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BIO 2459-Thursday class

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Final Report: GMO

Abstract:

In our last lab experiment we will learn more about genetically modified organisms (GMO). In particular we will be testing and analyzing certain food items from the grocery store for GMO presence. In addition to this, we will get some additional knowledge in the agarose gel preparation, PCR and gel electrophoresis. Lab test on GMO presence will help to increase confidence in our products and ensure that it is in compliance with applicable national and international legal standards. The methods of conducting the experiment are DNA extraction, a real-time polymerase chain reaction (PCR) that will help us to amplify specific required region, and Gel electrophoresis which will determine the absence or presence of genetically modified material in a sample by separating our DNA fragments. In addition, when DNA separated in the gel, the position of DNA can be observed using transilluminator. In order to extract DNA from our sample's (orange) tissue, methods such as dry bathing and micro-centrifuging were used. Our group hypothesized that our orange sample will not contain the 35S gene and therefore will be considered not genetically modified organism. After fallowing these simple techniques, the results confirmed the absence of 35S gene in the orange sample, supporting our initial hypothesis that our sample is not genetically modified.

Introduction:

A genetically modified organism (GMO) is an organism whose genotype has been modified through the introduction of genetic engineering techniques. GMO is created by

introduction of foreign genes into the DNA of animals, plants, or microorganisms. The first GMO was a bacteria that was resistant to the kanamycin antibiotic, which was created in 1972(Lander, 2000). In fact, GMO have become widespread in the biotechnology industry for the production of medicines, such as insulin, which was crucial for healthcare (Vujaklija, 2006). The first genetically modified plants and food were presented to the public in the 1990s, which gave rise to organisms that do not arise naturally (Buiatti, 2013). As a result, many of the products consumed daily have been modified and the number of GMO products used on a daily basis grew exponentially.

In general GMO plants have an increased yield and are resistant to herbicides, pests, and viruses. This makes it possible for a certain plant to be invulnerable to chemicals that are deadly to others. Of course, the economic benefits of growing genetically modified plants make it beneficial, and as a result, the production of food using genetically modified organisms (GMO) is growing extremely fast. Food with GMO do not differ from the organic ones, they have a normal taste, color, and smell. Genetically modified organisms are used for the food production, such as meat, cheese, fruits, vegetables, canned goods, oils, various sauces, chocolate, and you will be surprised but even baby food (Bawa, 2013). In the process of many scientific researches, it was found that mice that consumed genetically modified food were diagnosed with a weakened immune system (Krzyzowska, 2010). In addition, after consumption of GM food, there was a finding of abnormalities in the mice's stomach tissues, decreased volume of the brain, damage to the liver, spleen, intestinal tract, gonads, etc (Krzyzowska, 2010). Similarly, some scientific researches suggested that introduction of foreign genes into the plant DNA can be a source of allergic reactions in people who previously had no reaction to this product(Kim, 2006). For example, the transfer of Brazilian nut Gene into soy, which was done to increase the protein content, made it dangerous for people who are allergic to nuts, which was confirmed by experiments with mice (Kim, 2006). Also, important to realize that in the process of creating a

GMO the stability of the plant genome is disturbed, which could lead to the toxic protein production(Hammond, 2013). Even though a lot of people would wish to know what they eat, unfortunately labeling of genetically modified products is not mandatory in the US yet. However, on Jul 1st, 2016 Vermont state signed the bill which forbids all products labeled as natural, if they were produced with GMOs(McPherson, 2014).

Nowadays, there are a lot of techniques that have been used to produce GMOs. The first step is usually a detection of the desired fragment of DNA that contains required trait. The next step requires to cut the necessary fragment with restriction enzyme and transport it into a vector together with another regulatory sequences. The CMV promoter is usually used in mammals, which is extracted from cytomegalovirus. On the other side, the CaMV promoter is used in plants, which is derived from the 35S gene from cauliflower mosaic virus. The next step is usually require to transfer the vector into the cell and include it into the plant DNA (Maghari, 2011). To summarize, genetically modified foods have become one of the largest achievements of the bioengineering. But the main question, whether these products are safe for people, remain unanswered. Unfortunately, most people are not aware what GMO really is and the possible consequences of it.

