miniPCR™ GMO Learning Lab: Heart-Shaped Bananas

Newly-engineered GMO bananas can produce ß-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 p.7

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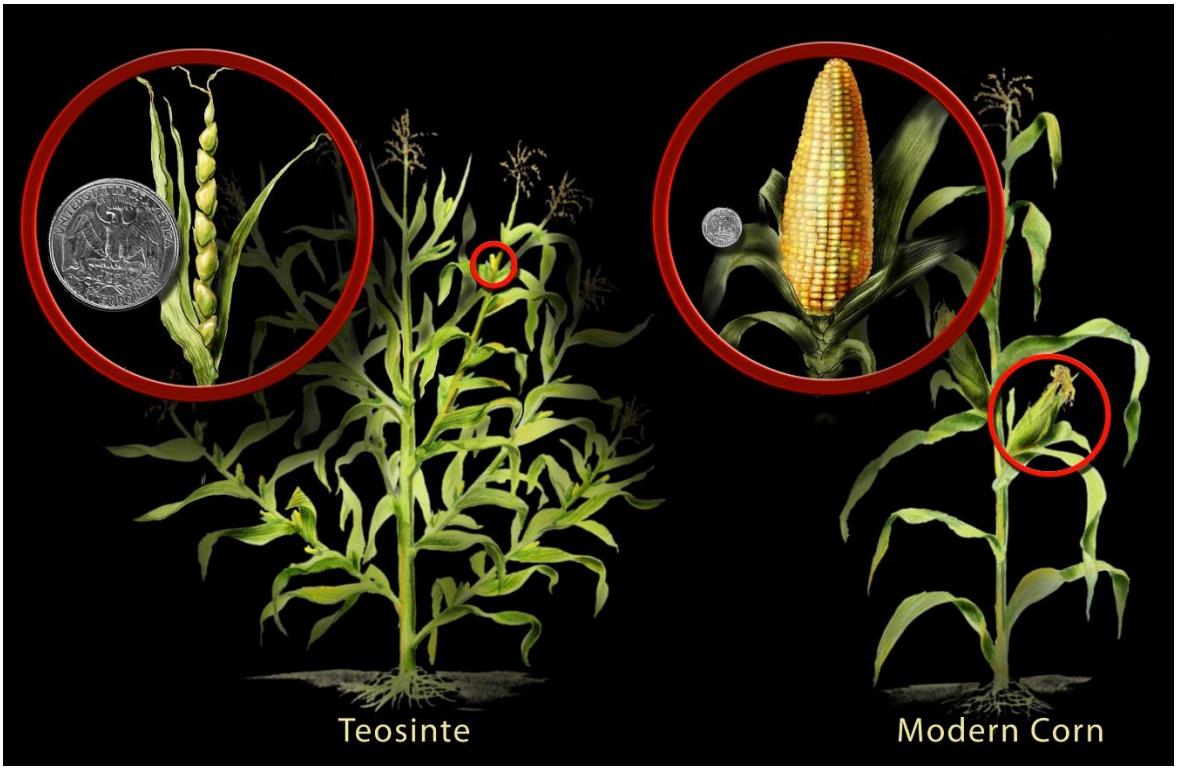
Genetic selection of crops

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)

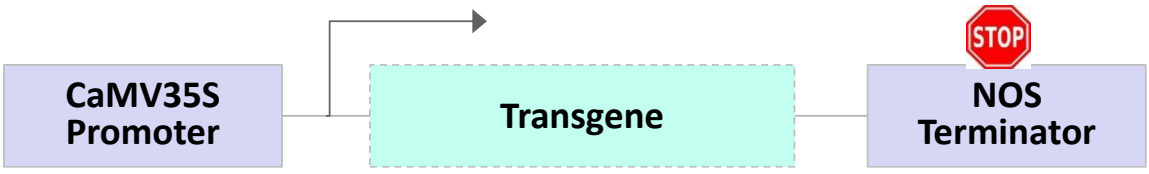
Methods for genetically engineering foods

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.

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.

