

Kansas Corn: GMO or GM-NO?



qrco.de/gmogmno

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Overview

Looking at the history of corn, a great story can be told about how people saved seeds and selected for specific traits to turn teosinte into the modern corn plant that we have today. This lab will enable students to use equipment that is not made available to very many. With the use of a Polymarese Chain Reaction (PCR) machine, students will start to see one of the final steps of identifying what a genetically modified organism is and what foods they consume today that contain a GMO. This lab can end the series of all biotechnology Seed to STEM labs, which enables a teacher to teach the science behind a genetically modified organization throughout the school year. GMO or GM-NO can provide a finale to these series of lessons.



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

Kansas College and Career Ready Standards

Science

- **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.



Language Arts (Common Core)

- **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.

Learning Objectives

- 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 engineered or genetically modified 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.
- Discuss implications of genetically engineered plants for society.

Materials

- MiniPCR GMO Learning Lab: Heart-Shaped Bananas (\$79)
- MiniONE Gel Electrophoresis
- MiniONE PCR Machine

Safety Considerations

Be aware of student allergies or seeds treated with chemicals.

Procedures for Instruction

Length of Time for Preparation: 20 minutes Length of Time for Classroom Teaching: 90 minutes

Preparation Procedure

See preparation pages 11-13 from MiniPCR GMO Lab Instructor's Guide.



Background Information

Pages 2-5 of the MiniPCR Lab Guide provides background information. Also, rely on previous Seed to STEM biotechnology labs for additional background information.

Classroom Discussion

Introduce the topic and assess students for prior understanding. See page 15 questions in Student MiniPCR Lab Guide to ask the following questions.

- How does genetic diversity of plants arise through natural selection?
- How and why do humans selectively breed crops?
- What is a GMO?
- How can new genes be introduced into a plant?
- Does the introduction of new genes into an organism ever occur in nature?

Procedure for Lab

MiniPCR GMO Lab

The MiniPCR GMO Lab procedure will be used with the following alterations of instructions from the miniPCR GMO Lab procedure.

- On page 3, Corn Crop Transgenic Modified 85%, the data table shows the percentage of specific crops that are transgenic. The table emphasizes corn, and that is what this lab will specifically be testing for as a transgenic food item.
- On page 4, Creating a GMO Product using Ti Plasmid [CaMV35S promotor/Transgene/NOS Terminator], there is an explanation of how the specific GMO cassette that we are going to attempt to test for is constructed in order to create a transgenic plant. We will be targeting the 35S promotor and the positive plant control (Tubulin) for this lab.
- On page 5, Explanation of the PCR and Electrophoresis Lab, we will follow these instructions to determine if common food items have been genetically modified, and this section will demonstrate how this complete lab will detect a GMO food.
- On page 6 (recommended foods to test: corn tortillas, tortilla chips, Doritos, Cheetos), we will specifically attempt to test just the corn products listed in that table.
- On pages 7-8, DNA Extraction Procedure, we will follow that exact procedure on DNA extraction from the selected food items being tested.
- On pages 8-9, Setting up the PCR Reactions, we will follow the directions to set up the PCR reactions.
- On pages 10-11, Setting up and Running the PCR Machine, we will follow the directions for setting up the MiniOne PCR machine (on pages 18-19 of the PDF) instead of the instructions for miniPCR listed on those pages.



- On page 12, Setting up and Running the Gel Electrophoresis, we will follow these exact instructions. Also, we will be using a 2.0% gel instead of the 2.4% gel the procedure calls for.
- On page 13, Photographing and Interpreting the Gel, we will follow this procedure to get an image of the completed gel and how to interpret the gel.
- On pages 15-17, Lab Questions and Extension Activities, we will be using the Lab Report Extension to assess what the students have learned from completing this lab activity.

Teacher Resources

www.gmoanswers.com provide numerous resources to help with this lab. Visit www.kansascornstem.com for additional resources.

Lab Analysis

See pages 12-14 of the miniPCR GMO Lab instructions to determine if you have detected any GMOs in the foods tested.

Reflection and Conclusion

See pages 16-17 of the miniPCR GMO Lab instructions and answer the following questions.

