

Detection of Genetically Modified Food

Abstract

Genetically modified foods are often in the news and widely grown in the United States. Three US government agencies (USDA, FDA, and EPA) work to regulate the introduction and production of genetically modified foods. These crops can provide agricultural, ecological and nutritional benefits, but there are also potential risks to the environment and consumers. As consumers and public interest groups around the world have become aware of these risks, there has been a call for more explicit product labeling and reliable methods for the detection of genetic modification in the foods we eat. This lab activity explores these issues by taking students through a three-part process to detect the presence of genetic modification in corn (maize) or soy food products. This lab uses one of the two methods for detection of genetic modification currently approved by the European Union.

Instructor Background

Introduction

Genetically modified foods are often in the news. While genetic modifications have made improvements in many crops and helped to increase yields, many groups have raised loud protest against “tinkering” with crop plants. In the US, however, genetically modified foods have been introduced to the market with little fanfare. For some crops in the US, over half of the acres planted are genetically modified varieties (USDA/NASS 2003) (Table 1). Much of the world, in contrast, has experienced strong and increasing resistance to the introduction of genetically modified foods to the market place. The European Union and other countries require certification that foods entering their countries be Genetically Modified Organism (GMO) free or contain minimal limits.

In the fall of 2000, genetically modified foods caught the attention of the US press when it was revealed by a watchdog group that Taco Bell® brand taco shells contained a type of genetically modified corn that was not approved for human consumption by the USDA. A nationwide recall of corn products was ordered after independent verification of the earlier results. Although the particular corn modification in these foods was not approved, the press largely ignored the fact that the USDA, FDA, and EPA already approve many genetically modified foods. With the availability of simple tests like this one to detect genetic modification in food products, public awareness will continue to increase.

Table 1.

Percentage of Acres Planted in the United States (All Genetically Modified Varieties)

	2000	2001	2002	2003	2004
Corn	25%	26%	34%	40%	45%
Cotton	61%	69%	71%	73%	76%
Soy Beans	54%	68%	75%	81%	85%

Source: (USDA/NASS 2003) United States Department of Agriculture / National Agricultural Statistics Service, Farmer Reported Genetically Modified Varieties. All Genetic Varieties: <http://jan.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0604.pdf>

Benefits of Genetically Modified Foods

People have been modifying crop plants since the dawn of agriculture. Year after year, ancient people selected and saved seeds from plants displaying specific traits. Later, with cross breeding and the development of hybrid plants, traditional plant breeding emerged. Today, modern techniques in biotechnology allow plant breeders to introduce very specific traits via particular genes into plants. Inserted genes may come from the same species of plant, from other plant species, or even from animals or bacteria.

Genetic modification of crops can produce three general benefits: 1) agricultural—increased yield, 2) environmental—reduced use of pesticides, herbicides, and fuel, and 3) nutritional—improved quality of foods and foods that prevent disease. Some genetic modifications may produce a combination of two or even all three of the benefits listed above.

Agricultural benefits include new methods to improve productivity and profitability, while at the same time, some modifications may improve the environment by reducing reliance on pesticides and herbicides (Table 2).

Table 2. Selected Genetically Modified Crops Currently Allowed in the US Food Supply
March 2000

Product	Company	Engineered Trait	Name & Year
Corn	Novartis	Bt toxin to control insect pests	Bt 11 1996
Corn	Novartis	Bt toxin to control insect pests	Knock Out™ 1995
Corn	Dow/Mycogen	Bt toxin to control insect pests	NaureGard 1995
Corn	Monsanto/DeKalb	Bt toxin to control insect pests	Bt-Xtra 1997
Canola	Monsanto	Resist glyphosate herbicide to control weeds	Roundup® Ready 1999
Cotton	Monsanto	Bt toxin to control insect pests	Bollgard® 1995
Cotton	Monsanto	Resist glyphosate herbicide to control weeds	Roundup® Ready 1996
Papaya	Cornell Univ. / Univ. Hawaii	Resist papaya ringspot virus	Sunup, Rainbow 1997
Potato	Monsanto	Bt toxin to control insect pests	NewLeaf 1995
Squash	Seminis Vegetable Seed	Resist watermelon mosaic 2 and zucchini yellow mosaic viruses	Freedom II 1995
Soybean	Monsanto	Resist glyphosate herbicide to control weeds	Roundup® Ready 1995
Sugarbeet	Aventis	Resist glyphosate herbicide to control weeds	Name unknown 2000
Tomato	Monsanto / Calgene	Altered ripening to enhance fresh market value	FlavrSavr™ 1994

