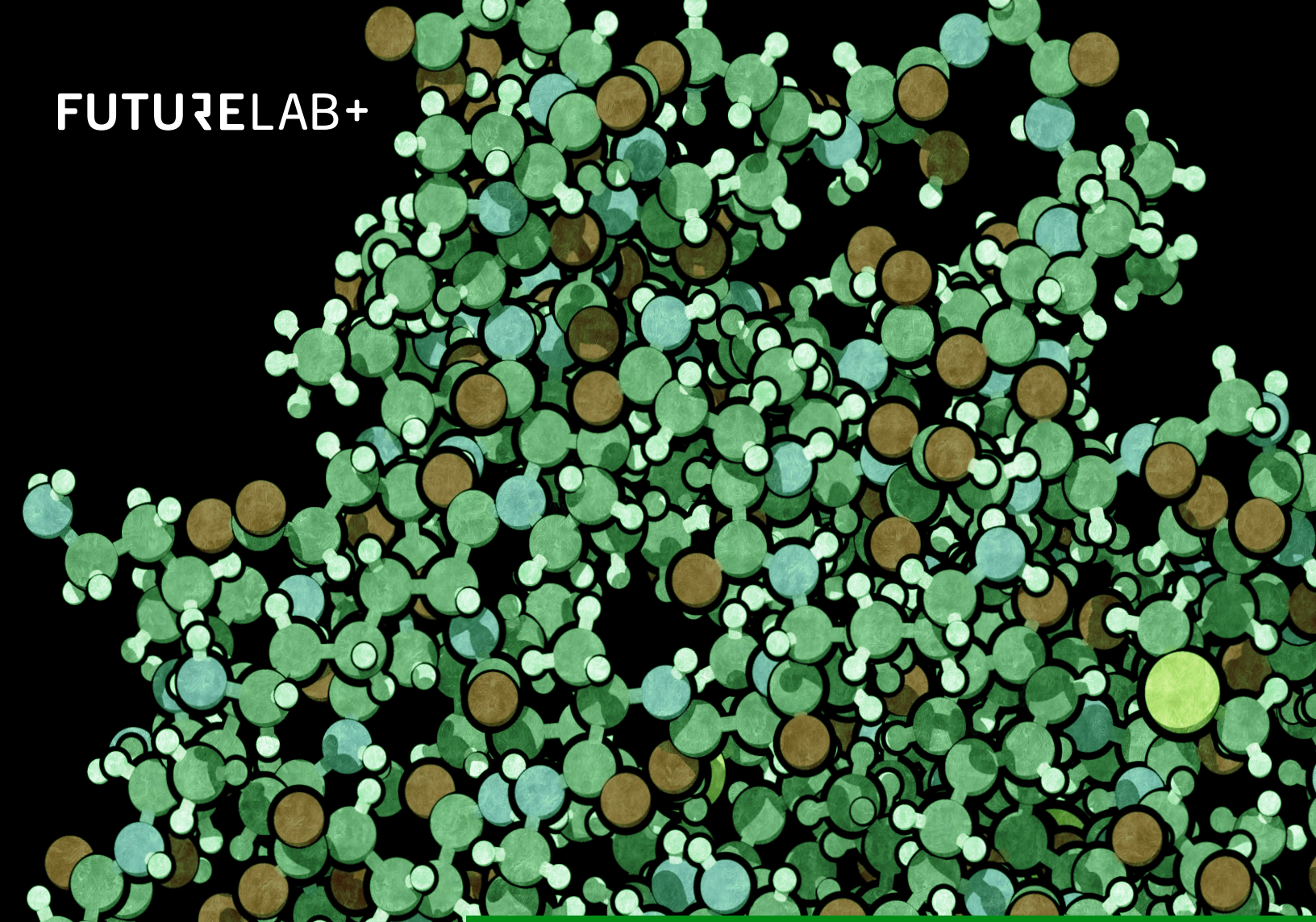
FUTURELAB+



AG/ENVIRONMENTAL

Alternative Proteins

Detecting Genetically Engineered Crops

Laboratory Investigation

Developed in partnership with:

Bay Area Bioscience Education Community

In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →

Cover Image
This ball-and-stick model of a whey protein in cow's milk is a common allergen. Could a genetically engineered modification help with digestion?

