

miniPCR™ GMO Learning Lab: Heart-Shaped Bananas

Newly-engineered GMO bananas can produce β -carotene, an essential nutrient and the primary dietary source of provitamin A especially needed by children. In this laboratory activity we will examine the genetic makeup of various foods, and weigh the evidence around the genetic engineering of crops.

Free

The DNA Times

Genetically Modified Bananas could solve world nutrition crisis

Transgenic bananas created by Australian scientists could help people produce vitamin A. Opponents contend the GMO fruit could expose eaters to unknown dangers. Public opinion goes bananas!

Cambridge, January 1, 2015. Bananas engineered by an Australian team contain a gene that helps them produce β -carotene (provitamin A), which our bodies can convert into vitamin A. In developing countries vitamin A deficiency is a major contributor to preventable childhood deaths. Researchers hope that the genetically engineered fruit can be a major breakthrough in the battle against malnutrition. Interestingly, these engineered bananas are of a variety that bends in an almost perfect heart shape.

As volunteers begin lining up for trials to test the GMO fruit's safety and effectiveness, opponents contend this could expose them to unknown dangers.

The engineered fruit has become a prominent topic in the GMO debate. Scientists say engineered crops can safely include important nutrients into the diet or other attributes important for agriculture. Skeptics worry that genetically modified foods could be dangerous and uncontrollable. Now, students equipped with cutting-edge molecular genetics tools are ready to weigh the evidence.



Missy Baker, town's favorite pastry chef, goes missing

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miniPCR™ GMO Learning Lab: Heart-Shaped Bananas

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1. Synopsis

Students will assess the presence of transgenic elements in processed foods and in plants by extracting DNA from food samples and analyzing DNA using the essential molecular biology techniques of PCR (polymerase chain reaction) and gel electrophoresis. This lab illustrates real-world applications of molecular biology in agriculture and the food industry such as the genetic engineering of plants for introduction of novel traits through recombinant DNA technologies.

- **Techniques utilized:** DNA extraction, PCR, gel electrophoresis, and DNA visualization
- **Time required:** Single 120 min. block or two 45-min. periods
- **Reagents needed:** 'miniPCR GMO Lab' reagents kit (available at miniPCR.com), gel electrophoresis reagents (See sections 5 and 9)
- **Suggested skill level:** Familiarity with DNA amplification concepts, basic familiarity with micropipetting techniques

2. Learning goals and skills developed

Student Learning Goals – students will:

- Understand the basic structure of DNA and its role in genetic inheritance
- Comprehend how DNA encodes traits that are passed across generations
- Understand what a genetically modified or genetically engineered organism (GMO) is
- Understand that PCR is a technique for amplifying specific parts of the genome
- Understand that seed selection and breeding can be used to alter traits in crops
- Understand how genetic engineering can be used to alter existing traits or introduce novel traits in crops in a faster and more targeted fashion
- Learn about agricultural and commercial impacts of genetically engineered crops
- Discuss implications of genetically engineered plants for society

Scientific Inquiry Skills – students should be able to:

- Formulate hypotheses and predict results
- Predict the presence or absence of genetically engineered sequences in plants and commercial food products
- Compare results to their predictions and draw conclusions based on hypotheses
- Engage in an evidence-based debate around GMO crops
- Generate data visualizations to present their results

Molecular Biology Skills:

- Micropipetting
- Principles and practice of PCR
- Preparation of agarose gels
- Agarose gel DNA electrophoresis
- Isolation of DNA from plant tissue and food products
- PCR amplification of transgenic sequences and their visualization
- Staining, visualization, and molecular weight analysis of DNA fragments

3. Standards alignment

Next Generation Science Standards - Students will be able to...

- HS-LS1-1 Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
- HS-LS2-6 Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.
- HS-LS2-7 Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.
- HS-LS3-1 Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS4-1 Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.
- HS-LS4-4 Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

Common Core English Language Arts Standards - Students will be able to...

- RST.11-12.1 Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.
- WHST.9-12.2 Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
- WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.
- SL.11-12.5 Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest.

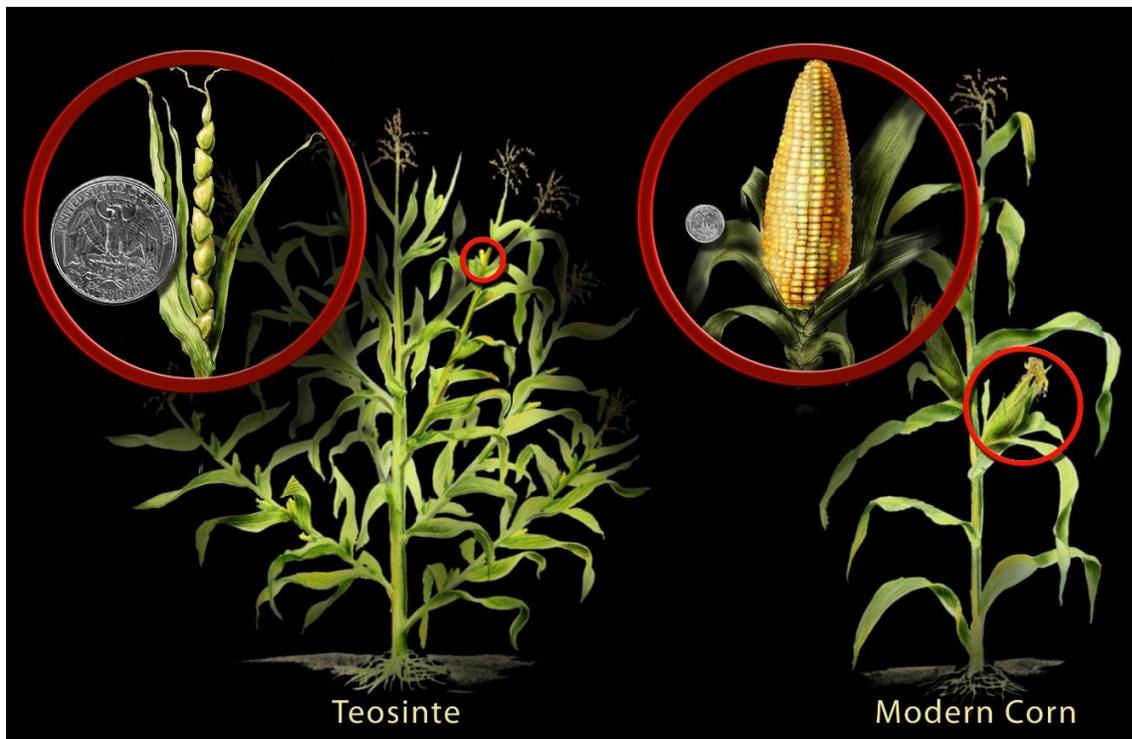
4. Background and significance

Genetic selection of crops

Humans have been modifying crop plants since the dawn of civilization. Ten thousand years ago human societies began to transition from hunting and gathering to agriculture. As of 4,000 years ago, early civilizations had completed the domestication of all major crop species upon which human survival is now dependent, including rice, wheat, and maize.

Year after year ancient peoples selected and saved seeds from plants displaying specific traits. Later, with cross breeding and the development of hybrid plants, modern plant breeding emerged. Most humans alive today have never seen and would not recognize the quirky wild plants that were the early progenitors of current crops.

For example, the ancestor of modern corn, teosinte, had small kernels each inside a tough husk. Teosinte plants had multiple stalks and long branches, while modern cultivated maize has a single stalk. During the domestication of maize, which began thousands of years ago, humans selected for large sheathed cobs containing large kernels without husks.



