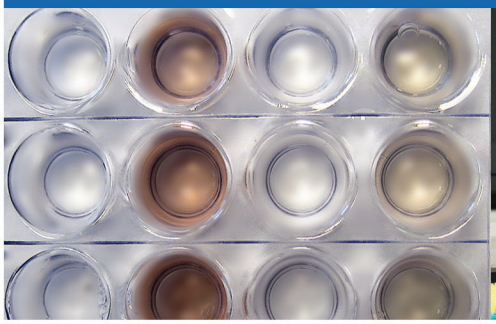
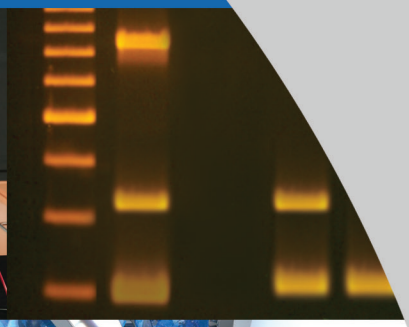
EDVOTEK WORKSHOP:

What's in My Lunch?

Using Biotechnology to Detect GMOs and Common Allergens

EDVOTEK®





www.edvotek.com

Introduction

Biotech got its first break with the domestication of animals and plants and today the field plays a major role in how our food is produced, tested, and consumed. Moreover, using common food items is a great way to engage students is real world science inquiry. This workshop explores Enzyme Link Immunosorbent Assays (ELISA) in the context of common food allergies and Polymerase Chain Reactions (PCR) in context of Genetically Modified Foods.

Background Information

WHAT ARE GENETICALLY MODIFIED ORGANISMS?

Over the past one hundred years, genetic research has expanded our understanding of the genome (the hereditary material of an organism encoded by its DNA) and its role as a blueprint for all processes within an organism. Variations in the DNA sequence, called mutations, can cause changes in the way an organism interacts with its environment. Most mutations result in negative effects for the organism; occasionally, a mutation grants an organism an advantage that promotes survival in its particular environment.

Humans have long recognized and taken advantage of genetic variation through traditional plant and animal husbandry techniques. For centuries, selective breeding and conventional hybridization have been used to increase crops' yields or give rise to other desirable qualities. While it used to take years of selective breeding to produce beneficial genomic changes, modern biotechnology techniques have accelerated this pace. Genetic engineering now allows scientists to directly manipulate a DNA sequence to generate desirable traits. These engineered genes, called transgenes, can be inserted, deleted or mutated in a matter of weeks using recombinant DNA technology. To be properly expressed in vivo, a transgene must include a promoter sequence, which recruits RNA polymerase to the transgene for transcription, and a terminator sequence, which signals RNA polymerase to end transcription (Figure 1).

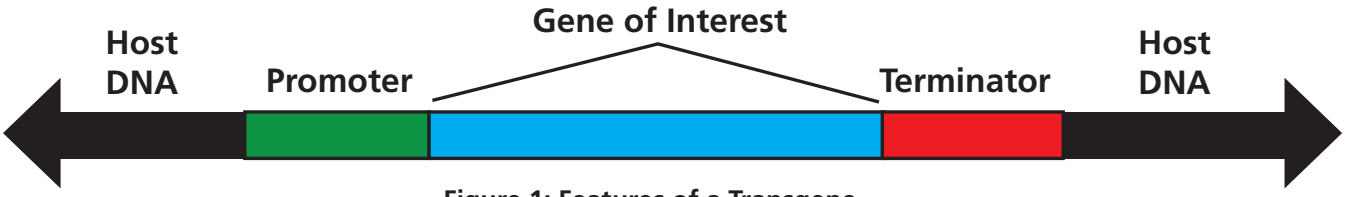


Figure 1: Features of a Transgene

Genetically modified organism like tomatoes, soybeans and corn were approved by U.S. agencies in the 1990s. Since then, the safety, efficacy and benefits of GM foods have been debated at a global level. Proponents of GM technology cite studies showing improved quantity and quality of plants, decreasing costs for growers, and benefits for the environment. Critics of GM technology fear the spread of transgenes to other crops, increased allergens, unanticipated health and environmental consequences, and the power that large GMO providers wield.

USING BIOTECHNOLOGY TO IDENTIFY GMOS

To determine whether food products have been genetically engineered, DNA is extracted from the samples and analyzed using the Polymerase Chain Reaction (PCR). This technique has revolutionized biological research because it quickly creates many copies of a specific region of DNA *in vitro*. This is accomplished by using short synthetic DNA molecules (primers) to target specific DNA sequences (Figure 2). In this way, PCR can differentiate between wild-type plants and those that have been genetically engineered by amplifying specific DNA sequences common in GM organisms.