Teachers [T] *and* Student Resources [S] *can be printed independently. Select the appropriate printer icon above to print either section in its entirety.*

Follow the tips below in the Range field of your Print panel to print single or a range of pages:

Single Pages (use a comma): T3, T6

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AG/ENVIRONMENTAL / ALTERNATIVE PROTEINS

Lab: Detecting Genetically Engineered Crops

DRIVING QUESTION

How can we detect genetically engineered crops in our food?

ACTIVITY DURATION

Five class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

How can we detect whether food contains ingredients that have been genetically engineered?

How can we use DNA extraction, Polymerase Chain Reaction (PCR), and gel electrophoresis to analyze DNA?

How can we use lateral flow to detect protein products?

OVERVIEW

Genetic engineering, though largely accepted for production of medicine such as insulin, remains controversial and often misunderstood for applications such as food production. Bt-corn is an example of a genetically engineered crop that is commonly grown in the United States. This corn contains genes from a soil bacterium called Bt (*Bacillus thuringiensis*), which codes for proteins that are poisonous to the European corn borer pest. As a result, Bt-corn is resistant to these insects without the use of external insecticides. Bt-corn has been available since 1996 and, as of 2020, makes up 82% of US corn crop acreage (USDA, 2020).

In this lab, students first choose a food product containing corn and extract its DNA. They then use Polymerase Chain Reaction (PCR) and gel electrophoresis to detect the presence or absence of the Bt gene *cry1Ab* in the DNA sample. They also perform a lateral flow test to detect the presence or absence of delta endotoxin proteins, one of which is encoded by the *cry1Ab* gene, that are responsible for the pest-resistant property of Bt-corn. Students then collect class data to determine the prevalence of Bt-corn in the foods they sampled and draw a scientific model explaining what happens inside Bt-corn at the molecular level.

BACKGROUND INFORMATION

This lesson utilizes the technique of PCR, to which students were introduced in Unit 5. Prior experience with micropipetting and gel electrophoresis is recommended before conducting this lab. It is also helpful for students to have a basic understanding of the structure and function of DNA and proteins.

Sources:

Recent Trends in GE Adoption

Use and Impact of Bt Maize

Have you ever wondered…

If your snack contains genetically engineered ingredients?

Genetically engineered crops are different from other crops in that their genetic material has been changed using tools of biotechnology. Crops such as corn that contain new genes to make them resistant to insects or herbicides are examples of GE crops that are present in many food products. The majority of corn crops used to make snacks such as corn chips in the United States have been genetically engineered.

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

Students will observe the ubiquitous nature of GMOs by testing corn products in the foods they eat.

How does this connect to careers?

Plant geneticists plan and conduct experiments to understand, improve, and create new varieties of plants or crops by modifying their DNA.

Research associates use basic lab techniques, such as PCR and gel electrophoresis. They follow written protocols as they collect samples and perform tests to analyze specimen DNA and other substances. They maintain clear records of their findings.

How does this relate to the product development life cycle?

Genetically engineered crops are examples of products developed by biotechnology companies. An important step in developing these crops is using techniques such as PCR and lateral flow to confirm a crop was successfully engineered to produce the desired new trait.

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students work cooperatively with a partner and group of four throughout the lab, helping them develop relationship skills.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lab provides students with an opportunity to analyze the DNA of a food of their choosing, including foods they eat or cook with on a regular basis. It also focuses on corn (maize), an integral part of the cuisine and agriculture of many cultures and nations.

COMPUTATIONAL THINKING PRACTICES

After performing PCR, gel electrophoresis, and lateral flow tests on their food products, students will collect and analyze data showing the presence or absence of genes and proteins associated with Bt-corn. Students will then find patterns in their class data to identify relationships between the food products tested and the use of Bt-corn.

OBJECTIVES

Students will be able to:

Describe how Bt-corn differs from corn labeled "USDA organic" using scientific text.

Extract, amplify, and **visualize** DNA from food products to determine if they contain genes present in Bt-corn using scientific protocols.

Detect proteins present in Bt-corn using lateral flow tests.

Identify patterns in the presence of Bt-corn genes and proteins in food products using experimental results.

Create a scientific model to explain what occurs inside Bt-corn in a food product to make it different from standard (non-genetically engineered) corn using experimental results.