<http://nrm101-summer2010.community.uaf.edu/2010/07/12/a-history-of-corn/>

Genetic engineering of crops

Today, modern biotechnology and genetic engineering allow scientists and breeders to confer very specific traits rapidly by introducing particular genes directly into plants.

Introduced genes (or *transgenes*) may derive from the same species of plant, from other plant species, or even from animals or bacteria. For example, the gene for the insecticidal toxin in transgenic cotton, potato, and corn plants comes from the bacterium *Bacillus thuringiensis* (Bt). One of the genes allowing vitamin A production in golden rice is derived from the bacterium *Erwinia uredovora*, commonly found in soil.

Crop	Traits	Modification	% modified (US)
Apples	Delayed browning	Genes added	Approved 2015
Canola	Tolerance of glyphosate herbicides. High laurate canola, Oleic acid canola.	Genes added	87% (2005)
Corn	Tolerance of glyphosate herbicides. Insect resistance. Higher ethanol production.	Genes, some from Bt, added	85% (2013)
Cotton	Insect resistance.	Genes, some from Bt, added	82% (2013)
Papaya (Hawaiian)	Resistance to the papaya ringspot virus.	Gene added	80%
Rice	Enriched with beta-carotene (a source of vitamin A).	Genes from maize and a soil bacterium	Not yet on the market
Soybeans	Tolerance of glyphosate. Reduced saturated fats. Insect resistance.	Herbicide resistant Bt gene added. Removed genes that catalyze saturation	93% (2013)
Sugar beet	Tolerance of glyphosate, glufosinate.	Genes added	95% (2010)
Tomatoes	Suppression of polygalacturonase (PG), retarding fruit softening after harvesting.	Antisense of the gene responsible for PG enzyme added	Taken off the market (commercial failure)

Examples of genetically engineered crops commonly grown in the US and their modifications, via Wikipedia.

Genetic engineering of foods usually pursues one or more of these benefits:

- **Agricultural:** increased yield or tolerance to suboptimal conditions (*e.g.* drought)
- **Environmental:** reduced use of herbicides, pesticides, or fertilizers (*e.g.* Bt cotton)
- **Nutritional:** higher quality, supplementation of diet deficiencies (*e.g.* Vitamin A)

Methods for genetically engineering foods

How do you get a plant to take up a foreign gene? At least three methods can be used to introduce foreign DNA into the host plant:

- **Biological vectors** (Ti plasmid from *Agrobacterium*)
- **Physical methods** (gene gun or electroporation)
- **Chemical methods** (polyethyleneglycol and calcium chloride). The biological vector system is the one most commonly used.

When using biological methods, two DNA vectors are typically introduced into the host cells. One vector contains the DNA to be introduced (the transgene). The second vector is the Ti plasmid of *Agrobacterium tumefaciens*, which contains genes encoding the mechanism necessary for the genetic transfer to occur to the host plant (McBride and Summerfelt, 1990).

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**. This grouping is called a gene cassette, where the promoter and terminator regulatory regions influence where and when a gene will be expressed. The most commonly used promoter in engineered plants is the **CaMV35S promoter** derived from the cauliflower mosaic virus (a virus that infects cauliflower plants). The **NOS terminator** from the Ti plasmid in *Agrobacterium tumefaciens* is the most common terminator. These regulatory regions enable strong and generalized transcription of the transgenes across all tissues of the host plant.



Schematic representation of a transgenic cassette used to generate GMOs. The regulatory elements CaMV35S Promoter and NOS Terminator are commonly used to drive expression of the transgene (inserted gene) in every plant cell and were selected because of their ability to be recognized in most plant species.

More recently targeted genome editing using nucleases has emerged as a novel and powerful genetic tool with potential to accelerate the engineering of plant (and animal) species. The most widely used system is the type II clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 (CRISPR-associated) system from *Streptococcus pyogenes* (Jinek et al., 2012). Interestingly, several independent groups have shown that the CRISPR/Cas9 system can introduce biallelic or homozygous mutations directly in the first generation of rice and tomato transformants, highlighting the exceptionally high efficiency of the system in these species. It was also shown in Arabidopsis, rice, and tomato that the genetic changes induced by Cas9/gRNA were present in the germ line and segregated normally in subsequent generations without further modifications. This has the potential to further accelerate plant research as well as plant breeding by providing the means to modify genomes rapidly in a precise and predictable manner over the coming years.

Today we will use PCR and gel electrophoresis to assess the presence of transgenes in plants and commercial food products. We will amplify these transgenic regulatory sequences by PCR to test foods derived from genetically engineered crops as well as “GMO” and “non-GMO” DNA samples. We will also test for endogenous plant “housekeeping” genes to confirm that we have extracted viable DNA.

5. Laboratory set-up manual

Reagent	Volume needed per lab group	Storage	Teacher's checklist
5X EZ PCR Master Mix, Load-Ready™ <ul style="list-style-type: none"> 5x Mix with <i>Taq</i> DNA polymerase dNTPs (included) PCR buffer with Mg²⁺ (included) Gel Loading Dye (included) 	25 µL	-20°C freezer	Supplied in Kit
Primer Mix <ul style="list-style-type: none"> GMO Lab primers 	100 µL	-20°C freezer	
Template DNA <ul style="list-style-type: none"> 'GMO Banana' DNA¹ 'non-GMO Banana' DNA² 	5 µL each	-20°C freezer	
DNA-EZ™ Extraction System <ul style="list-style-type: none"> Lysis Solution Neutralization Solution 	125 µL each 20 µL each	-20°C freezer	
DNA molecular weight marker <ul style="list-style-type: none"> 100bp DNA ladder, Load-Ready™ 	10 µL	-20°C freezer	
DNA staining agent, e.g. <ul style="list-style-type: none"> GreenView™ Plus (for Blue light) 	2 µL per blueGel™ agarose gel	Room temp, dark	
Agarose (2.4% gels) <ul style="list-style-type: none"> Electrophoresis grade 	0.48 g per gel (if using blueGel™)	Room temp.	
Electrophoresis buffer <ul style="list-style-type: none"> 1X TBE if using blueGel™ system 	Depending on gel apparatus	Room temp.	
Distilled or deionized H₂O	1 L (approx.)	Room temp.	

¹ Plasmid DNA containing CaMV 35S and tubulin sequences

² Plasmid DNA containing tubulin sequences

Equipment and Supplies	Teacher's checklist
PCR Thermal cycler: e.g. miniPCR™ machine	
Micropipettes: 2-20 µL and 20-200 µL are recommended	
Disposable micropipette tips	
PCR tubes: 200 µL microtubes, individual or in 8-tube strips (6 tubes per group)	
Plastic tubes: 1.5 or 1.7 mL tubes to aliquot reagents (6 tubes per group)	
Horizontal gel electrophoresis apparatus: e.g., blueGel™ by miniPCR	
DC power supply for electrophoresis apparatus (not needed if using blueGel™)	
Transilluminator: UV or Blue light (not needed if using blueGel™)	
Scale for weighing agarose	
250ml flasks or beakers to dissolve agarose gel	
Microwave or hot plate	
Microcentrifuge (optional)	
Gel documentation system (optional, or use cell phone camera instead)	
Other supplies: <ul style="list-style-type: none"> • UV safety goggles (if using UV transilluminator) • Disposable laboratory gloves • Permanent marker 	

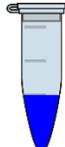
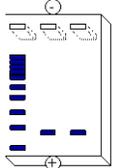
Planning your time

This experiment has 5 stages:

- A. DNA Extraction
- B. PCR Set up
- C. PCR Programming and monitoring
- D. Separation of PCR products by DNA electrophoresis
- E. Size determination of PCR products and interpretation

This lab is designed to be completed in two 45-min periods.