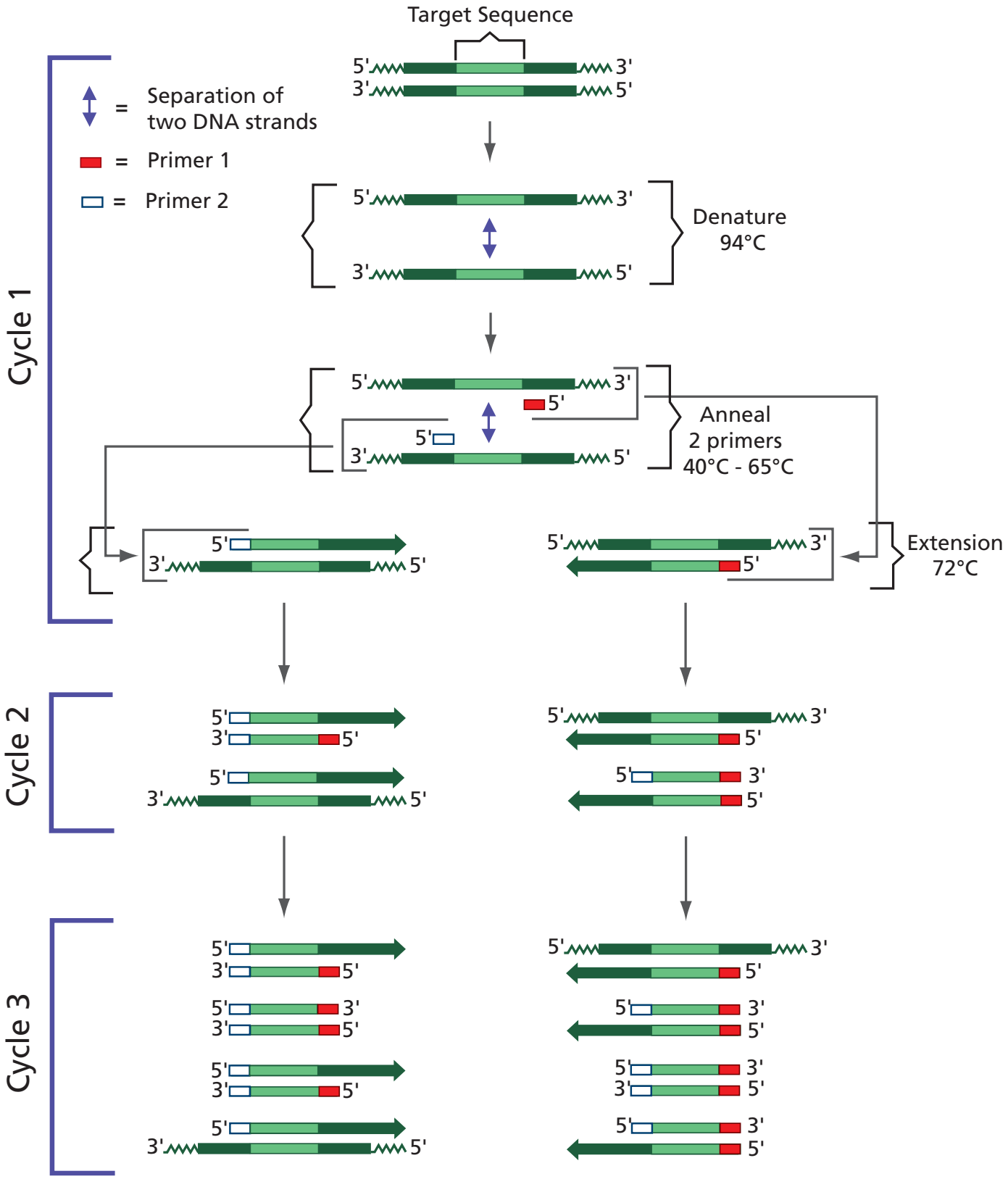


Figure 2:
DNA Amplification by the Polymerase Chain Reaction

Following PCR, scientists use a technique called agarose gel electrophoresis to separates DNA fragments according to size. The mixture of DNA molecules is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode. Small DNA fragments move through hole in the gel matrix easily, but large DNA fragments have a more difficult time squeezing through. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA.

UNDERSTANDING ALLERGIES

Allergies are one of the most common diseases of the immune system, occurring in up to 20% of people in developed countries. An allergic response occurs when the immune system overreacts to a foreign material, known as an antigen (short for antibody generator). Common antigens for allergy sufferers include infectious agents, such as bacteria or viruses, chemicals and various environmental materials such as pollen and food.

All allergies begin with sensitization, which starts when a normally non-hazardous antigen enters the body and encounters a lymphocyte cell (Figure 3A). For reasons still being researched, the lymphocyte cell registers this non-harmful particle as threatening, which triggers the production of novel immunoglobulin E (IgE) antibodies (Figure 3B). The highly specific IgE antibodies then attach themselves to immune cells, like mast cells and basophils, which circulate throughout the body (Figure 3C). The sensitization process can take between 6-10 days. After sensitization, IgE antibodies in the bloodstream can rapidly bind to their antigen, triggering immune cells to release mediator compounds such as histamine and proteoglycans into the body (Figure 3D). Once a person has become sensitized, small amounts of the antigen can trigger a full allergic reaction. Symptoms of an allergic reaction are varied, ranging from sneezing and itchy eyes to anaphylaxis.

