

Genetically Modified Organisms: General Biology Lab



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Introduction

It all began in the 1950's when chemical fertilizers, herbicides, and pesticides were being added to corn, rice, and wheat. About fifty years later, different genes, such as herbicide resistance, insect resistance, frost tolerance, drought tolerance were being added to plants that were in high demand. These plants that contain the added genes states above are known as foods containing genetically modified organisms (GMO's). In 1972, the first genetically modified organism was made. It was not until 10 years later that the first genetically modified plant was produced. There were roughly 2.8 million hectares of commercial GM crops grown in 1996, and that increased by 29 percent by 2004. Today, the most commonly planted GM crops are cotton, canola, papaya, corn, and soybeans. But, there are roughly at least 36 genetically modified food products (Millis, 2006).



GM food products are products that have specialized DNA that protects the crop. There is a special way for the gene to be inserted into the DNA of the GM product and it is actually quite a process. The gene being inserted contains a protein, which is the producer of the necessary characteristics. The first step is to introduce a promoter and a marker gene into the DNA of a parent organism. The promoter maintains the distribution and amount of the trait-determining gene while the marker is the test used to notice successful transformations (Llaguno, 2007).

This experiment used two different markers to test which food products were genetically modified and which were not. The two markers used were 35S and tubulin. Tubulin is used as control because all plants contain tubulin whether it is genetically modified or not. The 35S was used because it is the detector for the commonly used Roundup Ready, which is only present in genetically modified food products.

The purpose of this lab was to take several different samples including, wild type soy, Roundup Ready soybeans, soy bars, corn syrup, cornflakes, corn meal, and a fiber one bar and see which ones contained the Roundup Ready gene and which ones did not. The wild type soy is the negative control; it will not contain any of the Roundup ready, and the corn meal was thought to contain no Roundup Ready. The Roundup ready soybeans are the positive control group and will obviously contain the Roundup ready gene. The soy bars, corn syrup, corn flakes, and fiber one bars will all likely contain Roundup ready since corn and soy are very popular GM foods.



Methods

The experiment began by each group receiving a source: wild-type soy, Roundup Ready soybeans, soy bars, corn syrup, corn flakes, corn meal, or a fiber one bar. Then, using a micropipette, 100 ml of Edward's buffer was added to each tube containing the samples. The food located in the 1.5 ml tube was grounded for one minute by a pestle, and 900 ml of Edward's buffer was then added to each tube. Each tube was then placed on a vortexer for 5 seconds and then put into a water bath to boil for five minutes. The tubes then were placed into a microcentrifuge in a balanced configuration and spun for two minutes at 14,000 g, allowing the cell and food debris to pellet. While completely avoiding the supernatant, 350 ml was transferred into a new, clean tube. Using the micropipette, 40 ml of isopropanol was added to each of the tubes, and then, tubes were mixed and left at room temperature for three minutes. The tubes were once again be placed in a balanced configuration in the microcentrifuge and spun for 5 minutes at 14,000 g. All of the isopropanol was poured out of the tube, leaving the pellet behind. The pellet was left to dry for ten minutes or until all remaining isopropanol had evaporated. After, 100 ml of TE/ RNase A buffer was added to each tube and dissolved the



pellet by pipetting in and out. Then, the TE/RNase A solution sat at room temperature for five minutes before storing at a -20 degrees Celsius of a week.



After the samples sat for a week, two PCR tubes containing the Ready-to-Go PCR beads were obtained. These were labeled one tub (for tubulin) and the other 35S. Using the micropipette (with a fresh tip each time), 22.5 ml of the 35S primer was added to the correctly labeled tube, and 22.5 ml of tubulin primer was added to the other correctly labeled tube. Once again, using a micropipette with a clean tip, 2.5 ml of DNA was added to each tube. Samples were stored on ice until the thermal cycling began. After the tubes were placed in the thermal cycler, the lab instructor set it to do 40 cycles of each of the following steps: Denaturing, 30 seconds at 94 degrees Celsius, Annealing, 30 seconds at 60 degrees Celsius, and Extending 30 seconds at 72 degrees Celsius. Once all of these steps had been completed, there was a hold program at 4 degrees Celsius. Once again, the samples were stored at -20 degrees Celsius for a week.



The premade agarose gel was then placed into the electrophoresis chamber. 1XTBE buffer was placed in the chamber to cover the surface and fill in the wells. Using a micropipette, 10 ml of each sample was added into different wells of the gel. This was done at a slow pace to make sure the sample did not float within the gel and actually enter the well. The lab instructor previously added 10 ml of the molecular weight marker (pBR322/BstNI) into the very first well. She then ran the gels at 130 V for about thirty minutes and observed the light box to see which samples had the Roundup Ready and tubulin present.