An overview of the experimental plan is represented below:

Preparatory activity	Experimental stage
<p><i>Dispense reagents and prepare equipment</i></p> <ul style="list-style-type: none"> • 15 min 	<div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p>A DNA Extraction</p> <ul style="list-style-type: none"> • 10 min <p>B PCR set up</p> <ul style="list-style-type: none"> • 10 min <p>C PCR programming & monitoring</p> <ul style="list-style-type: none"> • 5 min PCR programming • 15 min PCR monitoring, discussion </div> <div style="width: 35%; text-align: center;">  </div> </div>
<p> <i>Possible stopping point. miniPCR will run for approximately 1h</i> <i>Store PCR product in fridge (up to 1 week) or freezer (longer term)</i></p>	
<p><i>Pour agarose gels</i></p> <ul style="list-style-type: none"> • 20 min 	<div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p>D Gel electrophoresis</p> <ul style="list-style-type: none"> • 10 min load samples • 25 min run samples <p>E Size determination & interpretation</p> <ul style="list-style-type: none"> • 5 min visualization • 5 min discussion </div> <div style="width: 35%; text-align: center;">  </div> </div>

Quick guide: Preparatory activities

A. DNA Extraction

- Each lab group can process up to two test foods for DNA extraction and analysis, and 2 control DNA samples provided with the kit
 - *Follow table on Page 14 with suggestions for foods to test*

 - For each lab group analyzing 2 test foods, label and dispense in microtubes:
 - DNA-EZ™ Extraction System
 - Lysis Solution 125 µL
 - Neutralization Solution 20 µL
-  *Avoid contact of Lysis solution with skin!*

B. PCR set up

- Thaw tubes containing EZ PCR Master Mix, primers, and DNA samples by placing them on a rack or water bath at room temperature
- Label and dispense the following tubes per lab group (for 4 PCR reactions):
 - 5X EZ PCR Master Mix, Load-Ready™ 25 µL
 - GMO Lab Primers 100 µL
 - 'GMO Banana' DNA³ 5 µL
 - 'non-GMO Banana' DNA⁴ 5 µL
- Each lab group will additionally need the following supplies:
 - Micropipettes (*2-20µL range is recommended*)
 - Disposable micropipette tips and a small beaker or cup to dispose them
 - 6 PCR tubes (thin walled, 200 µL microtubes)
 - Permanent marker (fine-tipped)

C. PCR programming and monitoring

- Ensure lab benches are set up with a miniPCR™ machine and power supply
- Ensure the miniPCR machines that are going to be monitored through the PCR reaction are connected to a computer or compatible smartphone/tablet

³ Plasmid DNA containing CaMV 35S and tubulin sequences

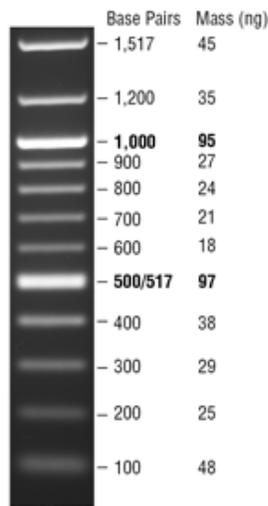
⁴ Plasmid DNA containing tubulin sequences

D. Gel electrophoresis

- Gels can be poured in advance of the second class
 - Pre-poured gels can be stored in the fridge, in a sealed container or wrapped in plastic wrap, and protected from light
- If running the gel on a different day than the PCR, completed PCR reaction tubes can be stored in the fridge for up to one week until they are used, or in the freezer for longer-term storage

E. Size determination and interpretation

- Have the banding pattern of the 100bp DNA Ladder handy to help interpret the electrophoresis results



100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel. Mass values are for 0.5 µg/lane. Source: New England Biolabs

6. Instructor laboratory guide

Which foods to test?

We will test “GMO Banana” DNA samples along with foods and plants of your choice. The table below may help in your choice of processed and fresh foods to test.

WE STRONGLY RECOMMEND TESTING ONE OR MORE **CORN** OR **SOY** DERIVATIVES, as these are some of the most abundant genetically engineered crops.

<p>Robust results</p>	<ul style="list-style-type: none"> • Fresh corn silk • Soy flour • Corn bread mix • Soy crisps 	<ul style="list-style-type: none"> • Corn tortillas • Tortilla chips • Tostitos, Doritos • Cheetos
<p>Feasible yet challenging</p>	<ul style="list-style-type: none"> • Quinoa grains • Tofu dogs • Veggie burgers • Fresh corn husk 	<ul style="list-style-type: none"> • Fresh soybeans • Fresh strawberries (stems, leafy parts)
<p>Best to avoid</p>	<ul style="list-style-type: none"> • Vegetable oil • Corn starch • Corn meal • Pop corn 	<ul style="list-style-type: none"> • Fresh corn kernel • Fresh fruits (pulp) • Breakfast cereal • Frito chips

A. DNA Extraction

1. **Label two 200 μ L thin-walled PCR tubes per lab group on the side, not cap, of the tube**
 - 1 tube labeled "F1": For DNA extraction from Food 1
 - 1 tube labeled "F2": For DNA extraction from Food 2



Also label each tube with the group's name on the side wall

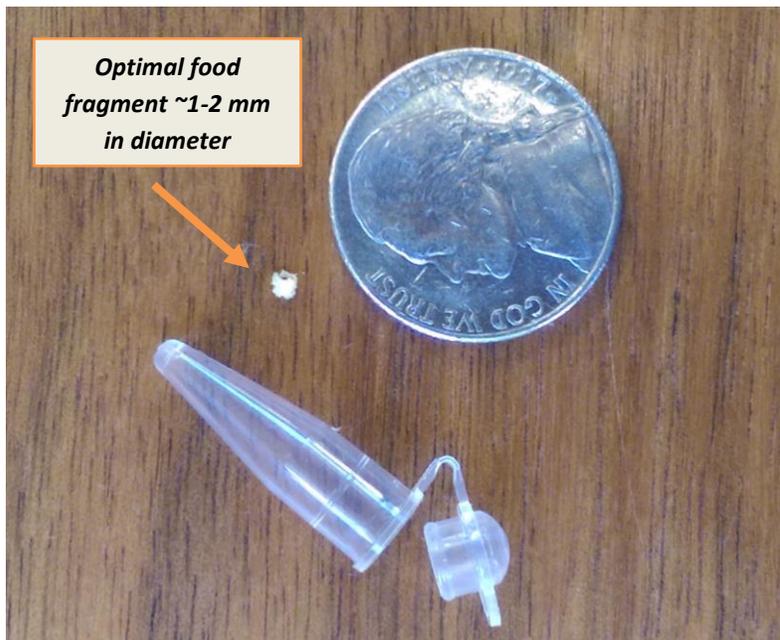
2. **Add 50 μ L of DNA-EZ™ Lysis Solution to each tube**



Avoid contact with skin!

3. **Prepare test foods or plant tissues for DNA extraction** (see p.14 for recommended foods)

- *From dried or processed foods* (e.g. corn chips): Crush the food into small pieces using your thumb and index finger. Alternatively, grind it to a fine powder using a mortar and pestle. Place a small amount, approximately 1 mm in diameter or less into a tube containing DNA-EZ™ Lysis Solution.