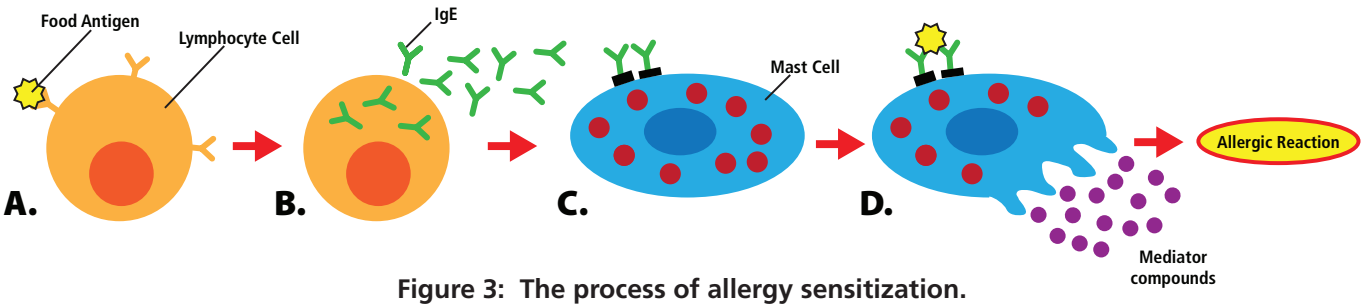


Figure 3: The process of allergy sensitization.

Food allergies are a serious and growing health issue that affects around 15 million people in the United States alone. For example, Cow's Milk Protein Allergy (CMPA) is the most common food allergy in children. Between 2 to 3% of the general population are diagnosed with CMPA, although many (79%) outgrow it by the age of 16. For sufferers, the main treatment for this condition is to eliminate cow's milk protein from their diet.

DETECTING FOOD ALLERGENS

In 2005 the FDA began requiring manufacturers to label the presence/absence of eight of the most common allergens (milk, eggs, fish, shellfish, peanuts, wheat, soybeans, and tree nuts) in their food products. This labeling helps consumers more easily identify and avoid potentially dangerous allergens. To detect these allergens in their product, food companies can use PCR, mass spectrometry, or immunochemical assays, although immunochemical assays tend to be the most popular due to their approachability and robustness.

One commonly used immunochemical assay is the Enzyme Linked Immunosorbent Assay (ELISA). ELISAs can determine the presence and concentration of specific antigens in complex solutions. To accomplish this, ELISAs rely on the ability of an antibody to recognize and bind to specific antigens. Most ELISAs use two different antibodies – a primary antibody that is specific to the antigen of interest and a secondary antibody that recognizes the antigen-antibody complex (Figure 4). This secondary antibody is coupled to an enzyme that reacts with a substrate to produce a signal. Since each secondary antibody can produce many molecules of the converted substrate ELISAs are very sensitive, even at extremely low levels of antigen.

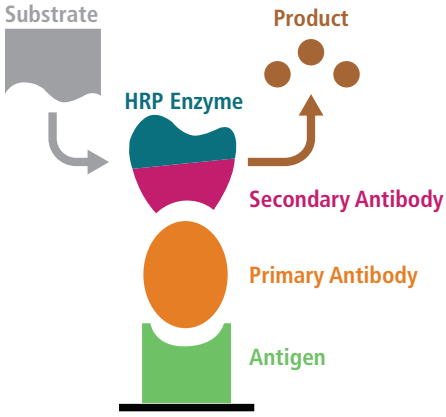
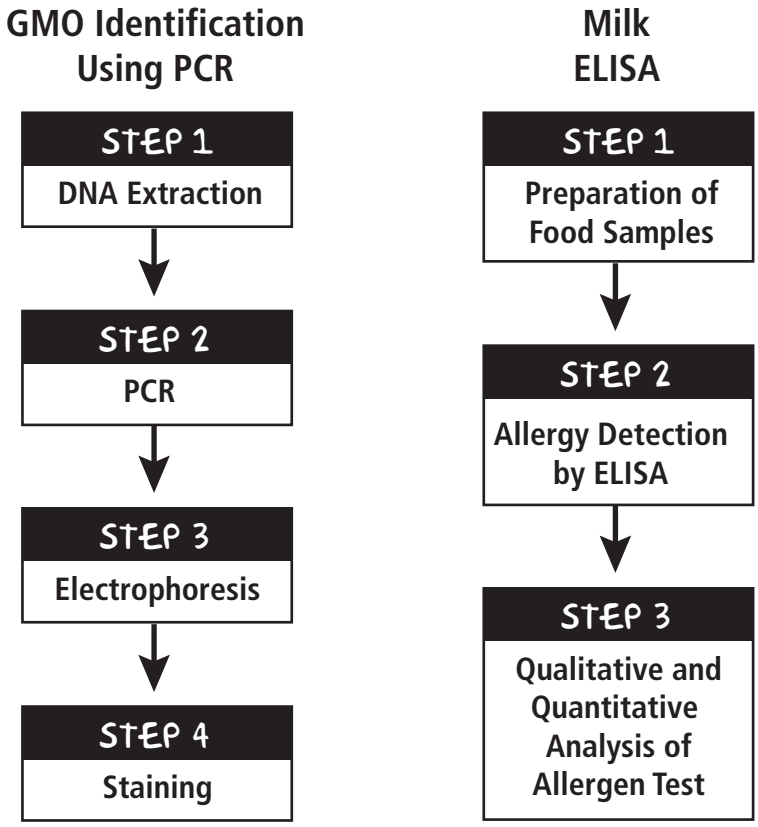