Source: US Food and Drug Administration (FDA 2000)

Several agricultural biotech companies have modified crops by introducing a gene that produces *Bt* toxin to control insect pests in lieu of pesticide application. The source of the gene is the bacterium *Bacillus thuringiensis* (*Bt*) which has long been used for insect control. Organic farmers use the bacterium because it kills plant-eating insects but not beneficial insects like bees. Insects ingest the *Bt* protein, which binds to the epithelial cells of the midgut killing the insect. Because the *Bt* protein is broken down in an acidic gut, vertebrates are not affected. *Bt* does not contaminate groundwater and is considered nontoxic to humans and livestock (Ramanujan 2000).

Another example of the agricultural benefits of modifying crops has been developed by the Monsanto Company. Monsanto has several products that contain a gene (CP4 EPSPS) that makes crop plants tolerant of the herbicide glyphosate (trade name Roundup®). Glyphosate kills plants by blocking the pathway for synthesizing essential amino acids (Clark 2000). The crops containing the herbicide tolerant gene are called Roundup Ready®. Before Roundup Ready® crops were developed, farmers would apply residual herbicides that stayed in the soil before and after the crops emerged. With Roundup Ready® crops growers apply glyphosate herbicide over the top of the crop without harming the growing crop. Roundup Ready® crops allow growers to reduce the amount of herbicide applied to fields, improve weed control and reduce crop damage from conventional herbicides (Monsanto Company 2003).

Further, scientists are working to engineer resistance to certain plant pathogens. For example, researchers at the University of Hawaii and Cornell University have

developed two new varieties of papayas, which are resistant to the papaya ringspot virus (PRSV). Papaya is Hawaii's second largest fruit crop and an important crop in the tropics everywhere. Worldwide, the disease is a serious threat because it is rapidly transmitted and can quickly destroy entire plantations. The modification used in papaya is called 'pathogen derived resistance,' where a gene from the pathogen is inserted to fight the pathogen itself (Tennant 1994).

Not only can genetic modification help to reduce pesticide use and crop diseases, it can also improve food quality. The first genetically modified whole food brought to market in the United States was the Flavr Savr™ tomato. The Flavr Savr™ tomato was developed by Calgene, a small California biotech start-up company; the Flavr Savr™ went on sale in spring 1994. The tomato contained an antisense gene that coded for an enzyme called polygalacturonase (PG), which involved the fruit ripening process. The Flavr Savr™ antisense gene shut down native PG, increasing the shelf life of ripe tomatoes (Martineau 2001). Flavr Savr™ tomatoes could be allowed to ripen on the vine creating a tastier tomato and still survive picking and shipment to market resulting in a firm and tasty tomato for consumers. In the beginning, Flavr Savr™ sales soared, but the success was short lived: Calgene was not able to supply enough tomatoes to meet high market demands.

Consumers also benefit by having foods available to them with improved nutritional content or even edible vaccines. Golden rice is an important example. The yellow-colored grains are produced by rice genetically altered to make beta-carotene, a pigment the body converts to Vitamin A. Vitamin A deficiency leads to blindness and immune system impairment, especially in children. This vitamin deficiency contributes to the death of more than a million children each year in Asia, Africa, and Latin America (Rusting 2000). To date, the developers of golden rice have not yet been able to distribute seeds to areas where it is needed. They are still in the process of obtaining permission from more than 40 patent and contract holders (Pollack 2003).