- What do the results suggest about your test foods?
 - Do they contain genetically engineered sequences?
 - Do they not?
- Are your results consistent with your expectations about these foods?
 - Are you a regular eater of these foods?
- Do you know the purpose of the genetic modification(s) you have detected?
 - Describe two transgenes that are commonly introduced into crops.
- 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?
- Describe three ways in which human health may be improved by GMOs.
 - How can they aid in nutrition?
 - How can they help feed a growing human population?
 - What might be the risks to manage?
- 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.



Extension Activity

There are only 10 GMO crops available in the United States today. Can the students name them? Corn, Soybeans, Cotton, Canola, Alfalfa, Sugar Beets, Papaya, Squash, Potato, and Apple

Food companies use labels on food to help market their products. Non-GMO labels are becoming common to help market food to consumer who are afraid of GMOs. Yet, many of these labeled food products were not made from foods that have a GMO variety.

Have students visit their local grocery store or have them look through their kitchen cabinets at home. Have them write down a list of 10 food products that are labeled as non-GMO. Have the students discuss their findings as a group.

- Were these products made from one of the 10 GMO crops available in the United States?
- It is appropriate to have the non-GMO label?
- Which products that do have a non-GMO label were made from a crop that has a GMO variety?

Assessments

To assess learning, the students will follow the extension instructions on page 17 of the miniPCR GMO lab instructions.

Lab Report - report on the findings of the written lab.

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

Science and Agriculture Careers

To learn more about agriculture careers, visit www.agexplorer.com. You can also find career profiles at www. kansascornstem.com.

Sources

MiniPCR GMO Learning Lab: Heart-Shaped Bananas

Any educator electing to perform demonstrations is expected to follow NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops, which are available at http://static. nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are under a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.



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A. DNA Extraction

- 1. Label two 200 µL thin-walled PCR tubes per lab group on the side, not cap, of the tube
 - <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</u> <u>diameter</u> or less into a tube containing DNA-EZ[™] Lysis Solution.



• *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



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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[™] <u>Neutralization Solution</u> 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
 - <u>1 tube labeled "T1"</u>: Test DNA extracted from Food 1
 - <u>1 tube labeled "T2"</u>: Test DNA extracted from Food 2
 - <u>1 tube labeled "G"</u>: 'GMO Banana' DNA provided in the kit
 - <u>1 tube labeled "W"</u>: '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



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.

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	DNA extract	Control 'GMO	Control 'Wild
	from Test	from Test	Banana' DNA	Banana' DNA
	Food 1	Food 2	supplied w/kit	Supplied w/kit
	2 μL	2 μL	2 μL	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)

miniPCR 1.6 File	
1 Protocol Library	
Protocol Name miniPCR 37C incubation miniPCR 37C incubation miniPCR annealing curve miniPCR Crime Lab miniPCR Crime Lab 10 miniPCR Crime Lab 15 miniPCR Crime Lab CSF miniPCR Forensics Lab miniPCR GMO Lab MtDNA Rockefeller Mullins Protool My unique DNA- D1S80 New Protocols Protocol Protocol Protocol Protocol Protocol Protocol Protocol C4 cycles QC 99 cycles Restriction digest Rocio & Joanna GMO Unicom's Majestical Fury USAFood Safety Lab	 3 Protocol Type PCR 4 Protocol Name miniPCR GMO Lab Block 5 Initial Denaturation Denaturation Annealing Extension Final Extension Temp (C) 94.0 94.0 55.0 72.0 72.0 72.0 Time (sec) 60 10 10 15 30 Heated Lid (C) ON Number of Cycles 35
New Protocol Make Copy Delete	6 Save Cancel
minìpcr _{1.6}	Upload to miniPCR

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



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- 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

miniPCR 1.4					
File					
Protocol Library	miniPCR Serial 190				
Protocol: DNA Forens	ics Lab				
Status: Running			Extension 00:08		
• • c					
			cycle 3 of 30		
	0:08:58				1:03:20
	Brotocol	Sottingo			
Udia	PTOLOCOI	Setungs			
	Sample Temperat	ture (C):72.1		Lid Temperature (C): 103.3	
			Sample temperature		
00:00:00	00	:16:40	00:33:20 Time (hh:mm:ss)	00:50:00	01:06:40
minipcria					

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: <u>10µL</u> 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)



<u>Note</u>: there is <u>no need to add gel loading dye to your samples</u>. The *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* come premixed with loading dye, and <u>ready to load on your gel</u>!

- 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