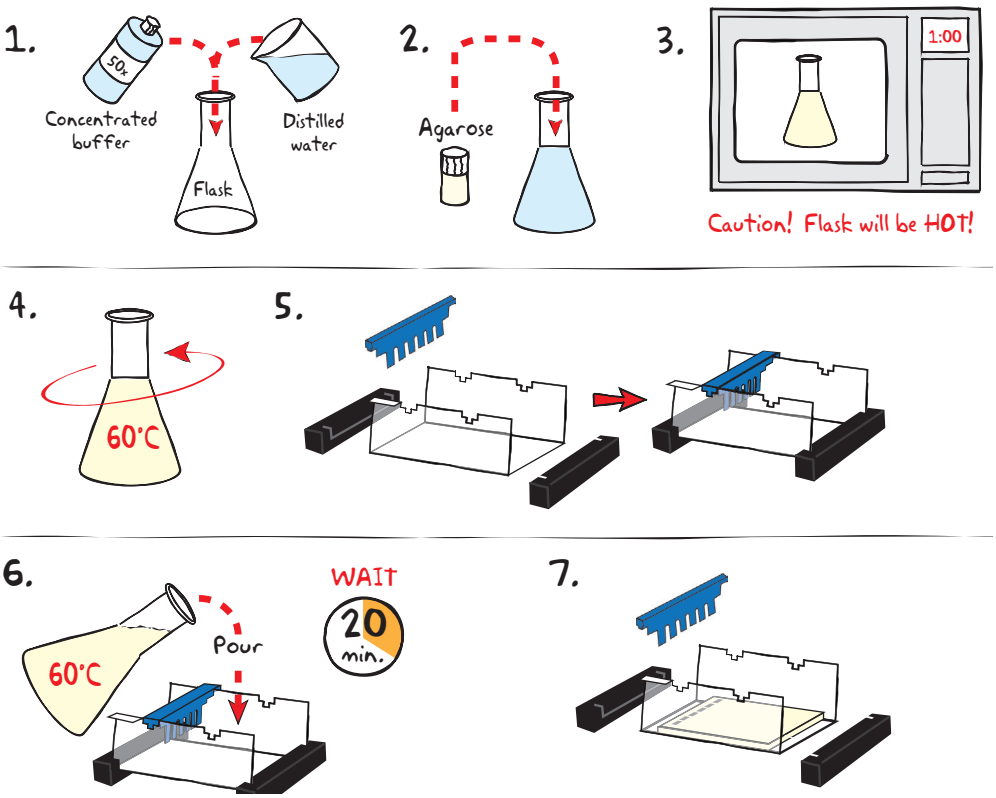
Figure 4: Overview of the ELISA.

ELISAs can be qualitative, in which case they indicate whether or not an antigen is present, or quantitative, in which case they also measure the antigen concentration. A quantitative ELISA requires that several wells be set-aside as standards. Each standard will contain a solution where the concentration of the antigen of interest is already known. The signal intensity in each well is measured and the values of the standards are plotted to create a standard curve. The intensity of the unknown samples can then be compared to the standard curve to determine an approximate protein concentration.

Experiment Overview



Separation of DNA by Agarose Gel Electrophoresis



NOTES:

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

Wear gloves and safety goggles

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table B).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

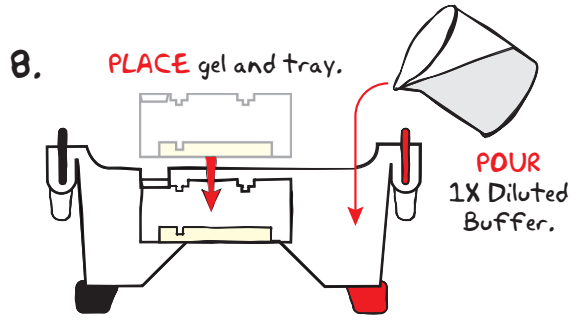
Table A — 1x Electrophoresis Buffer (Chamber Buffer)

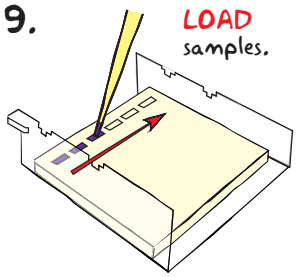
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table B — Individual 2.0% UltraSpec-Agarose™ Gel