Foods engineered to combat human disease offer enormous advances in public health. One-day children may get immunized by eating foods such as bananas, potatoes and tomatoes. The modified plants could be grown locally at low cost eliminating problems of vaccine transport and refrigeration. Edible vaccines would not require syringes or other equipment, which often contribute to infection and disease spread upon reuse (Landridge 2000). Researchers at Loma Linda University School of Medicine have already succeeded in inserting a gene from the cholera bacterium into potatoes. The modified potatoes produce a nontoxic component of the cholera toxin that triggers the production of antibodies against cholera when eaten (Arakawa 1998).

Finally, many modifications contribute to increasing the amount of food produced worldwide. As the world population continues to increase, it is undeniable that the problem of feeding everyone will require greater food production. Increased crop yields through genetic modification can work toward that end.

Risks of Genetically Modified Foods

People argue that along with the benefits of genetically modifying food come risks. Such risks may include: antibiotic resistant bacteria, exposure to possible allergens and toxins, the spread of introduced genes to non-target plants by out-crossing, and pollen drift and harm to the environment (Obrycki 2001).

Some people fear that genetically modified foods containing antibiotic resistance genes, as selectable markers, may promote drug resistant bacteria. A study, commissioned by the United Kingdom's Food Standards Agency, showed for the first time that bacteria in the human gut can take up DNA from genetically modified food. The study was performed on two groups of people, one group whose intestinal systems were fully intact and another group who had their colons surgically removed (ileostomy). When they examined stools from the group with intact intestinal systems, they found no evidence that gut bacteria had taken up the DNA. But when they examined waste products collected from ileostomy bags, they found that almost half of those samples contained bacteria that had taken up genetically modified DNA from food. To account for the difference, researchers speculate that DNA may survive the small intestine but gets completely digested by enzymes in the large intestine (New Scientist 2002).

The American Medical Association says that horizontal gene transfer from plants to environmental bacteria or from plant products consumed as food to gut microorganisms would be exceedingly rare but cannot be completely discounted (AMA 2000).

In November 2000, the Food and Drug Administration recalled 300 supermarket and restaurant products made with StarLink™ corn, a possible human food allergen. StarLink™, produced by Aventis, Research Triangle Park, N.C., contains the gene Cry9C, which protects the plants against insect pests. The EPA had approved StarLink™ corn in 1998 with the stipulation that it was not for human consumption. Studies had shown that the Cry9C protein produced in the modified corn was heat stable and resistant to stomach acids and enzymes, all characteristics of human allergens, hence the restriction on human use. Aventis failed to keep StarLink™ corn separate from approved and nongenetically modified corn so the unapproved corn entered the market initiating the massive recall (USDA 2000).

A different problem arose for the Terra Prima organic corn chip company in Hudson, Wisconsin, in 1995. Despite strict practices by its organic corn growers, it was discovered that some of Terra Prima's Apache Tortilla chips showed traces of *Bt* corn. Genetic testing revealed that pollen from a crop of Novartis *Bt* corn planted more than a quarter-mile away had contaminated an organic corn field of one of Terra Prima's suppliers. Because of the contamination by pollen drift, Terra Prima recalled and destroyed 90,000 bags of chips, a significant monetary loss to the small company (Ramanujan 2000).

In November of 2001, a controversial article appeared in the journal *Nature*. The paper claimed that genes from genetically modified plants had spread to native corn in the remote mountains of Oaxaca, Mexico. In 1998 the government of Mexico had banned the planting of genetically modified crops to preserve genetic diversity in the

country's native corn varieties and in the wild ancestor of corn, known as teosinte. The source of contamination is unclear. The contamination may have occurred before the ban was enacted or the ban on the planting of genetically modified corn was poorly enforced (Scientific American 2001).