Materials

Documents

Lab Preparation (for teacher)

Background Reading: Bt-Maize and Organic Crops (1 per student)

Background Reading: What is Polymerase Chain Reaction (PCR)? (1 per student)

Background Reading: Analyzing PCR and Lateral Flow Results (1 per student)

Vocabulary Tool (1 per student)

Student Protocol, Part 1: DNA Extraction and PCR (1 per pair)

Student Protocol, Part 2: Gel Electrophoresis (1 per pair)

Student Protocol, Part 3: Lateral Flow (1 per pair)

Student Guide (1 per student)

Reagents

Lab Part 1: DNA Extraction and PCR

- *Lysis buffer (1 mL per student)*
- *5M NaCl (40 µL per student)*
- *TE/RNase (100 µL per student)*
- *Organic cornmeal (non-Bt-corn) (1 tube)*
- *Conventional cornmeal (Bt-corn) (1 tube)*
- *91–100% Isopropanol (6 mL per group of four)*
- *70% Ethanol (2 mL per group of four)*
- *GE master mix (20 µl per student)*
- *GE primer mix (20 µl per student)*
- *Positive control DNA (15 µL PCR product per gel)*
- *dH_2O (15 µL PCR product per gel)*

Lab Part 2: Gel Electrophoresis

- *1X TAE running buffer (150–300 mL per group of four students—depending on which gel electrophoresis system you are using)*
- *2% agarose gel with DNA stain (1 per group of four)*
- *10X loading dye (1 per group)*
- *100 bp ladder (1 per group)*

Lab Part 3: Lateral Flow

- *Lysis buffer (2.2 mL per group of four)*
- *Lateral flow strips (6 per group of four)*
- *Conventional cornmeal (Bt-corn) (1 tube)*

Continued>

Materials

Equipment and Consumables

Lab Part 1:
DNA Extraction and PCR

- *P1000 micropipettes (1 per pair)*
- *P1000 tips (1 box per pair)*
- *P200 micropipettes (1 per pair)*
- *P200 tips (1 box per pair)*
- *P20 micropipettes (1 per pair)*
- *P20 tips (1 box per pair)*
- *1.5 mL microtubes (3 per student)*
- *PCR tubes (1 per student + controls)*
- *Microtube rack (1 per pair)*
- *PCR tube rack (1 per pair)*
- *Centrifuge (1–2 per class)*
- *Thermal Cycler (1 per class)*
- *Heat block set at 99°C (1 per class)*
- *Cap locks (1 per student)*
- *Permanent marker (1 per pair)*
- *Dry waste beaker (1 per group of four)*
- *Sink or wet waste beaker (1 per group of four)*
- *Paper towel (1 per student)*
- *Crushed ice (1 per group of four)*
- *Plastic micropestles (1 per student)*

Lab Part 2:
Gel Electrophoresis

- *P20 micropipettes (1 per pair)*
- *P20 tips (1 box per pair)*
- *Microtube rack (1 per pair)*
- *Dry waste beaker (1 per group of four)*
- *Sink or wet waste beaker (1 per group of four)*
- *Electrophoresis gel setup (1 per group of four)*
- *UV light source and UV safety goggles (if needed for electrophoresis equipment)*

Lab Part 3:
Lateral Flow

- *P1000/P200 micropipettes (1 per pair)*
- *P1000/P200 tips (1 box per pair)*
- *1.5 mL microtubes (10 per group of four)*
- *Microtube rack (1 per pair)*
- *Permanent marker (1 per pair)*
- *Dry waste beaker (1 per group of four)*
- *Plastic micropestles (1 per student)*

Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

Predict whether different products are made by genetic engineering using example foods.

Extract DNA from a corn product of their choice using scientific protocols.

Describe how Bt-corn differs from corn labeled "USDA organic" using scientific text.

Teacher Note > *Before the lab, every student needs a food product that contains corn. You may bring in a variety of corn products for students to choose from or they may bring in any of their choice. To increase the chances of a successful DNA extraction and lateral flow, we recommend using dry, minimally processed foods such as cornmeal, cornflour, or corn chips.*

Before class, aliquot reagents, set up heat block and centrifuge station(s) around the room, and set up lab stations using the Lab Preparation document.

Whole Group (10 minutes)	
1	Pass out the *Student Guide* and *Background Reading: Bt-Maize and Organic Crops.*
2	Warm Up: Bring in one bag of corn chips labeled "USDA organic" and one without this label (or display images of each) for students to observe. Label one "A" and one "B." Have student pairs discuss the similarities and differences, as well as which product(s) they believe are made from genetically engineered corn.
3	Have students record their predictions and what they think is happening inside a genetically engineered food product on *Student Guide, Part 1: Pre-Lab* questions #1–2.
4	Have students share responses with a partner or use equitable practices for choosing students to share with the class.
5	Introduce the lab to students by sharing that today they will extract DNA from a corn product of their choice so that they can test whether or not it was made from genetically engineered corn. Have students select a corn product from the options you have presented or they can use the product they brought.