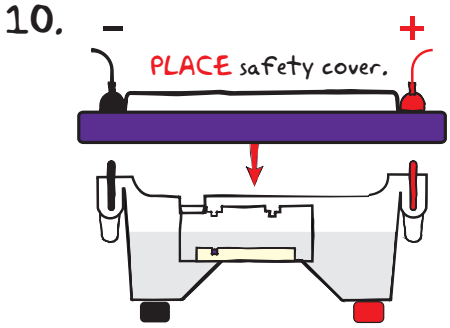
Size of Gel Casting Tray	1X Diluted Buffer	+ Amt of Agarose
7 x 7 cm	25 ml	0.50 g
7 x 14 cm	50 ml	1.0 g

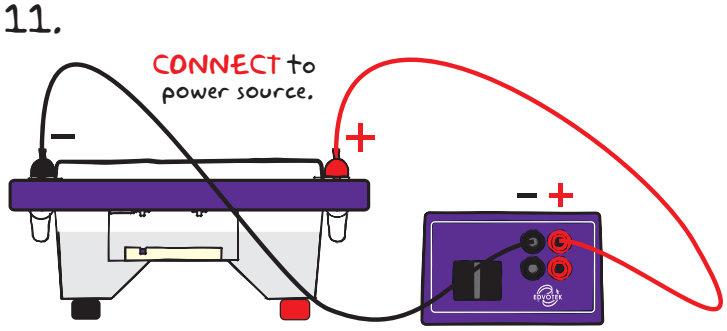
Separation of DNA by Agarose Gel Electrophoresis, continued





Reminder:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.





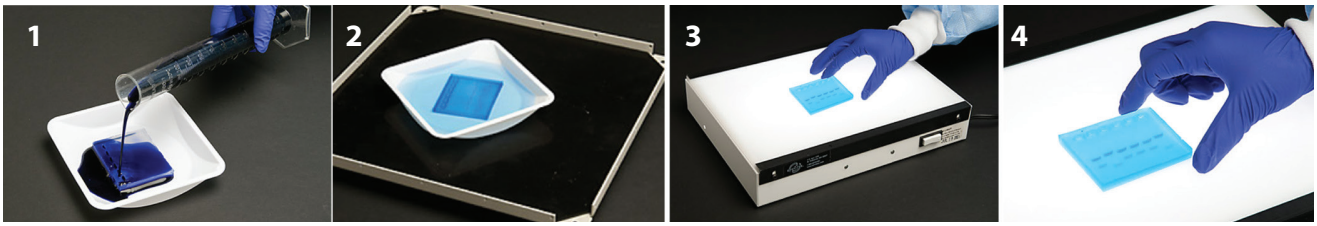
8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table A for recommended volumes). The gel should be completely submerged.
9. **LOAD** the entire sample (30 µl) into the well.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

Table C — Time and Voltage Guidelines (2.0% Agarose Gels)

Volts	Time: 7 x 7 cm gel ~4.0 cm migration	Time: 7 x 14 cm gel ~6.5 cm migration
125	30 min.	60 min.
70	60 min.	120 min.
50	90 min.	150 min.

Lane	Label	Sample Name
1	A	Standard DNA ladder
2	B	GMO Negative Control
3	C	GMO Positive Control
4	D	Corn Sample
5	E	Wheat Sample
6	F	Soy Sample

FlashBlue™ Stain • Simple & Rapid • Stain in Less Than 5 Minutes!



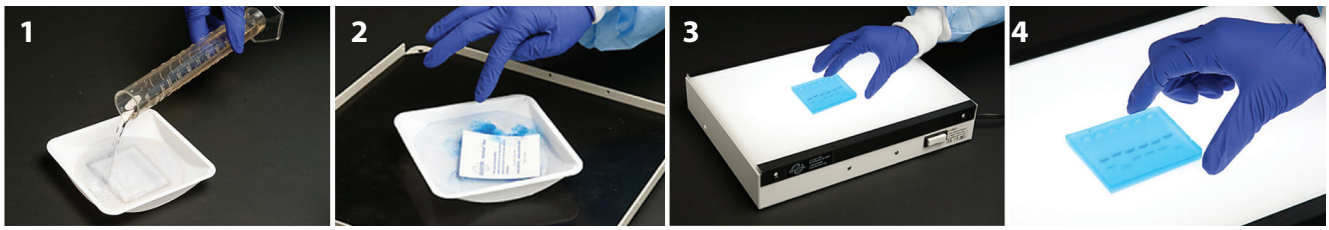
After electrophoresis, wear gloves and place the gel in a small gel staining tray. Pour 75 ml of 1x FlashBlue™ stain into the tray, enough to cover the gel. Allow the gel to stain for no longer than 5 minutes.

Transfer the gel to another container with 250-300 ml distilled water. Gently agitate container every few minutes or place on a shaking platform. Destain for at least an hour (longer periods will yield better results).

After destaining, gel should have a light blue background and well-stained DNA bands.

For optimal visibility, examine the gel on a white light visualization system.

InstaStain® Blue • Saves time & minimizes chemical waste!