In December of 2002, officials from the USDA, working with the FDA, imposed a fine against ProdiGene, Inc. of College Park, TX. In Iowa, ProdiGene test plots of biopharmaceutical corn were allowed to cross-pollinate with corn grown for human consumption. And in Nebraska, ProdiGene allowed volunteer corn plants from a previous years biopharmaceutical field test to contaminate 2002 soybean fields intended for human consumption (Fox 2003). Since the biopharmaceutical corn may be considered a drug and not widely approved for human or animal consumption, a new problem with pollen drift and possible environmental contamination had arisen.

It is likely that more field tests of crops containing biopharmaceuticals and industrial chemicals will occur. Can companies and government regulators guarantee the integrity of the consumer food supply?

Methods for Genetically Modifying Foods

In order to genetically modify food crops, one needs a reliable means of introducing new genetic material into the host. There are three main methods for introducing foreign DNA: biological vectors (Ti-plasmid from *Agrobacterium*), physical methods (particle gun, microinjection and electroporation), and chemical methods (polyethyleneglycol and calcium chloride) (Hemmer 1997).

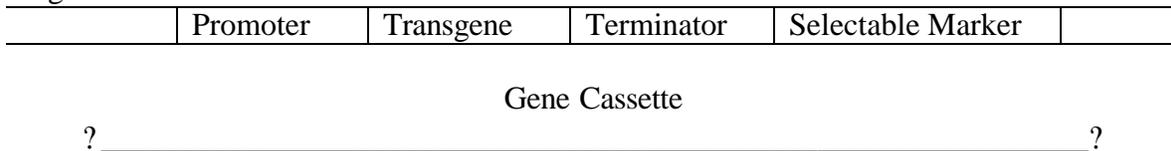
Of the three methods, the biological vector system is used most often. This is a binary vector system. One vector contains the DNA to be transferred (the transgene) and is introduced along with the second vector, the Ti plasmid of *Agrobacterium tumefaciens*, which contains genes encoding the necessary mechanism for the genetic transfer to take place (McBride 1990). This method utilizes the natural transformation capabilities of the soil bacterium, *Agrobacterium tumefaciens*, to insert selected DNA from the Ti plasmid into plants.

In nature, *Agrobacterium tumefaciens* causes the formation of a gall on the crown of the plant it infects. This is known as crown gall disease. The bacterium has the ability to insert its own T-DNA from the Ti plasmid into the DNA of the host plant where it is expressed by the infected host. Essentially, the bacteria forces the plant to produce substances the bacteria needs to grow (Watson 1983).

Genetic engineers can manipulate the *Agrobacterium tumefaciens* vector system, so that instead of the bacteria inserting its own Ti plasmid DNA into the plant that ordinarily causes the gall to form, the gall forming DNA is removed with restriction enzymes and replaced with a gene cassette. The gene cassette (Figure 1) is composed of a promoter, transgene, terminator and a selectable marker. The *Agrobacterium tumefaciens* containing the new gene cassette is then incubated under specific conditions with the plant cells and the gene cassette is transferred into the plant cells.

In order for the transgene to work effectively in its new host, it needs to be controlled by a promoter sequence and a terminator sequence. Many potential promoter elements have been identified, but the most commonly used is the CaMV35S promoter derived from the phytopathogenic cauliflower mosaic virus (*Caulimovirus*) (Spath 2000). The NOS terminator from the Ti plasmid in *Agrobacterium tumefaciens* is the most common terminator.

Figure 1.



Researchers need to be able to identify which plant cells have been transformed or have incorporated the new DNA into their chromosomes. A selectable marker is needed. Antibiotic resistance genes are commonly used as selectable markers. When the plant cells are grown on media containing the antibiotic, only the cells containing the antibiotic resistant gene can multiply and develop into full-grown plants.

Regulation of Genetically Modified Foods

The US government works to ensure that new agricultural biotechnology products are safe for animal and human health and safe for the environment (USDA 2000). Three agencies are involved: the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and United States Department of Agriculture (USDA) / Animal and Plant Health Inspection Service (APHIS).