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Day 1

Continued

Procedure

Small Group (30 minutes)	
1	Place students into lab groups of four, then pass out lab stations and ask groups to complete steps #1–15 of *Student Protocol, Part 1: DNA Extraction and PCR*.
2	Point out where the heat block and centrifuge station(s) are and share the following safety precautions and guidelines:
	a. Do not touch heated blocks. (They may be extremely hot, even if off!)
	b. Always make sure that the centrifuge is balanced with a counterweight before running.
	c. Wash your hands before and after the lab.
	d. Do not eat or drink during the lab.
	e. Keep your lab station clean and clear of clutter.
3	During wait times, instruct students to complete the *Background Reading: Bt-Maize and Organic Crops* and related questions.
4	Have students clean up and give you their tubes of isolated corn DNA to store in the freezer until next class. Make sure students have clearly labeled their tubes so they can easily retrieve them.

Individual (5 minutes)	
1	Exit Ticket: Can products made with Bt-corn be labeled 'USDA Organic'? Explain.
2	Ask students to write their own sentence for each of the first five words in the *Vocabulary Tool* for homework.

Day 2

Procedure

LEARNING OUTCOMES

Students will be able to:

Amplify the *rbcL* and *cry1Ab* genes using PCR.

Describe how PCR can be used to amplify specific regions of DNA using scientific text.

Teacher Note > *Before class, set up lab stations using Lab Preparation and remove students' partially completed DNA extractions from the freezer. Place a Thermal Cycler Grid next to the thermal cycler for students to record PCR tube placement. You will also need to set up the positive and negative control PCR reactions today. Once the PCR reaction is complete, store tubes in the freezer.*

Whole Group (10 minutes)

1. Have students review their *Background Reading: Bt-Maize and Organic Crop* individually and then review answers with the whole class.

2. Have students discuss with their lab groups whether or not they think the corn product they are testing contains a GE crop. Have students record their responses in *Student Guide, Part 1: Pre-Lab* Question #3.

Small Group (35 minutes)

1. Have a student from each lab group retrieve their DNA from the freezer.

2. Share the following safety reminders with students.
   - **a.** Always make sure that the centrifuge is balanced with a counterweight before running.
   - **b.** Wash your hands before and after the lab.
   - **c.** Do not eat or drink during the lab.
   - **d.** Keep your lab station clean and clear of clutter.

3. Ask students to complete steps #16–30 of *Student Protocol, Part 1: DNA Extraction and PCR*.

Teacher Note > *Tell them to answer the questions and then check their answers with the key.*

4. During wait times they should complete the *Background Reading: What is Polymerase Chain Reaction (PCR)?*

Teacher Note > *This reading is the same as in Unit 5 Lab: Detecting Wolbachia: A Microbe to Control Disease.*

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Day 2

Continued

Procedure

5	Once ALL students have placed their PCR tubes in the Thermal Cycler and recorded the positions of their tubes in the Thermal Cycler Grid, run the program described in *Lab Preparation* (do not forget the positive and negative control reactions). PCR will take approximately two hours to run. (DO NOT save tubes to run later.)

Individual (5 minutes)

1	Exit Ticket: What is the purpose of the DNA extraction and PCR you performed? What is the next step in the lab and why?
2	Ask students to write their own sentence for each of the last three words in the *Vocabulary Tool* for homework.

Day 3

Procedure

LEARNING OUTCOMES

Students will be able to:

Visualize the amplified DNA from their PCR reactions to determine if their food product contains Bt-corn using gel electrophoresis.

Describe how gel electrophoresis can be used to determine if a food product contains Bt-corn using scientific text.