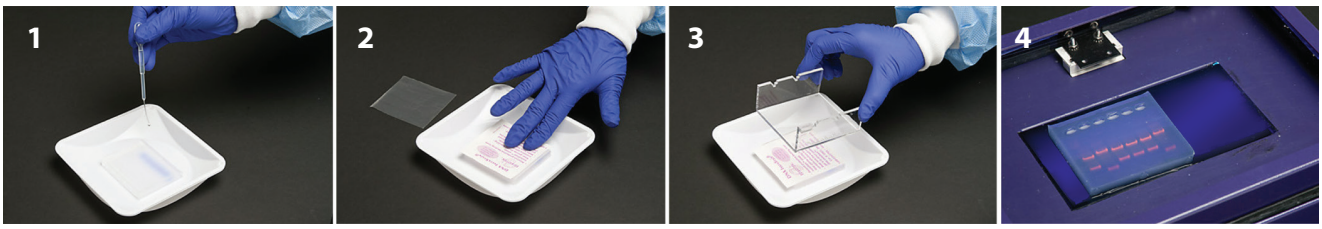
Remove the agarose gel from its bed and totally submerse the gel in a small, clean tray. To stain a 7 x 7 cm gel, add 75 ml of distilled or deionized water. The agarose gel should be completely covered with liquid.

Gently float a card of InstaStain® Blue with the stain side facing the liquid (blue side down). The gel will be ready for visualization in approx. 1 hour. For best results, cover the gel and let soak in the liquid overnight.

The gel is now stained, destained and ready for photography.

For optimum visibility, transfer the gel to a white light visualization system.

InstaStain® Ethidium Bromide • Minimizes chemical waste • Stain in 3-5 Minutes!



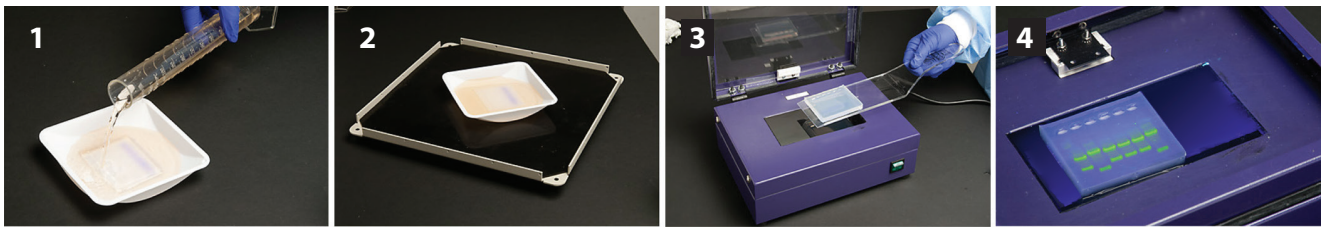
After electrophoresis, wear gloves and place the gel in a small gel staining tray. Moisten with a few drops of buffer.

Remove clear plastic protector and place the unprinted side of the InstaStain® Ethidium Bromide card on the gel. Firmly run your fingers over the entire surface of the card. Do this several times.

Place a small weight on top to ensure the card maintains direct contact with the gel. Stain the gel for 3-5 minutes.

After staining, remove the InstaStain® card. View gel on a UV transilluminator. Be sure to wear UV protective goggles.

SyBr® Safe DNA Stain • Non-mutagenic • Ultra-sensitive • Safe • Stain in 10-15 Minutes!



After electrophoresis, wear gloves and place the gel in a small gel staining tray. Add approx. 75 ml of 1x SYBR® Safe stain to the tray, enough to cover the gel.

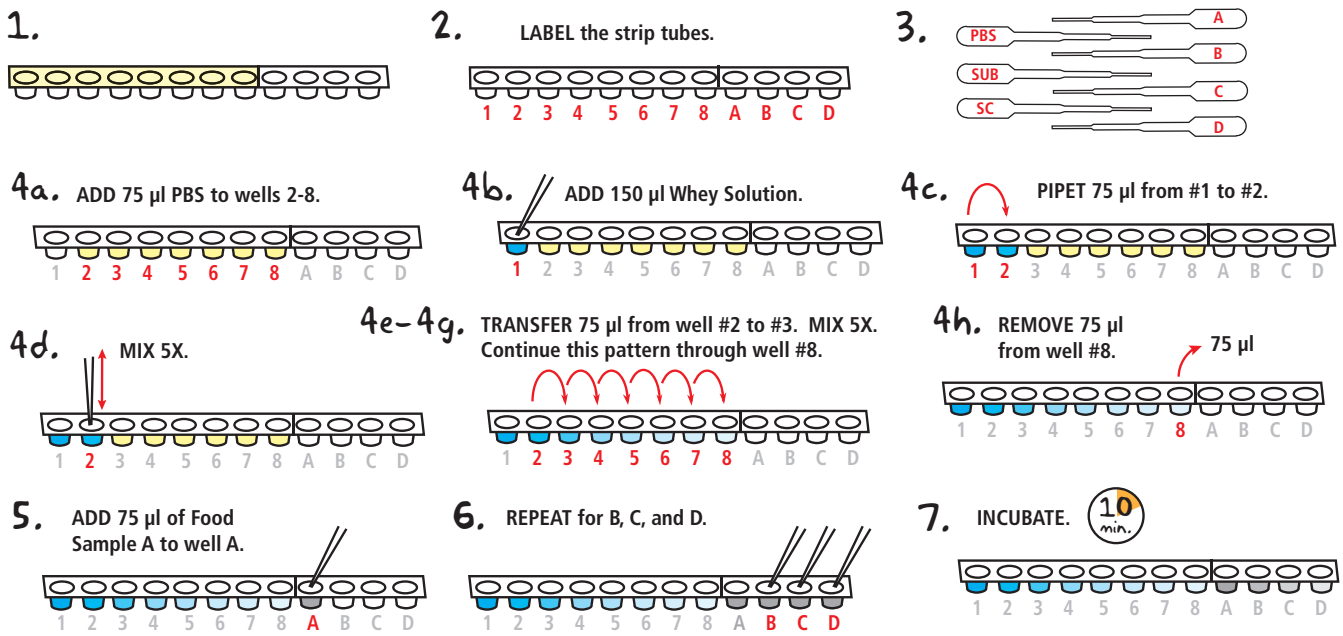
Allow the gel to stain for 10-15 minutes. (Agitation is optional.)