Under FDA regulations, companies are legally obliged to ensure that any food product meets the safety standards of the law. This applies equally to both conventional and genetically modified food. The FDA will recall products if they do not meet safety standards (USDA 2000). The safe use of pesticide and herbicide substances is regulated by the EPA. The EPA reviews and approves bioengineered food that contain herbicide or pesticide elements (USDA 2000). Companies wishing to field test or move biotechnology derived plants must obtain APHIS approval under USDA regulations. An APHIS “determination of non-regulated status” must be obtained by companies before a crop can be produced on a wide scale and sold commercially (USDA 2000). Over 5000 field trials have been conducted since 1987 with APHIS approval. About 40 GMO products have met all federal regulatory requirements and are sold commercially (USDA 2000).

In other countries regulations for GMO foods vary. Some countries have adopted labeling regulations. Such regulations necessitate appropriate techniques to identify the presence or absence of genetic modification so food can be labeled properly (Zimmermann 1998). Many countries and communities are developing and standardizing methods for the detection of genetically modified foods.

In the US, the USDA Grain Inspection, Packers and Stockyards Administration (GIPSA) is establishing a biotech reference laboratory in Kansas City, MO. The reference lab will evaluate and verify analytical techniques for the detection of genetically enhanced grains and grain products. The lab will also accredit independent analytical laboratories that test for the presence of genetic modification. This new reference lab will alleviate testing problems that occur because currently there is no standardization of reference materials, sampling methods, or extraction procedures for detection tests (USDA/ERS 2000).

Detection of Genetically Modified Foods

Several companies in the United States and Europe test foods for genetic modification. Methods vary for the different companies and countries, but there are two basic means for detecting genetic modification. One method tests food for the product of the transgene, usually a protein. The other method tests for the presence of DNA from the transgene or another portion of the gene cassette.

Proteins are assayed using an ELISA (Enzyme Linked ImmunoSorbent Assay). The protein is assayed by first forming a complex with an antibody that is linked to an enzyme. This complex is then detected when the enzyme produces a color change in a chemical marker. ELISAs usually cost less than DNA tests, offer quicker results, and can sometimes be done on site. The big drawback is that ELISAs do not work well on processed foods because heat during processing can destroy the protein.

In contrast, DNA tests are more expensive, cannot be done on site, and take several hours to complete. Importantly, however, DNA tests are very accurate, work on processed foods, and can be quantified.

The British government is considering forcing biotech companies to encode a specific, detectable DNA sequence or barcode into genetically modified organism to make identification easier. The new DNA barcodes would make it easier for regulators to trace genetically modified food or detect which genetically modified strains have possibly contaminated other foods or crops. The DNA barcode would simplify detection methods currently used. To date all genetically modified food crops use the same CaMV35S promoter but this convention could change. With the barcode method, all genetically modified organisms would contain the same unique DNA sequence and a simple DNA polymerase chain reaction (PCR) test could identify any product as genetically modified or not. More specific DNA barcodes could be added to provide detailed information about the product, when it was produced and by which company (New Scientist 2003).

Detection Laboratory Background

The following lab uses a DNA PCR test to detect foreign DNA in genetically modified food. The lab uses primers which are designed to amplify DNA from the

CaMV35S promoter component of the gene cassette. Amplification of this promoter yields DNA fragments 195 base pairs (bp) in length.

Since all transgenic crops contain the CaMV35S promoter, it offers a good target for DNA testing. A positive test for this promoter is not always conclusive, however. Plants in the cabbage family (*Brassicaceae* formerly *Cruciferae*) should be treated carefully because they may be naturally infected with the *Caulimovirus*, the source of the CaMV35S promoter. In such cases further PCR tests should be run with primers designed to amplify the specific transgene DNA. For plants from other families, the risk of infection by the *Caulimovirus* is very small (DG JRC 1998).

The following lab activity has been modified for use in the pre-college or college classroom. The method of DNA isolation and PCR are based on currently validated tests used in the European Union (DG JRC 1998). This lab is designed to be used with corn products and certified reference standard maize powder from the Institute for Reference Materials and Measurements. Efforts have been made to keep costs to a minimum; however, PCR lab activities are generally not considered to be shoestring. This activity assumes the instructor has previous experience and knowledge of PCR and the necessary preparation involved. Instructors should allow several lab periods to complete this lab activity.