IMPORTANT!

- Avoid large food fragments as they will result in inefficient DNA extraction
- Ideal food fragment is the size of a pinhead - Less is more!!
- If available, use a mortar and pestle to grind food into a fine powder

- *From fresh plant tissue* (e.g. corn or papaya): puncture the fruit or vegetable a few times with a yellow tip attached to a pipette until a small amount of tissue

adheres to the inside or outside of the tip. Place the tip inside a tube with DNA-EZ™ Lysis Solution labeled in step 1, then pipette up and down several times.

4. **Tightly cap the 200 µL tubes** containing DNA-EZ™ Lysis Solution and the test foods
 - Ensure that food fragments are well mixed into the Lysis Solution
5. **Incubate the food mix in DNA-EZ Lysis Solution 5 minutes at 95°C**
 - Conduct this incubation in a miniPCR™ machine in Heat Block mode, or use a 95°C heat block or water bath
6. **Remove tubes from heat** and let them rest in a tube rack at room temperature
 - Ensure the tubes remain steady and in vertical position
7. **Add 5 µl of DNA-EZ™ Neutralization Solution to each tube**
 - Pipette up and down to mix well
 - The DNA extract should be used *immediately* for PCR
8. **If a microcentrifuge is available, spin down debris before PCR**
 - 10,000 RPM for 2 minutes

PCR set up

1. **Label 4 clean PCR tubes** (200 µL thin-walled tubes) per group on the side wall
 - 1 tube labeled "T1": Test DNA extracted from Food 1
 - 1 tube labeled "T2": Test DNA extracted from Food 2
 - 1 tube labeled "G": 'GMO Banana' DNA provided in the kit
 - 1 tube labeled "W": 'non-GMO Banana' DNA provided in the kit



Also label each tube with the group's name on the side wall

2. **Add PCR reagents** to each 200 µL PCR tube

	Tube T1	Tube T2	Tube G	Tube W
GMO Lab Primers	20 µL	20 µL	20 µL	20 µL
5X EZ PCR Mix	5 µL	5 µL	5 µL	5 µL



Use a micropipette to add each of the reagents.
Remember to change tips at each step!

3. Add DNA samples to each tube, using a clean tip



CRITICAL STEP

- i. **Tubes T1 and T2 (Food DNA extracts):**
 Add 2µL of DNA extract avoiding large food particles, as these will clog your pipette tip. If clogging occurs, pipette up and down to unclog.
- ii. **Tubes G and W (controls supplied with kit):**
 Pipette 2µL of 'GMO Banana DNA' and 'non-GMO Banana' samples supplied with the miniPCR GMO Lab kit

	Tube T1	Tube T2	Tube G	Tube W
Template DNA	DNA extract from Test Food 1 2 µL	DNA extract from Test Food 2 2 µL	Control 'GMO Banana' DNA supplied w/kit 2 µL	Control 'Wild Banana' DNA Supplied w/kit 2 µL
FINAL VOLUME	27 µL	27 µL	27 µL	27 µL

4. Cap the tubes

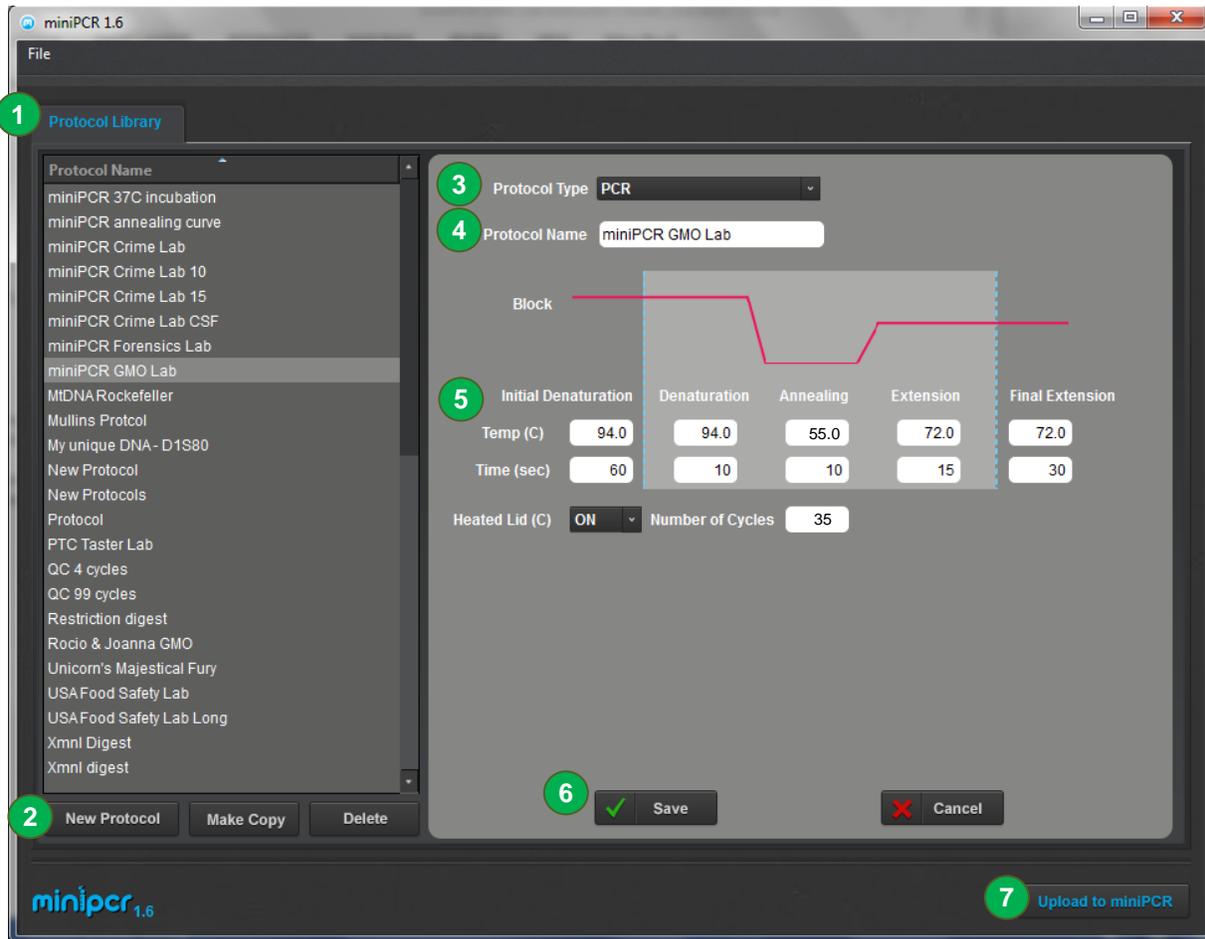
- Make sure all the liquid volume collects at the bottom of the tube
- If necessary, spin the tubes briefly using a microcentrifuge (or flick them)

5. Place the tubes inside the PCR machine

- Press firmly on the tube caps to ensure a tight fit
- Close the PCR machine lid and gently tighten the lid