Wearing gloves, carefully remove the gel and transfer to a short/mid-range UV Transilluminator or blue bright light box.

Be sure to wear UV protective goggles. while visualizing DNA bands.

Allergen Detection by ELISA

This is a simulation of our milk allergy ELISA kit to save time we have combined the antigen, primary antibody, and secondary antibody steps. You will be testing four food samples: an energy bar (A), soy milk (B), dairy free ice cream (C), and dairy free yogurt (D).



1. **ORIENTATE** your strip so that the longer box is on the left.

2. **LABEL** the left box wells 1 through 8 and the right box wells A through D.

3. **LABEL** pipets "PBS", "SUB", "SC", "A", "B", "C", "D".

4. Prepare the standard curve.
   a. **ADD** 75 µl of PBS to wells 2-8 using a fixed or adjustable volume pipet.
   b. Change tips and then **ADD** 150 µl of Whey Solution in well 1. This solution is provided in a starting concentration of 30 µg/ml.
   c. **TRANSFER** 75 µl of the solution in well 1 to well 2.
   d. Fully **MIX** the sample by pipetting up and down 5 times.
   e. **TRANSFER** 75 µl of the solution in well 2 to well 3.
   f. Fully **MIX** the sample by pipetting up and down 5 times.
   g. **CONTINUE** serially diluting the remaining samples through well 8.
   h. **REMOVE** and discard 75 µl of the diluted antigen from well 8.

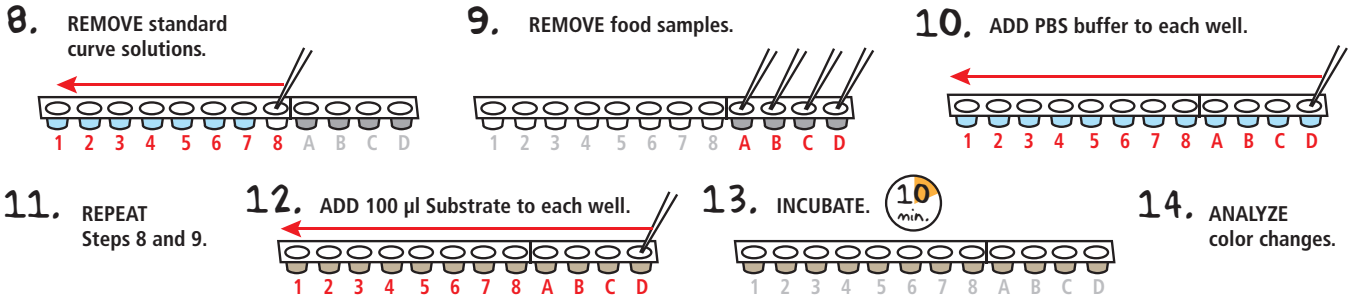
5. With a new pipet tip, **ADD** 75 µl of food sample A to well A.

6. **REPEAT** step 5 for food samples B, C, and D.

7. **INCUBATE** for 10 minutes at room temperature.

Continued

Allergen Detection by ELISA, continued



8. Using the "SC" transfer pipet, **REMOVE** all standard curve samples starting with well 8.

9. **REMOVE** all food samples using the appropriately labeled transfer pipet.

10. Using the PBS transfer pipet, **ADD** PBS buffer to each well until it is almost full – around 9 drops.

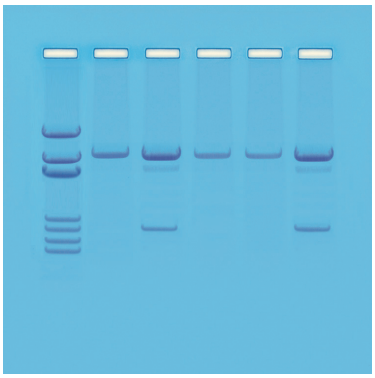
11. **REPEAT** steps 8 and 9 to remove the PBS solution.

12. Using the SUB transfer pipet, **ADD** 100 µl (4 drops) of substrate solution to all wells.

13. **INCUBATE** for 10 minutes at room temperature.