Robust results	• Fresh corn silk • Soy flour • Corn bread mix • Soy crisps	• Corn tortillas • Tortilla chips • Tostitos, Doritos • Cheetos
Feasible yet challenging	• Quinoa grains • Tofu dogs • Veggie burgers • Fresh corn husk	• Fresh soybeans • Fresh strawberries (stems, leafy parts)
Best to avoid	• Vegetable oil • Corn starch • Corn meal • Pop corn	• Fresh corn kernel • Fresh fruits (pulp) • Breakfast cereal • Frito chips

A. DNA Extraction

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

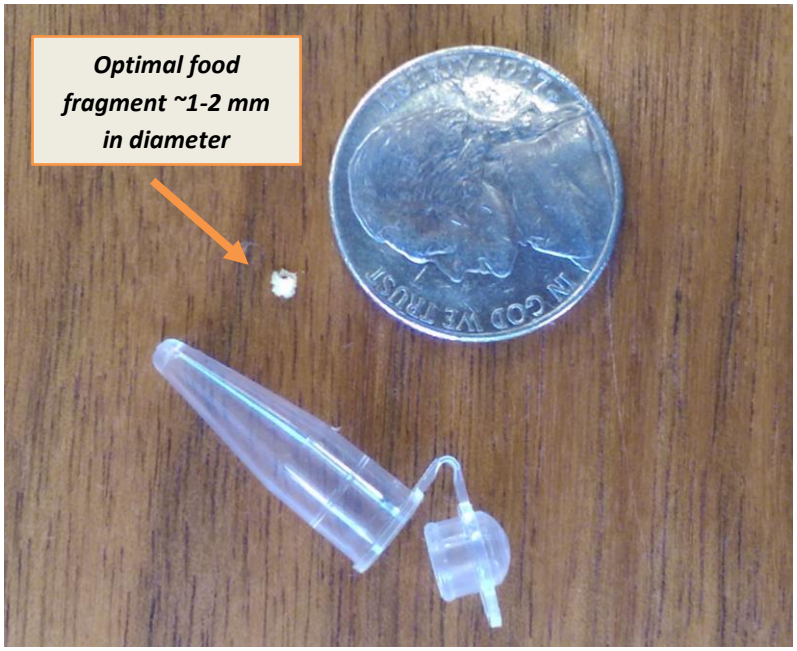
   Also label each tube with the group's name on the side wall

2. **Add 50 µL 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, <u>*approximately 1 mm in diameter*</u> 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
4. **Remove tubes from heat** and let them rest in a tube rack at room temperature
    - Ensure the tubes remain steady and in vertical position
5. **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
6. **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