Teacher Note > *Before class, set up lab stations using Lab Preparation and remove students' PCR reactions from the freezer. Each group of four students will need one agarose gel with at least seven wells for loading their samples. You may pre-pour gels for students or have each group pour their own. Suggest preparing additional pre-poured gels in case students puncture the wells.*

When helping students interpret their gels, note that the rbcL *and* cry1Ab *bands will be very close together and may be difficult to distinguish. It is also possible that one band is much brighter than the other. Because students are looking for a positive or negative result, any presence of the* cry1Ab *band indicates the presence of Bt-corn.*

Whole Group (5 minutes)	
1	Warm-Up: Ask students to jot down all the things they remember about gel electrophoresis. *For example: What is it used for? What are the important components? How do you load and run a gel?*
2	Call on students and have a volunteer record responses on the board.
3	Share that today students will use electrophoresis to visualize their PCR products from last class to determine whether or not their food products contain Bt-corn.
4	Pass out one copy of *Student Protocol, Part 2: Gel Electrophoresis* per pair and *Background Reading: Analyzing PCR and Lateral Flow Results* per student.

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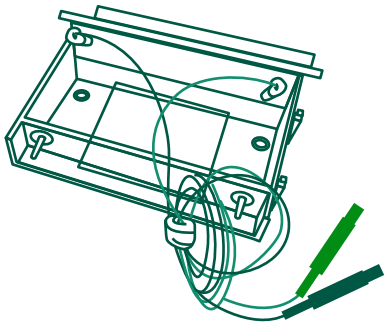
Day 3

Continued

Procedure



Small Group (35 minutes)

1. Ask students to retrieve their PCR reactions and go to their lab stations (four students will share one gel).

2. Share the following safety reminders with students and ask them to complete the steps of *Student Protocol, Part 2: Gel Electrophoresis*.
   - **a.** Be careful with liquid agarose—it is extremely hot!
   - **b.** If using non-minione electrophoresis equipment, turn off the power supply before opening the gel box.
   - **c.** If using non-minione electrophoresis equipment, wear UV goggles when looking at the gel under UV light.

3. While the gel is running, students can complete the *Background Reading: Analyzing PCR and Lateral Flow Results* and questions.

4. After the gel has run, ask students to take a picture with their phones (or take a picture for them) and attach it to their *Student Guide, Part 2: Lab* Question #1 and label the lanes.

Individual (5 minutes)

1. Ask students to complete *Student Guide, Part 3: Data Analysis* Question #1.

Teacher note > *Before starting Day 4, it is recommended to finish Unit 6 Lesson 1 and start Lesson 2 through Days 1-2.*

Day 4

Procedure

LEARNING OUTCOMES

Students will be able to:

Detect the presence or absence of Cry1A and Cry2A proteins in their food product to determine if it contains Bt-corn using a lateral flow test.

Teacher Note > *Before class, set up lab stations using Lab Preparation.*

Whole Group (5 minutes)

1. Warm-Up: Tell students to review their PCR results in *Student Guide, Part 3: Data Analysis* Question #1. They should make a prediction about which, if any, delta endotoxin proteins they will observe in the lateral flow test today (Cry1Ab/Ac and/or Cry2A). Ask them to explain their predictions.

2. Ask students to share with a partner. Invite them to share one of their partner's responses to the class.

3. Share the learning outcomes for today and pass out one copy of *Student Protocol, Part 3: Lateral Flow* per pair.

Small Group (30 minutes)

1. Ask students to retrieve their food products and go to their lab stations.

2. Share the following safety reminders with students and ask them to complete the steps of *Student Protocol, Part 3: Lateral Flow*.
   - **a.** Wash your hands before and after the lab.
   - **b.** Do not eat or drink during the lab.
   - **c.** Keep your lab station clean and clear of clutter.

3. After the strips have developed, ask students to dispose of the bottom part in the trash and attach the rest to their *Student Guide, Part 2: Lab* Question #2 and label the lines (or upload a picture).

Individual (10 minutes)

1. Ask students to complete *Student Guide, Part 3: Data Analysis* Questions #2–3.

Day 5

Procedure

LEARNING OUTCOMES

Students will be able to:

Identify patterns in the presence of Bt-corn genes and proteins in food products using experimental results.

Create a scientific model to explain what occurs inside Bt-corn in a food product to make it different from standard (non-genetically engineered) corn using experimental results.

Whole Group (5 minutes)

1 Warm-Up: Look at the drawing you made in *Student Guide, Part 1: Pre-Lab* Question #2 to explain what happens inside genetically engineered food. List some features you could add or change about your model based on what you have learned this week.

2 Ask students to share with a partner and share one of their partner's responses with the class.

3 Share the learning outcomes for today.

Whole Group (10 minutes)

1 Collect class data using a spreadsheet or on the board (only collect data from successful DNA extractions/amplifications—lanes that show a band at 599 bp—and valid lateral flow strips—those that show the control line).