14. **ANALYZE** the strip.

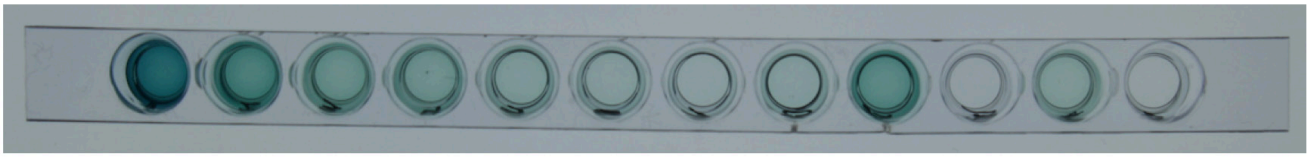
Experimental Results and Analysis



The DNA Standard Marker sizes are:
6751, 3652, 2827, 1568, 1118, 825, 630

Lane	Sample	Result	Molecular Weights
1	Standard DNA Marker	---------	---------
2	GMO Negative Control	GMO Negative	4282
3	GMO Positive Control	GMO Positive	4282 2872 1282
4	Corn Sample	GMO Negative	4282
5	Wheat Sample	GMO Negative	4282
6	Soy Sample	GMO Positive	4282 2872 1282

Plant Chloroplast: 4282
CaMV: 2872
Nos: 1282



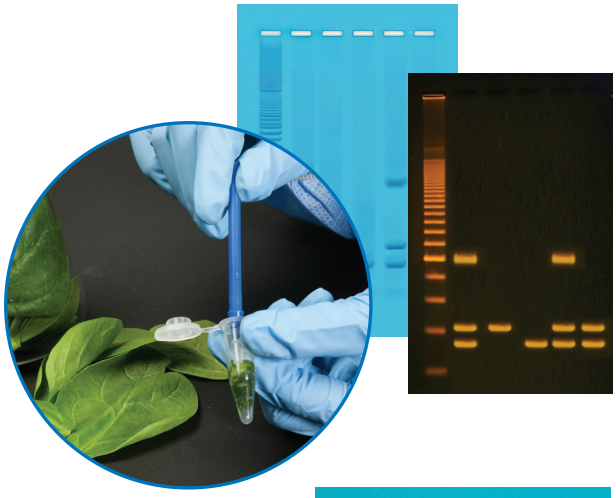
Well	1	2	3	4	5	6	7	8
Dilution	---	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Concentration	20 µg/ml	10 µg/ml	5 µg/ml	2.5 µg/ml	1.25 µg/ml	0.625 µg/ml	0.3125 µg/ml	0.15625 µg/ml

The color intensity of each well reflects the initial concentration of whey. In this simulation the energy bar (A) showed the highest concentration of why antigen. The dairy free ice cream (C) also showed trace levels of whey. Soy milk (B) and dairy free yogurt (D) had negative results. The concentration of positive samples can be estimated using the standard curve wells. Alternatively, densitometry analysis (quantitative measurement of light absorption) can be carried out with access to a digital cameral and computer.

Related Products

Cat. #962

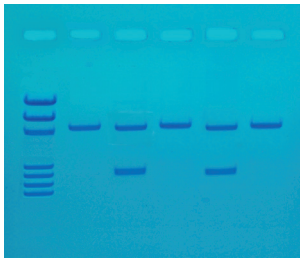
Identification of Genetically Modified Foods Using PCR



For 10 Groups. Some foods contain raw materials from genetically modified organisms (GMO). Examples include tofu, corn flakes and corn meal. In this experiment, your students will extract DNA from food or plant material and perform PCR to determine if any GM indicator genes are present. Amplified DNA is separated and sized by agarose gel electrophoresis.

Cat. #122

Detection of the Influenza Virus



For 8 Groups. The influenza virus, or "the flu," is a common contagious disease that affects the respiratory system. In this simulation, students will perform two common tests (RIDT, RT-PCR) used to diagnose the flu in a clinical setting.

Cat. #266

What's In My Lunch? Quantitative Milk Allergy ELISA

For 10 Groups. Milk proteins are the most common food allergens in children. Accurate detection and labeling is vital to inform consumers about potentially dangerous foods. In this inquiry-based experiment, students will master the concepts behind the enzyme-linked immunosorbent assay (ELISA). Students will perform an ELISA to detect the presence and measure the concentration of whey protein in various food products.

Cat. #5067

Classroom PCR LabStation™

Supports up to 25 Students.

- 6 Cat. #502/504 M12 Complete™ Package (7 x 14 cm Tray & 7 x 7 cm Trays (2)
- 3 Cat. #509 DuoSource™ 150 (75/150 V, for 1 or 2 units)
- 6 Cat. #590 Variable MicroPipet (5 - 50 µl)
- 2 Cat. #534 Piccolo Microcentrifuge
- 1 Cat. #541 EdvoCycler™ (25 x 0.2 ml)
- 1 Cat. #558 Midrange UV Transilluminator (7.5 x 7.5 cm filter)
- 1 Cat. #539 1.8 L Waterbath