

Title: Protein Isolation and Identification in Genetically Modified Food

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This biotechnology unit is directed toward upper level science research students or AP Biology students. Students research and write a paper on a specific genetically modified food of their choosing and genetically modified foods in general. As part of their research the students must find the protein(s) that have been inserted into the organism and why, and determine the molecular weight of the protein. Students then confirm that a food sample is genetically modified by extracting DNA from modified and unmodified samples, amplifying the samples with polymerase chain reaction, and then running gel electrophoresis. The genetically modified sample is then subjected to protein extraction through vertical SDS-gel electrophoresis and protein in-gel digestion, followed by mass spectrometry MALDI-TOF MS to find the molecular weight of the protein. Protein identification will be attempted by entering the data from the accumulated spectrum into the Protein Search Engine on the Molecular Detective website: <http://www.moleculardetective.org>. Student assessment will be a lab report with a discussion section, a conclusion, and future studies.

When placing science research students in the categories they will be competing in it becomes apparent that few if any students are in the biology/biochemistry category. Similarly there are very few students enrolled in the AP Biology class at my school, while the other AP sciences have no difficulty attracting students. When I heard that Biotechnology is a rapidly growing field, especially in the area of bioinformatics and proteomics, I was excited to have my science research students do some research and lab activities in these areas. This lab was created to promote student interest in biology and biotechnology.

Students will write a 1,500-2,000 word research paper on a genetically modified food of their choosing such as corn, tomato, or a dairy product. Through their research students must uncover which protein or proteins have been inserted into the organism's (GMF) genome. Students will research the protein's function, the role it will play in the modified food, and the desired outcome of the modification. The students will also need to find the molecular weight of the protein to use as a guide for removing the correct band after SDS-gel electrophoresis.

When all background research is completed students will verify that their food sample is indeed genetically modified. Students will accomplish this by extracting and comparing DNA samples

from their genetically modified food sample and a sample of the same food type that has not been genetically modified. Both DNA samples will be amplified using a polymerase chain reaction kit (there is a thermal cycler in the classroom already), then gel electrophoresis will be performed on both samples. The samples will be compared to determine if one sample is indeed genetically modified. If the sample is not genetically modified then DNA extraction would be repeated on a new sample(s).

If the electrophoresis conforms that one of the samples is indeed genetically modified, that sample will then be subjected to protein extraction through vertical SDS-gel electrophoresis and protein in-gel digestion. The band of interest will be sent to Dr. Chen's lab for protein in-gel digestion using the microwave digestion apparatus. The completed sample will undergo mass spectrometry MALDI-TOF MS in Dr. Chen's lab.

Protein identification will be attempted by entering the data from the accumulated spectra into the Protein Search Engine on the website: <http://www.moleculardetective.org>. Students will be required to justify their protein identification.

Upon completion of the experiment students will write formal lab reports which will include their methods, a discussion of the results, a conclusion that summarizes their experiment, and ideas for future studies.

Dr. Chen and Dr. Polfer were the impetus for this activity. I have a science research student who has done research projects the past 2 years on genetically modified foods, and the protein extraction and identification is the logical next step. The student will contact Dr. Chen and plan on travelling to UF at Dr. Chen's convenience, preferably in the month of November.

I will encourage the student to present the results of her research as a presenter or speaker at JSEHS.

In addition, after the Regional Science Fair competition in February, the high school research students will be implementing Mission Biotech for 2-3 weeks in the classroom. I will also be presenting Mission Biotech to science teachers at a mini-conference in Brevard County on September 13th.

Budget: Mini-PROTEAN Tetra Cell for vertical gel electrophoresis- \$299.25

Lesson Plan for Protein Isolation and Identification in Genetically Modified Food

Objectives:

- Students will research and write a research paper on genetically modified foods (GMOs).
- Students will identify and research a protein inserted into a GMO.
- Students will extract DNA, amplify the DNA with PCR, and run gel electrophoresis.
- Students will perform protein extraction, and use mass spectrometry to isolate the protein.
- Students will use a Protein Search Engine to identify the protein they isolated.

Weeks 1 and 2:

Students will write a 1,500-2,000 word research paper on a genetically modified food of their choosing such as corn, tomato, or a dairy product. Through their research students must uncover which protein or proteins have been inserted into the organism's (GMF) genome. Students will research the protein's function, the role it will play in the modified food, and the desired outcome of the modification. The students will also need to find the molecular weight of the protein to use as a guide for removing the correct band after SDS-gel electrophoresis.

Week 3 Day1:

Review in depth the steps of DNA extraction, PCR, gel electrophoresis, and equipment handling.

Week 3 Days 2-5

Perform DNA extraction, PCR, and gel electrophoresis on foods the students have procured. One sample should be genetically modified, the other will not be. Both foods must be of the same type, example tomatoes.

If the foods are determined to be genetically different based on gel electrophoresis, proceed to the next step. If not, continue DNA extraction etc. until a GMO food is found.

Subject the GMO to protein extraction through vertical SDS-gel electrophoresis and protein in-gel digestion. Arrange to have mass spectrometry MALDI-TOF MS performed on the protein of interest (based on its molecular weight) in another lab (Dr. Chen, UF).

Week 4 Day 1

Enter the accumulated spectrum data into the Protein Search Engine on the website <http://www.moleculardetective.org>. to attempt protein identification.

Week 4 Days 2 and 3

Students will write formal lab reports which will include their methods, a discussion of the results, a conclusion that summarizes their experiment, and ideas for future studies.

S.S .S. addressed:

SC.912.L.16.In.e: Identify ways that biotechnology has impacted society and the environment, such as the development of new medicines and farming techniques.

SC.912.L.16.Su.d: Recognize that new medicines and foods can be developed by science (biotechnology).

SC.912.N.1.In.a: Identify a problem based on a specific body of knowledge, including life science, earth and space science, or physical science, and do the following: 1. Identify a scientific question 2. Examine reliable sources of information to identify what is already known 3. Develop a possible explanation (hypothesis) 4. Plan and carry out an experiment 5. Gather data based on measurement and observations 6. Evaluate the data 7. Use the data to support reasonable explanations, inferences, and conclusions.

SC.912.N.1.In.d: Identify that scientists use many different methods in conducting their research.

SC.912.N.1.Pa.b: Recognize a process used in science to solve problems, such as observing, following procedures, and recognizing results.