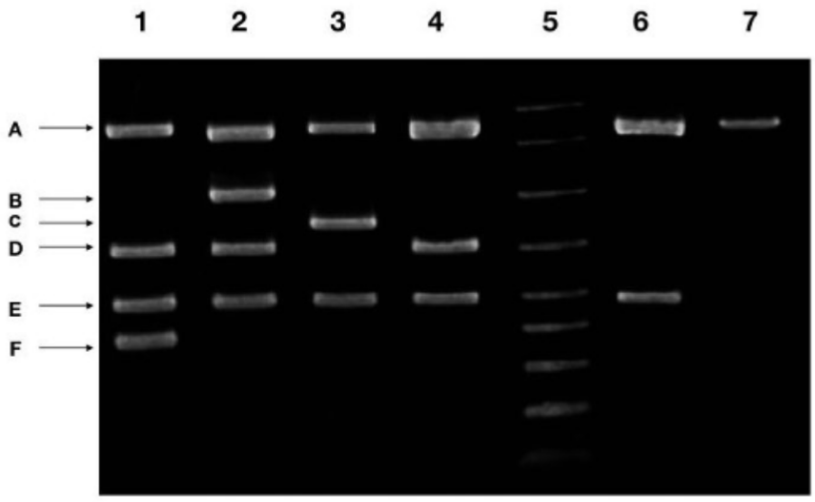


Image 3





1= ladder
2= tofu
3= Apples
4=Corn
5= Potatoes
6= Beyond meat
7= NA

D= Plant primer
F= GMO

Table

	Ladder	Tofu	Apple	Corn	Potatoes	Beyond Meat
Plant Primer	+	+	-	+	+	-
GMO	+	-	-	-	+	-

Analysis

In these experiments, data was unsatisfactory. Several of the foods tested negative for plant primer. Results were scattered as the plant primer was the positive control and did not turn out as positive to several of the foods. This error most likely occurred when loading the samples

into the gel. This technique was quite difficult. Realistically the forth picture represented a more accurate experiment with desired results. The DNA ladder estimates the size of DNA molecules that were separated during gel electrophoresis. DNA was isolated from a plant sample using several techniques and solutions. Chelex 100 beads were added to the food sample to extract DNA for PCR based testing. It was important to use a thermally stable DNA polymerase because of the annealing process. In the synthesizing stage of PCR, DNA polymerase will synthesize only when there is a template and a primer. Without the primer DNA polymerase will not synthesize daughter strands. Since DNA tested is negatively charged, the DNA moves towards the positive end when the electrical current is applied. DNA will move at different rates due to the differing size of fragments of DNA molecules. Larger fragments will not move as fast and smaller ones. This is how the data is separated during gel electrophoresis. During this process the electrophoresis buffer solution was used to produce ions that carry a current through the gel and to maintain a constant pH. The DNA staining dye was used to make the DNA glow under a ultraviolet light so data can be recorded. The bands seen on the gel represent a positive result of the amplified DNA. If no band was visualized then a negative result was recorded. The plant primer tested positive in tofu, corn, and potatoes. GMOs were found in the potatoes. It seems that potatoes were used as a positive control for the GMO as it is the only food positive for GMO.

Conclusion

This experiment's goal was to gain knowledge of the polymerase chain reaction and agarose gel electrophoresis methods utilized in molecular biology. This was done to determine which foods were genetically modified. However, the results did not correspond to the actual genetically modified foods. A significant majority of the samples containing plant primers did

not test positively as they should have, despite a few of the positive controls showing positive results. PCR has developed into a crucial element in the medical and forensic industry. PCR is commonly used at crime scenes to amplify, synthesize, and identify DNA. PCR is the perfect technological tool for identifying genes and DNA that has undergone genetic modification due to its improved sensitivity and speed. The PCR method is a reliable method that is commonly used to detect GMOs.

References

Boyles. R., & Areda, D. (2017) Application of Molecular Biology Techniques. In Grand Canyon University (Ed.), *Laboratory manual for General Biology I* (2nd ed.). Retrieved from https://lc.gcumedia.com/bio181l/laboratory-manual-for-general-biology-i/v3.1/#/chapter/10

Marmiroli, N., Maestri, E., Gullì, M., Malcevschi, A., Peano, C., Bordoni, R., & De Bellis, G. (2008). Methods for detection of gmos in food and feed. *Analytical and Bioanalytical Chemistry*, *392*(3), 369–384. https://doi.org/10.1007/s00216-008-2303-6

Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., Jackson, R., & Campbell, N. A. (2011). Campbell Biology. Retrieved 10 April 2022, from https://viewer.gcu.edu/24WWXP