The lab has three parts: 1) isolation of DNA from food, 2) PCR, and 3) visualization of the results using agarose gel electrophoresis.

DNA Isolation Background

During steps 1-3 of the DNA Isolation, the food is hydrated with nuclease free water and homogenized with a stick.

In steps 4-6, extraction buffer, guanidine-HCl and proteinase K are added to the hydrated, homogenized mixture. Cells contain three major classes of biologically important molecules: proteins, lipids, and nucleic acids. The components in the extraction buffer, along with proteinase K and guanidine-HCl work to free the cellular nucleic acids from the cell lipids and proteins while keeping the nucleic acids intact.

The extraction buffer contains four components. Tris keeps the mixture buffered at an optimal pH of 7.5. EDTA binds divalent cations in the lipid bilayer, weakening the food cell membranes and also limits DNA degradation by binding Mg⁺⁺ ions that are a necessary cofactor for nucleases (Micklos 1990). SDS is a detergent that dissolves the lipid components of cell membranes. NaCl salt maintains an optimal ionic strength for the extraction mixture (Seidman 2000).

Proteins and nucleases released when the cells are disrupted are removed by digestion at 55-60 °C with proteinase K, which is active against a broad spectrum of native proteins (Manniatis 1982). Guanidine-HCl is a chaotropic salt. Chaotropic salts increase the solubility of DNA in water; they also denature cellular proteins like DNase, but not DNA. (BD Biosciences Clontech 2002)

In step 7, centrifugation separates the solid phase of degraded and denatured particles from the aqueous phase.

Next in steps 8-14, the DNA contained in the aqueous phase is allowed to pass through and bind to a proprietary silica-based resin (Promega 1996). Any remaining unbound aqueous phase components pass through the resin or are removed during the 80% isopropanol wash.

The principle of the method is based on selective adsorption of DNA to the resin in the presence of high concentrations of chaotropic salts and allows for the elution of the DNA with low ionic strength solutions such as TE buffer or water, steps 15-17 (Promega 1996).

Polymerase Chain Reaction (PCR)

In 1993, Kerry Mullis received the Nobel Prize in chemistry for his discovery of the Polymerase Chain Reaction (PCR) technique in the mid 1980's. Basically, PCR is a technique for making an exponential number of copies of a portion of DNA in just a few hours.

PCR became easier to perform with the discovery of an enzyme found in the bacterium *Thermus aquaticus* (*Taq*) which lives at a temperature of 75°C in water, the enzyme is stable even at 94°C. DNA polymerase is an enzyme that builds new strands of DNA along the strand in DNA replication. *Taq* DNA polymerase is heat stable and can be added just once at the start of a reaction and will remain active through a complete set of amplification cycles. Originally, DNA polymerase from *E. coli* was used in the PCR, but it was not stable at high temperatures, and fresh enzyme needed to be added manually for each cycle. With the discovery of *Taq* DNA polymerase, automation of PCR was made possible by using thermalcycler machines.

There are three basic steps in PCR that are repeated over and over again.

Step 1. Separation

In this step, double-stranded DNA molecules are heated to a temperature of 94°C, which separates or denatures them so that they become single-stranded. These single strands of DNA then become the templates for the new DNA strands that are made.

Step 2. Annealing

The *Taq* DNA polymerase must have something to hook onto to start building a new strand of DNA. A primer is used to start the process. Primers are short (10 to 30 base pairs long) pieces of single stranded DNA that will anneal, or stick, to the separated DNA template strand. Scientists use specific primer sets that flank the portion of the DNA they are interested in amplifying. In order for the primer to anneal to the template strand, the temperature must be lowered to a temperature that will allow a double stranded complex to form to start making a new strand.