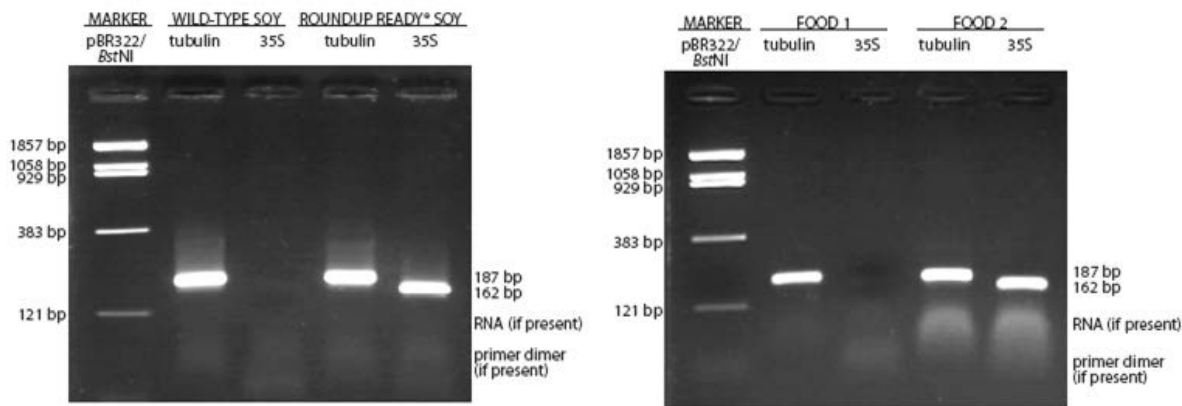
Results



After the final step (gel electrophoresis) was completed, results were observed. When observing the gel, the bands of all of the samples were observed to determine which samples had

the marker DNA showing the Roundup Ready and tubulin or not (see figure 1). The wild-type soy had tubulin present. The Soy Joy also only had the tubulin gene present in its DNA. Corn meal, soy plant, and corn flakes all had both the 35S and tubulin gene present in its DNA. The data is also shown in table 1. The trends noticeable were the “natural” foods, such as wild-type soy and Soy Joy did not contain the 35S gene, but every other sample contained tubulin.

Figure 1 This figure is an example of Gel Electrophoresis of Soy and Food Products PCR Products.



Key:

Roundup Ready® Soy = Genetically Modified (GM) Soy

Food 1 = Corn Meal

Food 2 = Corn Flakes

This gel is a sample of a different test done with the same samples and markers. It still shows that all samples contained the tubulin, but only the Roundup Ready Soy and Corn flakes contained 35S.

Table 1 This table shows which samples had tubulin and which samples had 35S and the size of the bands.

	Wild-type	Soy plant	Corn Flakes	Corn Meal	Soy Joy
35S	NO	YES(162bp)	YES(162bp)	YES(162bp)	NO
Tubulin	YES (187bp)	YES(187bp)	YES(187bp)	YES(187bp)	YES(187bp)

This table indicates that every sample contained tubulin. All plants contain tubulin, which is why it was the control. The right hand column indicates that not all of the samples contained 35S.

Discussion

The results from this experiment do not support the hypothesis that everything would contain the 35S marker, except the wild-type soy. The Soy Joy also did not contain the 35S marker. The corn meal was also unexpected because it did contain the 35S marker, which is the exact opposite of what was predicted. Therefore, if repeating the experiment one may state: The wild-type soy and Soy Joy bar contains only the tubulin, while corn meal, corn flakes, and the soy plant contained the 35S marker. Also, when testing the Fiber One bar, something must have gone wrong with the group's experiment, which prevented accurate results to be shown.

There are a few possible sources of error pertaining to this lab. One may disturb the pellet while trying to extract the supernatant, which in fact could ruin the DNA extraction. Another may be filling the wells too fast, so the DNA ends up flowing or actually mixing with the gel not allowing for the entire process of gel electrophoresis to finish properly. Another source of error could be, when placing the supernatant in the PCR tube, the pellet almost fell out, so when trying to catch it, the cap accidentally cut it in half, which may have allowed some of the pellet to actually leave the tube.

Genetically modified organisms are a very controversial topic; some believe that they are absolutely horrible for us, while others think there are many benefits. There are about one billion people suffering from malnutrition in this world, and GM products may be able to help by producing a surplus of products. Food production, nutritional value, and insect resistance could all improve. For example, an added gene of salinity-resistance was combined with rice in India that allowed it to grow in water much saltier than seawater. Another rice had specific genes added to it, so it would be able to help prevent vitamin A deficiency. A specific rice has milk proteins that can be used to treat infant diarrhea. Corn is also used to treat cystic fibrosis, duckweed to treat hepatitis, and safflower to treat diabetes (Reece, 2012).

But, even though there are many great benefits with genetically modified organisms, there are many things that are problematic with GMO's. One major concern is that the development of a new pathogen may arise from attempting to make these GMO's. It is also very possible that these genes coming from other species might be hazardous to the environment or more importantly human health. Since so many organisms on this earth have survived through evolution, the addition of GMO's may kill off species because they will not be able to readily adapt to weather or new insects because their genes are preset (Reece, 2012). Herbicides, a common modifier used to protect plants, are causing great risks to human health. Since these herbicides have to be sprayed in abundance, the glyphosate is noticeable in the air, water, and food. If herbicides continue to be used as much as they do on these crops, the residue is also going to become more common in meat, milk, and other animal products (Benbrook, 2012).

Since some of our results were questionable, the corn meal containing the 35S marker and the Fiber One bar not being tested correctly, it would be beneficial to do the experiment over to see if the same results occurred. It may be beneficial if these tests were completed by a

different group following the same instructions to see if the results change in any way. It seems that experiments involving GMO's are fairly straightforward, but more experiments should be done on how they affect humans and animals. It would also be interesting to do another experiment to see how many foods one eats daily are genetically modified. It would benefit a people if they knew exactly what are consuming. Overall, this experiment allowed one to actually notice how everyday crops are being genetically modified, which leads to questions about how much food we consume is genetically modified.

References

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