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

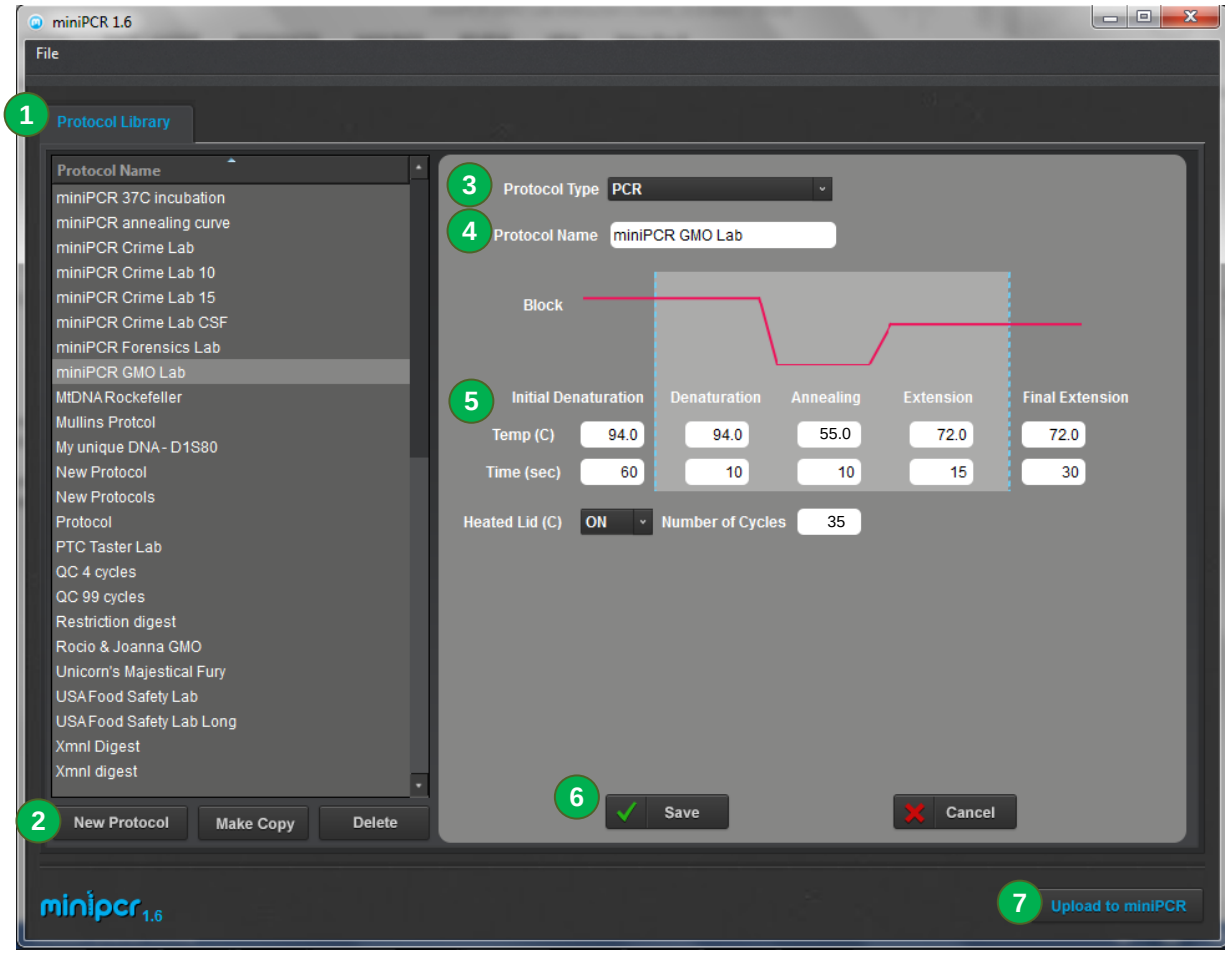
	Tube T1	**Tube T2**	**Tube G**	**Tube W**
Template DNA	DNA extract from Test Food 1 2 µL	DNA extract from Test Food 2 2 µL	Control '*GMO Banana'* DNA supplied w/kit 2 µL	Control '*Wild Banana'* DNA Supplied w/kit 2 µL
FINAL VOLUME	***27 µL***	***27 µL***	***27 µL***	***27 µL***

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

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:

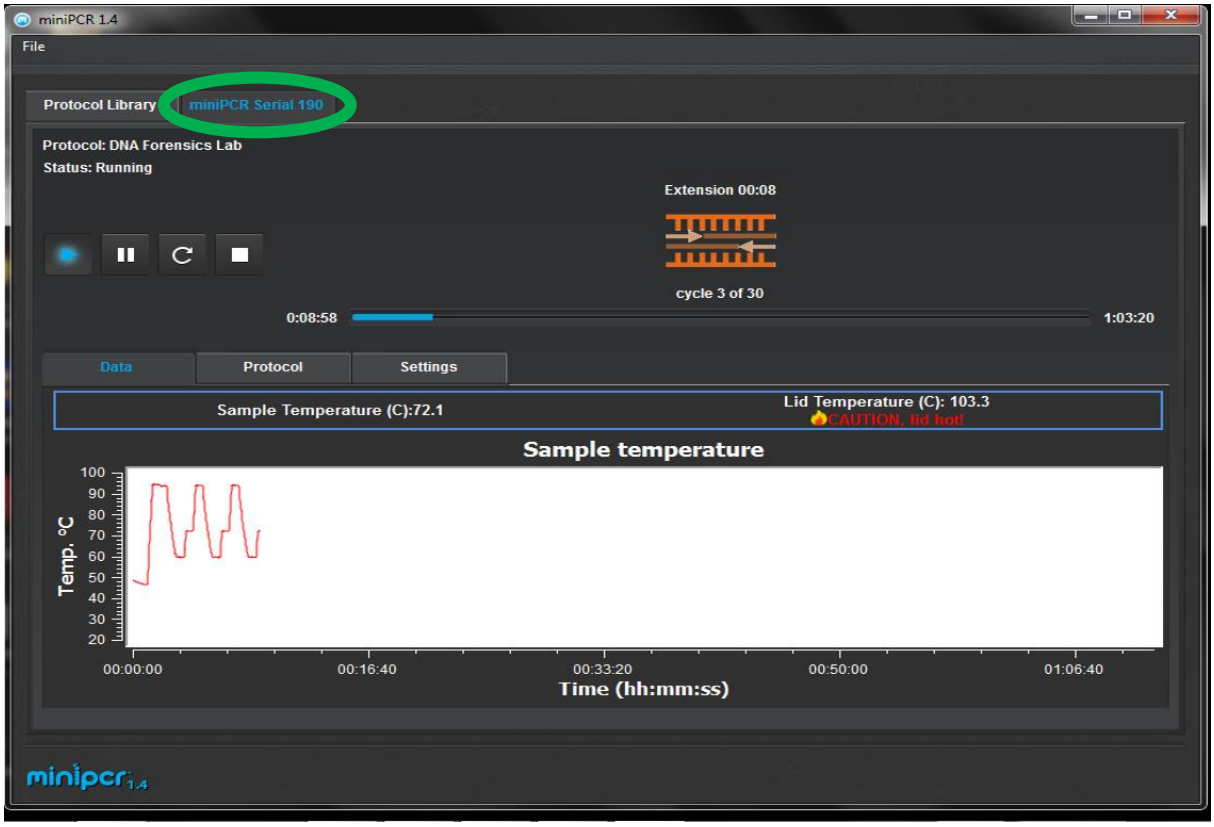
   - **Initial Denaturation** 94°C, 60 sec
   - **Denaturation** 94°C, 10 sec
   - **Annealing** 55°C, 10 sec
   - **Extension** 72°C, 15 sec
   - **Number of Cycles** 35
   - **Final Extension** 72°C, 30 sec
   - **Heated Lid** ON