Step 3. Extension

During this step of the cycle, the *Taq* DNA polymerase will extend the primer by bringing in complementary nucleotides as it moves along the template strand. The *Taq*

DNA polymerase works best at temperatures between 72 to 75°C. All four nucleotides are added to the reaction mixture so that the *Taq* DNA polymerase builds a new strand complementary to the template strand.

These three steps are repeated over and over again, the newly made strands are separated from the template strands by heating. The new strands then go on to serve as template strands for new strands to be made in each new cycle. This is called amplification. The reaction may be kept going for 20 to 50 cycles.

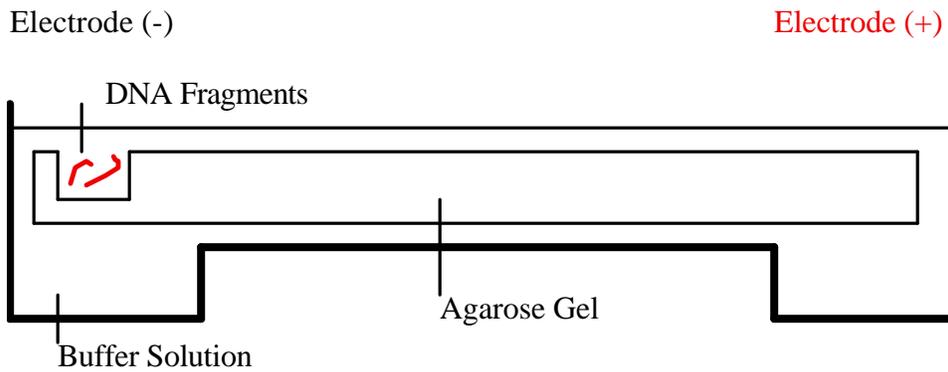
Agarose Gel Electrophoresis

Agarose gel electrophoresis separates mixtures of molecules in two ways: by electrical charge and by size. The technique was developed by a team of scientists at Cold Spring Harbor Laboratories in 1973 to separate DNA fragments and is widely used in molecular biology research today (Micklos 1990).

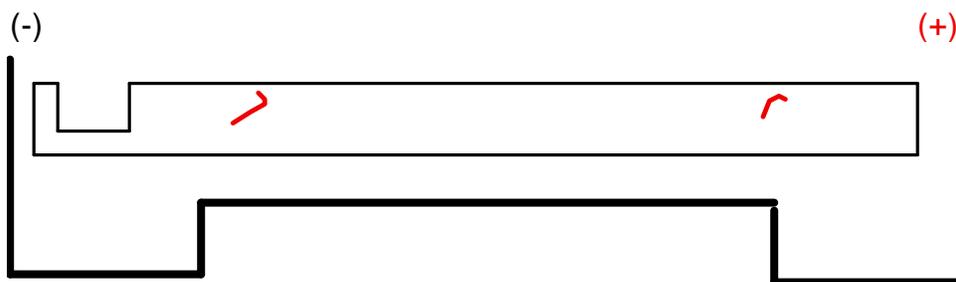
Electrophoresis means literally *to carry with electricity*. When charged molecules are placed in an electric field, positively charged molecules are attracted toward the negative pole (anode) and negatively charged molecules are attracted toward the positive pole (cathode) (Micklos 1990).

Agarose gel electrophoresis is performed with a highly purified form of agar. The agarose/buffer matrix conducts electricity and acts as a molecular filter through which small molecules can move more easily than larger ones (Micklos 1990). The agarose separates molecules by size. Four main constraints influence the migration rate of molecules in agarose gel electrophoresis. The constraints include agarose concentration, conformation and size of the molecules and the voltage applied (Maniatis 1982).

Agarose Gel Electrophoresis of DNA



Electrophoresis Chamber
A.



Electrophoresis Chamber
B.

Figure 2.

A. DNA fragments before electrical current is turned on. B. Shows how far the DNA fragments have migrated after the electrical current is applied. Smaller fragments of DNA migrate farther than larger fragments.

Graphic Adapted from DNA Science by Micklos & Freyer (Micklos 1990)

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