B. PCR programming and monitoring (illustrated using miniPCR™ software)

1. Open the miniPCR software app and remain on the "Protocol Library" tab



2. Click the "New Protocol" button on the lower left corner

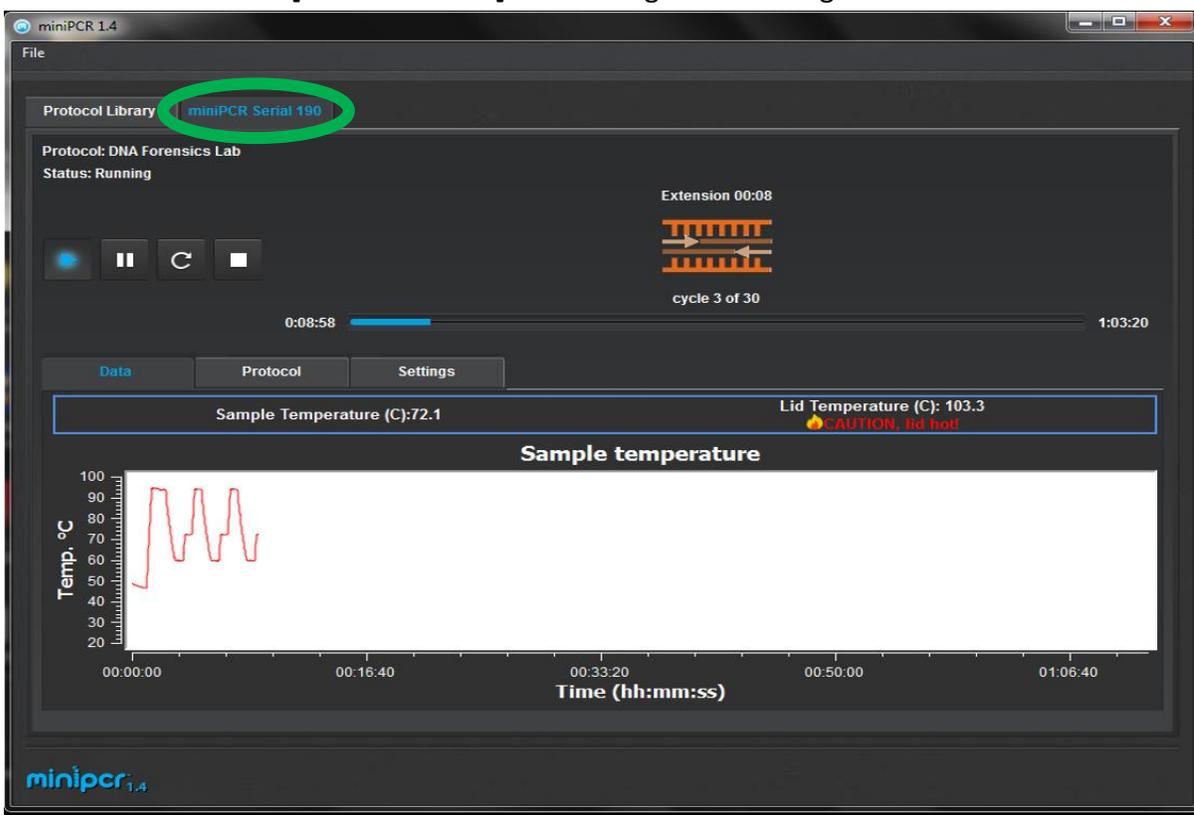
3. Select the PCR "Protocol Type" from the top drop-down menu

4. Enter a name for the Protocol; for example "Group 1 – GMO Lab"

5. Enter the PCR protocol parameters:

- **Initial Denaturation** 94°C, 60 sec
- **Denaturation** 94°C, 10 sec
- **Annealing** 55°C, 10 sec
- **Extension** 72°C, 15 sec
- **Number of Cycles** 35
- **Final Extension** 72°C, 30 sec
- **Heated Lid** ON

6. Click "Save" to store the protocol
7. Click "Upload to miniPCR" (and select the name of your miniPCR machine in the dialogue window) to finish programming the thermal cycler.
8. Make sure that the **power switch in the back** of miniPCR is in the **ON** position
9. Click on "miniPCR [machine name]" tab to begin monitoring the PCR reaction



The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, and to export the reaction data for analysis as a spreadsheet.

Once the PCR run is completed (approximately 60 min), the screen will display: "Status: Completed". All LEDs on the miniPCR machine will light up.



You can now open the miniPCR lid and remove your PCR tubes.
Be very careful not to touch the metal lid which may still be hot

PCR products can now be stored for up to 1 week in the fridge or 1 year in a freezer.

C. Gel electrophoresis – Pouring agarose gels (Preparatory activity)



If the lab is going to be completed in a single time block, agarose gels should be prepared during the PCR run to allow the gels to settle.

If the lab is going to be performed over two class periods, gels can be prepared up to one day ahead of the second period and stored in a refrigerator, covered in plastic wrap and protected from light.

1. Prepare a clean and dry agarose gel casting tray
 - Seal off the ends of the tray if needed for your apparatus
 - Place a well-forming comb at the top of the gel (5 lanes or more)

2. For each lab group, prepare a 2.4% agarose gel using electrophoresis buffer
 - For example, add 0.48 g of agarose to 20 ml of TBE buffer (for blueGel™)
 - Adjust volumes and weights according to the size of your gel tray
 - Mix reagents in glass flask or beaker and swirl to mix

3. Heat the mixture using a microwave or hot plate
 - Until agarose powder is dissolved and the solution becomes clear
 -  Use caution, as the mix tends to bubble over the top and is very hot

4. Let the agarose solution cool for about 2-3 min at room temperature.
 - Swirl the flask intermittently

5. Add gel staining dye (e.g. GreenView™ Plus)
 - Follow dye manufacturer instructions
 - e.g., 2µL of GreenView Plus 10,000X per 20 mL blueGel mix.
 -  Note: We recommend the use of safe alternatives to ethidium bromide such as GreenView™ Plus (available at www.miniPCR.com).

6. Pour the cooled agarose solution into the gel-casting tray with comb

7. Allow gel to completely solidify (until firm to the touch) and remove the comb
 - Typically, ~10 minutes for blueGel™ gels

8. Place the gel into the electrophoresis chamber and cover it with run buffer

Gel electrophoresis – Running the gel

1. Make sure the gel is completely submerged in electrophoresis buffer
 - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged)
 - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to cover the gel and wells

2. Load DNA samples onto the gel in the following sequence
 - **Lane 1:** 10µL DNA ladder
 - **Lane 2:** 15µL PCR product from **Test Food 1** (tube T1)
 - **Lane 3:** 15µL PCR product from **Test Food 2** (tube T2)
 - **Lane 4:** 15µL PCR product from '**GMO Banana**' (Tube G)
 - **Lane 5:** 15µL PCR product from '**non-GMO Banana**' (Tube W)



Note: there is **no need to add gel loading dye to your samples.**

The *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* come premixed with loading dye, and ready to load on your gel!