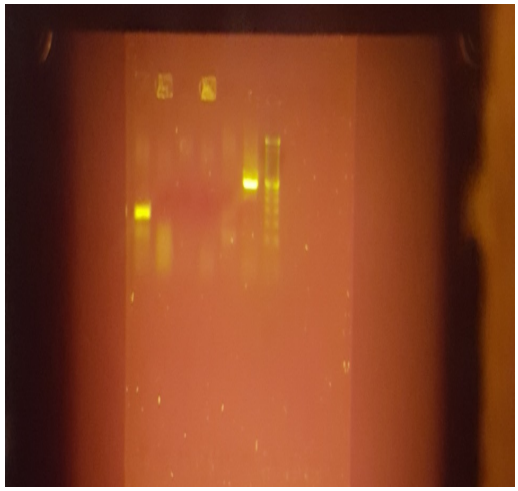
Methods and Results:

On a day one of this experiment our group was informed that we need to bring the food sample from the grocery store that will be tested for GMO presence. Two 1.5ml microcentrifuge tubes were obtained and labeled. Tube one was labeled as “non-GMO” and the other was labeled as “test food”. After that we were informed to place a crumb of “non-GMO”, that was provided by our professor, into the corresponding tube. After that 50ul of dH2O was added to each tube. In order to grind the crumb a clean plastic pestle was twisted against the inner surface of the tube for

couple of minutes. To avoid contamination a different pestle was used for each sample. The next step was to add 300ul of InstaGene matrix to each tube. The procedure was followed by incubation for five minutes in a dry bath. After incubation was completed all tubes were removed and microcentrifuge for another 5 minutes in order to separate the DNA. In addition, we were informed by our professor that after centrifugation DNA will be find in the supernatant and will have white color. PCR set up was the following step in this experiment. By mixing 3 g of agarose with 100 ml of TBE buffer we were able to prepare 3% gel. After microwaving it for 1min, we were informed to place 10 ul of SYBR solution which will help with the DNA visualization. Since the flask with gel was hot, we were informed to wear the protective gloves as a precaution. After all the comb with 6 wells was placed in to the gel apparatus. After tout agarose gel completely cooled down and dissolved, we poured it into a gel tray that contained previously placed comb with 6 wells. To expedite solidification the gel was placed in to refrigerator for 20 minutes. While gel was in the fridge, we had time to prepare 6 tubes and label them. The following week we were able to do gel electrophoresis by loading our samples into separate compartments of an agarose gel and running it for 20min at 120V. To avoid contamination, fresh pipet tip was used each time we added 20ul of each PCR sample and 5ul of the DNA ladder into the wells. The last step was to analyze the banding patterns of our samples by using transilluminator and determine if our orange sample was genetically modified. It was concluded that our food sample was not genetically modified. All data was recorded and analyzed.

Tube	Primer Mix (Forward, reverse, loading dye)	DNA
1	22ul Plant Primer Mix	3ul non-GMO control DNA
2	22ul GMO Primer Mix	3ul non-GMO control DNA
3	22ul Plant Primer Mix	3ul test food DNA

4	22ul GMO Primer Mix	3ul test food DNA
5	22ul Plant Primer Mix	3ul GMO positive control DNA
6	22ul GMO Primer Mix	3ul GMO positive control DNA



Discussion:

By performing this lab, our group was able to learn more about genetically modified organisms (GMO). In particular we were testing and analyzing orange sample bought in the grocery store for GMO presence. In addition to this, we got some additional knowledge in the agarose gel preparation, PCR and gel electrophoresis, which could benefit us in the future. This Lab test on GMO helped us to increase confidence in our products and ensure that it is safe and not genetically modified. After performing analysis of the banding patterns of our food sample by using transilluminator, we determined that there was no band present, which supported our hypothesis that the orange was not genetically engineered. Our sample did not possess the CaMV 35S sequence, which is usually present in most genetically modified organisms and in general ~200bp in length. Since we were able to extract DNA from our sample and determine that orange

was not genetically modified, we can conclude that for the most part performed lab experiment was successful and experience we gained will definitely benefit us in the future.

Reference

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