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.

B. Gel electrophoresis – Running the gel

1. Make sure the agarose 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
    - Ensure the positive and negative electrode terminals fit into place

4. Press the power button ON and conduct electrophoresis for 25 minutes, or until the colored dye has progressed to at least three quarters of the gel
    - Check that small bubbles are forming near the terminals in the box
    - Longer electrophoresis times will result in better size resolution

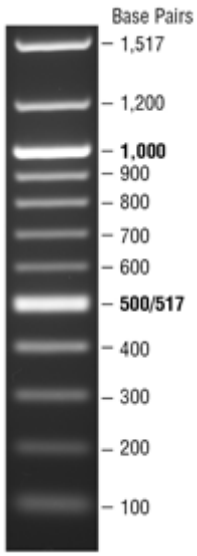
5. Once electrophoresis is completed, turn the power off and remove the gel from the box

C. Size determination and interpretation

1. Turn on the blue-light transilluminator

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

Results and discussion

- All DNA samples were subjected to PCR for two separate targets:
    - CaMV 35S Promoter present in the majority of genetically engineered crops
    - Tubulin gene as an endogenous plant control

- Tubulin is present in all plans and is used as an internal positive control for the quality of the DNA extraction
    - All successful DNA extractions followed by good PCR should show the 150 or 180 base pair band corresponding to the tubulin gene
    - This should be true for all samples regardless of genetic modifications

- When a transgene is present (GMO food) we expect to see the 125 base pair band corresponding to the CaMV 35S promoter
    - These lanes should also display the larger band corresponding to the tubulin gene (DNA extraction control)
    - Absence or presence of the 125 bp band in Test Foods 1 and 2 serves to reveal their GMO status

Study questions

Questions before experimental set-up

1. How does genetic diversity of plants arise through natural selection?
2. Artificial selection: How and why do humans selectively breed crops?
3. What is a genetically modified organism (GMO)?
4. How can new genes be introduced into a plant?
5. Does the introduction of new genes into an organism ever occur in nature?

Questions about DNA extraction

1. What is the purpose of boiling the food samples (95°C incubation)?
2. What other molecules besides DNA are released from the tissue?
3. What is the role of alkaline pH in the EZ-DNA™ Extraction System?

Questions during PCR run

1. What is happening to DNA molecules at each step?
    - **Denaturation**:
    - **Annealing**:
    - **Extension**:
2. What is the purpose of the following PCR mix components?
    - **dNTPs**
    - **Mg^{+2}**
    - ***Taq* DNA polymerase**
3. What temperature do you think is optimal for most enzymes?

4. What feature of *Taq* DNA polymerase makes this enzyme unique?

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?

6. How will we know whether the PCR has worked?

7. How can the PCR product be recovered?

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.

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)