3. Place the cover on the gel electrophoresis box

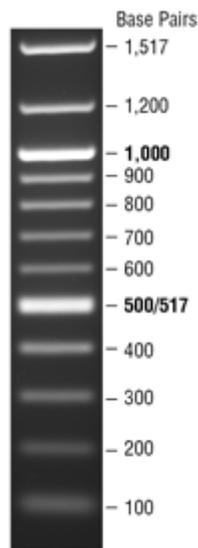
4. Insert the terminal leads into the power supply (not needed for blueGel™)

5. Conduct electrophoresis for ~25 minutes, or until the colored dye has progressed to at least half the length of the gel
 - Check that small bubbles are forming near the terminals in the box
 - Longer electrophoresis times will result in better size resolution
 - Lower voltages will result in longer electrophoresis times

Once electrophoresis is completed, turn the power off and remove the gel from the box

D. Size determination and interpretation

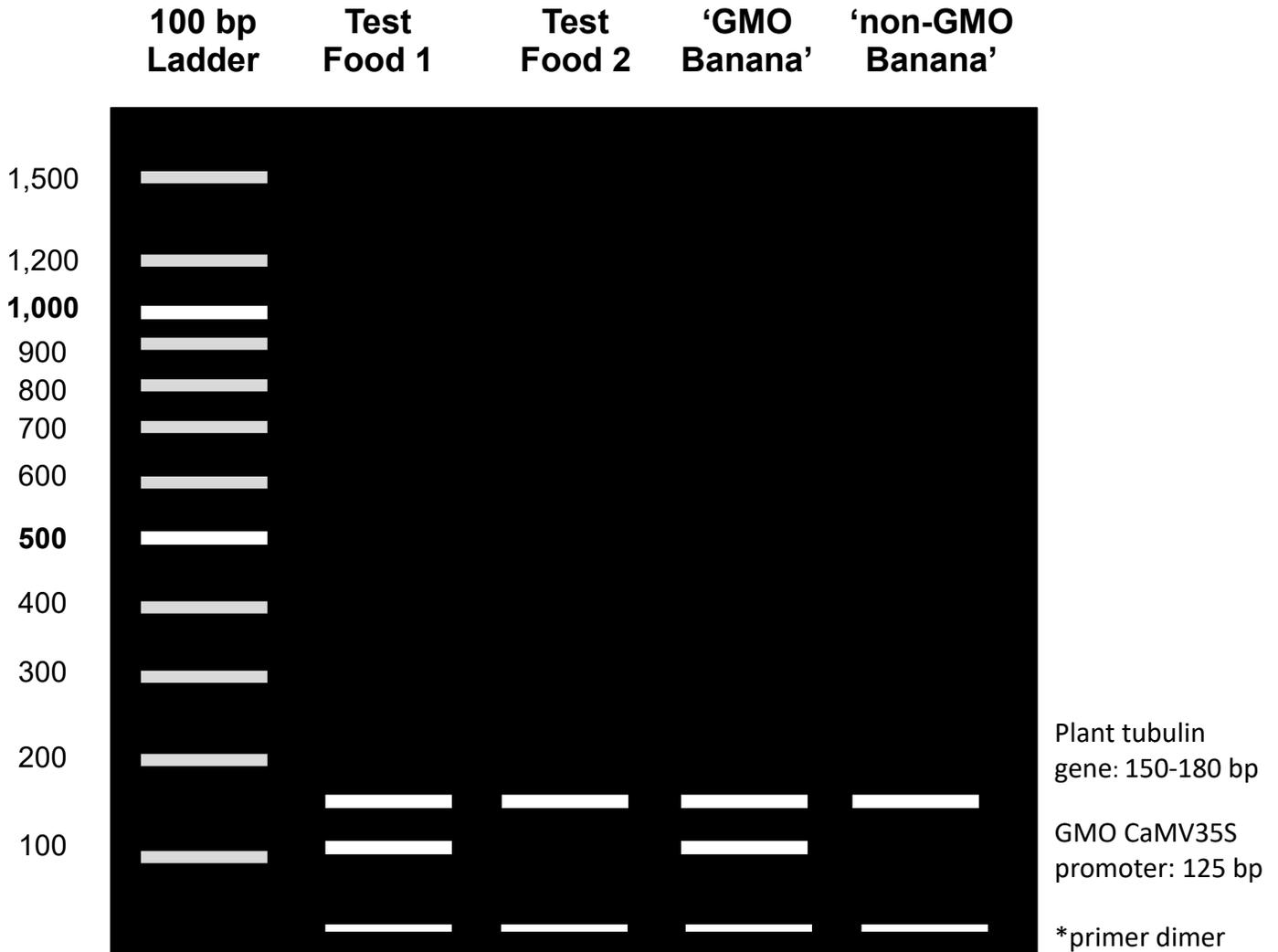
1. Turn on the blueGel™ blue light illuminator
 - Or place the gel on a transilluminator if not using blueGel™
2. Verify the presence of PCR product
3. Ensure there is sufficient DNA band resolution in the 100-300 bp range of the 100bp DNA ladder
 - Run the gel longer if needed to increase resolution
 - DNA ladder should look approximately as shown



*100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.
 Source: New England Biolabs*

4. Document the size of the PCR amplified DNA fragments by comparing the PCR products to the molecular weight reference marker (100bp DNA ladder)
 - Capture an image with a smartphone camera
 - If available, use a Gel Documentation system

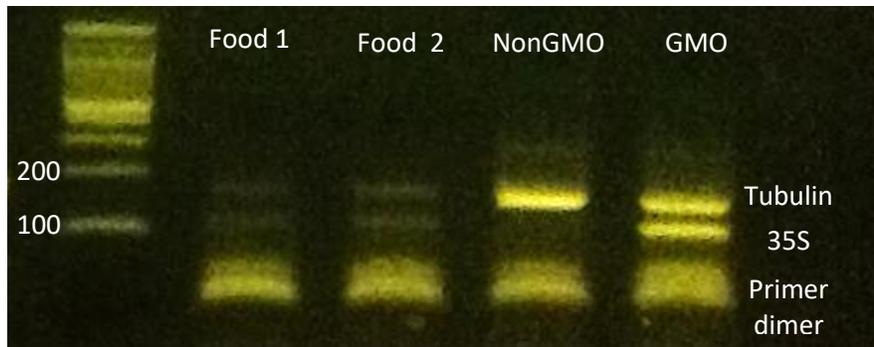
Expected experimental results



This schematic image shows idealized results of possible experimental outcomes:

- **~150-180 band:** endogenous DNA from plant tubulin gene. All foods.
Expect ~180bp from corn DNA and ~150bp from soy DNA.
- **~125 band:** 35S promoter from CaMV. GMO foods.
Expected in GMO tissues only.
- **<100 band:** primer dimer.
Non-specific result, arising from primers annealing to each other.

Troubleshooting



Food 1 and Food 2 lanes show results from suboptimal DNA amplification. The quality of your experimental results will usually vary with:

- Quality of DNA extraction
 - The tubulin band serves as an internal control (expected from all foods)
 - If the band is absent, try repeating your DNA extraction varying conditions: grind the food more finely, add more/less food, extend the incubation time at 95°C to 10 minutes.
- PCR efficiency
 - Try increasing the number of PCR cycles: compare 30, 35, and 40 cycles
 - Increase duration of the initial denaturation step, to reduce primer-dimer amplification
- Electrophoresis time and voltage
 - Run your gel until you achieve sufficient separation in the 100-200 bp range
 - If necessary, increase the concentration of agarose to 3%

Discussion

- All DNA samples were subjected to duplex PCR for two independent targets:
 - CaMV 35S Promoter present in the majority of genetically engineered crops
 - Tubulin gene as an endogenous plant control
- Tubulin is present in all plants and serves as internal control for DNA extraction
 - All samples should show the 150 or 180 base pair tubulin band
- When a transgene is present (GMO food) we expect to see the 125 base pair band
 - Absence or presence of the 125 bp band in Test Foods 1 and 2 serves to reveal their GMO status (presence of CaMV 35S promoter).

7. Study questions

Questions before experimental set-up

1. **How does genetic diversity of plants arise through natural selection?**

DNA is a molecule found in the nucleus of cells that has encodes genetic information necessary for organisms to function and reproduce. The genetic code contained in the nucleotide sequence of DNA is unique for every non-clonal organism. The sum of genetic differences between organisms is called genetic diversity; the greater the diversity, the more differences there are between organisms.

DNA mutations create genetic differences that can be minor or major in scale. Minor mutations include single nucleotide polymorphisms (SNPs) where a single nucleotide is swapped for another. Major mutations include insertion/deletion mutations where pieces of the genetic code are added or removed completely, translocation mutations where parts of chromosomes change locations, and duplication mutations where sections of the genetic code are repeated. As DNA is transcribed to RNA, which is then translated to proteins, differences in the genetic code can lead to differences in the resulting proteins. Changes in protein structure or function can lead to a variety of phenotypic differences.

Natural selection, or the differential survival and reproduction of organisms based on their phenotypes, can lead to certain mutations becoming more or less common in the genome. Some mutations can confer an organism a better chance of reproducing; the organism's offspring will likely inherit the mutation and will also have a greater chance of reproducing. If the mutation continues to be of benefit and the organisms that have the mutation reproduce more successfully than their non-mutated counterparts, the mutation will grow in frequency across the population (i.e. a greater percentage of organisms will have the mutation).

2. **Artificial selection: How and why do humans selectively breed crops?**

For centuries, humans have bred plants and animals selectively so that they contain desirable traits. To use an agricultural example, if you were to find a ear of corn that you liked because it was exceptionally sweet, you would try to plant seeds that came from that plant. Those seeds would produce some plants that were sweet and others that were not. If over the next 10 years, you again only planted seeds from the sweetest corn from each year, you would find that, after 10 years, more of the plants produced sweet corn than before you began to selectively plant only the sweetest plants.

This process is known as *artificial selection*. However, artificial selection could take many years and humans could only select based on the traits that the plants showed, created by mutations that occurred randomly in nature. Once genetic research led to the ability to introduce novel sequences of DNA into existing genomes, scientists began to use those technologies to create organisms that had genes originally found in others. This is the process known as *genetic modification* or *genetic engineering*.

3. What is a genetically modified organism (GMO)?

An organism containing genes that originated in another species is called a transgenic organism. There are multiple techniques that can be used to add a new piece of DNA into an organism's genome. The first step in the process is to identify a protein that you want included in the novel organism. Say for example that we want to add the delta-endotoxin protein to a crop of corn. The delta-endotoxin protein is poisonous to many insects and therefore, any plant that produces this protein is largely protected against insect predation. However, the gene that codes for the delta-endotoxin protein is not found naturally in corn plants which means that, in order for the plants to start producing the protein, the gene that codes the protein must be inserted into the plant's genome. Scientists use genetic engineering techniques to introduce desirable genetic modifications to species that would be otherwise almost impossible to acquire.

4. How can new genes be introduced into a plant?

There are a number of steps required to introduce a foreign gene into the genome of a host plant. The first is to isolate the gene and affix proper promoter and terminator regions to each end of the gene. The method of isolating the gene is called cloning and starts by identifying the gene in its entirety. Then the gene is isolated and purified meaning that only the coding region of the gene is retained. A promoter and a terminator are then added to opposite ends of the purified gene. Promoters are sequences that are used by the host cell to identify where transcription should begin, and one of the most commonly used promoters in genetically engineered plants is the 35S promoter from the cauliflower mosaic virus (CaMV35S). Terminators are DNA sequences that indicate where transcription should end.

The next step is to insert the gene into the genome of the host species. There are several techniques that scientists can use to insert a novel gene into a host's genome, a process called transformation. In plants, the most common technique is using the bacterium *Agrobacterium tumefaciens*, which naturally infects plant cells and has a small molecule of DNA called a plasmid that has the ability to insert itself into the genome of the host cell

where it is then transcribed to RNA. Scientists use this to their benefit by replacing the DNA that the plasmid inserts (known as T-DNA where T stands for transfer) with the gene that they want the plant to express. They then put the engineered plasmid back into the *Agrobacterium tumefaciens* and allow bacteria to infect plant cells naturally while inserting the novel gene into the host's genome. A second technique uses something called a gene gun that takes tiny gold particles coated with many copies of the engineered plasmid and fires the particles into the cell at high velocity such that they penetrate the cell wall and plasma membrane. Although many cells are completely destroyed in the process, some are not and the engineered DNA has the opportunity to insert into the host genome. A third technique utilizes electric current to open pores in the cell membrane where the engineered plasmids can pass through and find their way to the nucleus where they insert into the host DNA.

Once the transformation process is complete, which is not simple and often fails, the transformed cells are grown into an adult plant. If the transformation was successful, all of the cells in the plant will carry the engineered gene. This plant can then be bred with existing crops. To bring the engineered gene to fixation (100% allele frequency) in the entirety of the crop takes many years.

5. Does the introduction of new genes into an organism ever occur in nature?

The use of *Agrobacterium* in the biotechnology lab to transfer new genes to plant crops has resulted in public opinion controversy and in some cases consumer distrust of the safety of transgenic foods. However, *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens* are plant pathogenic bacteria capable of transferring DNA fragments bearing functional genes into the host plant genome in the wild. Recently published work (Kyndt, Quispe, et al., 2015) has shown that virtually every cultivated variety of “non-GMO” sweet potato available for study contains in its genome one or more transfer DNA (T-DNA) sequences originally from *Agrobacterium* species. In other words, “non-GMO” sweet potato is naturally transgenic while being a widely and traditionally consumed food crop.

Agrobacterium infection in these crops seems to have occurred in evolutionary times. One of the T-DNAs is apparently present in all cultivated sweet potato clones, but not in the crop's closely related wild relatives, suggesting the T-DNA provided a trait or traits that were selected for during domestication. This finding draws attention to the importance of plant–microbe interactions, and given that this crop has been eaten for millennia, it may help change perceptions around the “unnatural” status of transgenic crops.

Questions about DNA extraction

1. What is the purpose of boiling the food samples?
 - Serves to lyse the cells. Essentially, break up extracellular material, extra- and intracellular membranes to release DNA into solution to be accessible for PCR.

2. What other molecules besides DNA are released from the tissue?
 - All sorts of biological macromolecules (*e.g.* lipids, structural proteins, enzymes) as well as free ions are released into solution.

3. What is the role of pH in the EZ-DNA™ Extraction System?
 - Alkaline conditions provided by the Lysis Solution help break up the cells, releasing DNA. Alkaline pH also serves to denature the genomic DNA, converting the double-stranded DNA into single stranded DNA. Addition of the Neutralization Solution restores neutral pH conditions, necessary for enzymatic activity during PCR.

Questions during PCR run

1. What is happening to DNA molecules at each step?
 - **Denaturation:** DNA strands of template separate through breakage of H bonds between nitrogenous bases on opposing strands
 - **Annealing:** primers bind to complementary sequence on template DNA through H-bond formation between primer and template complementary bases
 - **Extension:** elongation of primers, from their 3' ends, through the addition of complementary nucleotides catalyzed by Taq DNA polymerase and formation of phosphodiester bonds

2. What is the purpose of the following PCR mix components?
 - **dNTPs:** provides a source of deoxynucleotides for primer elongation, and consequently, new strand synthesis
 - **Mg⁺²:** a co-factor necessary for Taq DNA polymerase function
 - **Taq DNA polymerase:** catalyzes the primer elongation

3. What temperature do you think is optimal for most enzymes?
 - Many enzymes display optimal activity at temperatures in the 36-39°C, the body temperature of most mammals

4. What feature of *Taq* DNA polymerase makes this enzyme unique?
 - *Taq* DNA polymerase was discovered from a thermophilic bacterium, *Thermus aquaticus*, that lives in thermal vents like the geysers in Yellowstone National Park

5. How many additional DNA molecules are there at the end of each PCR cycle? If the PCR consists of 30 cycles, how much more DNA will there be after the PCR completes?
 - During every PCR cycle, the strands of each DNA molecule separate, primers anneal and elongate to yield two DNA molecules. So, each PCR cycle doubles the number of DNA molecules.
 - The amount of additional DNA after a certain number of PCR cycles can be calculated using the mathematical expression 2^N , where N is the number of cycles. So, for 30 cycles, $2^{30} = 1,073,741,824$, or just over a billion (109) times more molecules than the initial amount.

6. How will we know whether the PCR has worked?
 - The '*GMO Banana*' Control run alongside the student reactions serves as a positive control for the PCR. The positive control reaction contains a known DNA template that should produce two PCR products:
 - a 125 bp band corresponding to GMO DNA,
 - and a 150 bp band corresponding to tubulin DNA from soy.
 - If the positive control PCR yields products with the expected lengths, as visualized on the agarose gel, then the PCR worked.

7. How can the PCR product be recovered?
 - DNA fragments amplified by PCR can typically be recovered before or after agarose gel electrophoresis and utilized further for sequencing or cloning. Techniques include column purification, ethanol precipitation, and gel extraction. These procedures enable the separation of the PCR product from excess primers and dNTPs.

Questions after gel electrophoresis and visualization

1. What do the results suggest about your Test Foods?
 - Do they contain genetically engineered sequences?
 - Do they not?

2. Are your results consistent with your expectations about these foods?
 - Are you a regular eater of these foods?

3. Do you know the purpose of the genetic modification(s) you have detected?
 - Describe 2 transgenes that are commonly introduced into crops

4. Describe three ways in which genetically engineered crops may protect the environment
 - Describe three ways in which they may harm the environment.
 - How do you think growing GMOs can accelerate the selection of herbicide-resistant weeds?

5. Describe 3 ways in which human health may be improved by GMOs
 - How can they aid nutrition?
 - How can they help feed a growing human population?
 - What might be the risks to manage?

6. Pollen contains the complete genetic material of the plant and is airborne. Describe ways in which the spread of transgenes via pollen can be contained.

7. Many countries, especially in Europe, oppose growing and consuming genetically modified crops. Why do you think these countries' perception of GMOs is so different from that in the US?

8. How is genetically engineering crops different from selective breeding? Similar?

9. Describe your position regarding the generation and use of GMOs.

8. Student-centered investigations and extension activities

Student Centered Investigations

Investigation 1: Ask students to propose and experimentally test new PCR conditions

- Increase denaturation times (e.g. 60 seconds)
- Increase the annealing temperature (e.g. 65°C)
- Increase number of PCR cycles (30, 35, 40 cycles)

Make predictions for the outcome of each intervention.

Investigation 2 (*Advanced*): Amplify and sequence endogenous plant tubulin genes and the CaMV 35S promoter

- Isolate each individual amplified DNA band from the agarose gel
- Purify the DNA using a gel extraction protocol
- Clone samples, send them out for sequencing

Post-Lab Extension Activities

Lab Report – report on the findings of the written lab or further investigations

- Title
- Introduction
- Materials
- Procedure
- Results
- Discussion

Student writing exercise – write a persuasive article about the benefits and risks of genetically engineered crops. Briefly describe the process used to regulate GMO production in the US and the roles of each regulatory agency involved, from research planning to field production:

- Animal and Plant Health Inspection Service (APHIS) of USDA
- Food and Drug Administration (FDA)
- Environmental Protection Agency (EPA)

Additional teacher resources:

- *Yes, we have no GMO bananas. For now.* Tony Leys, The Des Moines Register. USA Today, 2015.
<http://www.usatoday.com/story/news/nation/2015/01/13/iowa-trial-of-gmo-bananas-is-delayed/21678303/>
- *These vitamin-fortified bananas might get you thinking differently about GMOs.* Nathanael Johnson, Grist.org, 2016.
<https://grist.org/science/these-vitamin-fortified-bananas-might-get-you-thinking-differently-about-gmos/>
- *CAN GMOS END HUNGER IN AFRICA?* Elizabeth Lopatto, The Verge, 2015.
<http://www.theverge.com/2015/2/18/8056163/bill-gates-gmo-farming-world-hunger-africa-poverty>
- *The Molecular Genetics of Crop Domestication.* John Doebley et al. Cell, 2006.
<http://www.sciencedirect.com/science/article/pii/S0092867406015923>
- *A Hard Look at GM Crops.* Natasha Gilbert, Nature, 2013.
http://www.nature.com/polopoly_fs/1.12907!/menu/main/topColumns/topLeftColumn/pdf/497024a.pdf
- *Genetically Engineered Crops in the United States.* Jorge Fernandez-Cornejo et al. USDA, 2013.
<http://www.ers.usda.gov/media/1282246/err162.pdf>
- *Improved binary vectors for Agrobacterium-mediated plant transformation.* McBride, K., and K. Summerfelt. Plant Molecular Biology 14: 269-276, 1990.
- *The genome of cultivated sweet potato contains Agrobacterium T-DNAs with expressed genes: An example of a naturally transgenic food crop.* Kyndta, T., Quispea, D., et al., Proceedings of the National Academy of Sciences Early Edition, April 21, 2015.
<http://m.pnas.org/content/early/2015/04/14/1419685112.full.pdf>
- *A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.* Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. Science, 337 (2012), pp. 816–821.

9. Ordering information

To request miniPCR™ GMO Lab reagent kits, you can:

- Call (781)-990-8PCR
- email us at orders@minipcr.com
- visit www.minipcr.com

miniPCR™ GMO Lab kit (catalog no. KT-1003-01) contents:

- 5X EZ PCR Master Mix, Load-Ready™
 - including *Taq* DNA polymerase, dNTPs, PCR buffer, and gel-loading dye
- GMO Lab Primers
- DNA-EZ™ Extraction System
 - Lysis Solution
 - Neutralization Solution
- 'GMO Banana' DNA sample (plasmid DNA containing 35S and tubulin sequences)
- 'non-GMO Banana' DNA sample (plasmid DNA containing tubulin sequences)
- 100bp DNA ladder, Load-Ready™ (50µg/ml)

Materials are sufficient for 8 lab groups, or 32 students

All components should be kept frozen at -20°C for long-term storage

Reagents must be used within 12 months of shipment

Other reagents available from miniPCR.com (not included):

- | | |
|--|------------|
| • Agarose, electrophoresis grade | RG-1500-02 |
| • DNA intercalating agent (e.g. GreenView™ Plus) | RG-1501-01 |
| • 20X TBE electrophoresis buffer | RG-1502-02 |

10. About miniPCR Learning Labs™

This Learning Lab was developed by the miniPCR™ team in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop miniPCR Learning Labs™ to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The guiding premise for this lab is that a ~2-hour PCR-based experiment can recapitulate a real-life biotechnology application and provide the right balance between intellectual engagement, inquiry, and discussion. The design of this lab has simplified certain elements to achieve these goals. For example, we use plasmid DNA instead of banana genomic DNA as the control PCR templates in order to increase the protocol's robustness, rather than implausibly procuring "real" GMO banana DNA.

Starting on a modest scale working with Massachusetts public schools, miniPCR™ Learning Labs have been well received, and their use is growing rapidly through academic and outreach collaborations across the world.