	Food Product	Labeled Organic? (Y/N)	*cry1Ab* gene? (Y/N)	Cry1A protein? (Y/N)	Cry2A protein? (Y/N)
1					
2					
3					
4					
	Totals Positive Results				
	% positive results (total positive / total tested)				

Continues next page >

Day 5

Continued

Procedure

2 Ask students to identify three to five patterns in the data and record their answers under *Student Guide, Part 3: Data Analysis* Question #4.

Optional If time allows, ask students to work with their partner or group of four to make a graph or other visualization of the class data on a whiteboard or poster paper. Then have students view each others' graphs, choose one they think is best, and share why they think it is the best representation of the data.

Small Group (20 minutes)

1 Share with students that to synthesize the information they have learned during the lab, they will draw a scientific model. Share the following features of a scientific model and suggest showing an example by searching for *NGSS model examples*.

- **a.** Represents a system or a phenomenon
- **b.** Context-rich and specific
- **c.** Pictorial (drawings and pictures) and written (text, labels, equations, etc.)
- **d.** Observable and unobservable features (make the "invisible" visible!)
- **e.** Revisable over time

2 With their partner or whole lab group, ask students to start *Student Guide, Part 4: Make a Model* Question #1. Suggest having students draw on large whiteboards or poster paper.

3 Ask students to switch models with another pair or group and provide feedback:

- What aspect of the model is the most helpful to understanding the science behind the lab?
- What aspect of the model is confusing or could be clearer?
- What suggestions do you have for improving the model?

4 Ask students to modify their model to incorporate at least one piece of feedback they received and describe limitations of their model in *Student Guide, Part 4: Make a Model* Question #2.

National Standards

Next Generation Science Standards

LS1.A: Structure and Function

All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.

LS3.A: Inheritance of Traits

Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function.

Science and Engineering Practices

Developing and Using Models

Develop, revise, and/or use a model based on evidence to illustrate and/or predict the relationships between systems or between components of a system.

Crosscutting Concepts

Cause and Effect

Cause and effect relationships can be used to explain and predict behaviors in complex natural and designed systems.

Math

MP.4 Model with mathematics.

Identify patterns in prevalence of Bt-corn-specific genes and proteins in tested food products.

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National Standards

Continued

Career and Technical Education (CTE)	**A3.3** Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.
	A3.5 Predict outcomes of DNA and protein separation protocols.
	A8.1 Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.
	A8.6 Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances.
	A8.7 Determine which equipment is appropriate to use for a given task and the units of measurement used.

Lab

Preparation

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Quick Tips	
1	Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab.
2	Before the lab, every student needs a food product that contains corn. Students may bring in any food product of their choice, but to increase the chances of a successful DNA extraction and lateral flow, we recommend using dry, minimally processed foods.
3	We recommend having students complete this in a group of four with each student individually extracting DNA from their own food product, setting up their own PCR reaction, and performing their own lateral flow strip test.
4	*Virtual Learning Options* for this lab, including digital-only resources, are provided.

Preparing Food Products

1	Any time before the lab	30 min
☐	Because it is often difficult to successfully extract DNA from many food products, we recommend having each student group extract DNA from the conventional and/or organic cornmeal provided in the reagent kit along with a product of their choice.	
☐	Cut blue pipet tips at an angle to make a small scoop for students to pick up a small amount of their crushed food product.	
☐	Clean micropestles with ethanol if they have been used before.	

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Lab

Continued

Preparation

Preparation, Part 1: DNA Extraction and PCR

1 — 1–2 days before the lab — 30 min

☐ Aliquot reagents for DNA extraction.

Example for a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
Lysis Buffer	4 students X 1 mL X 1.1 (overage)	4.4 mL
5M NaCl	4 students X 40 µL X 1.1 (overage)	176 µL
TE/RNase	4 students X 100 µL X 1.1 (overage)	440 µL
Isopropanol	4 students X 800 µL X 1.1 (overage)	—
70% Ethanol	4 students X 400 µL X 1.1 (overage)	—

Note > *Suggest setting up 3–8 beakers with ~50 mL of each alcohol in ice buckets near group lab stations (day of lab). Crushed ice is ideal to keep the beakers of alcohol level.*

2 — 1–2 days before the lab — 30 min

☐ Aliquot reagents for PCR:

Example for a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
GE Master Mix	4 students X 20 µL X 1.1 (overage)	88 µL
GE Primer Mix	4 students X 20 µL X 1.1 (overage)	88 µL
Water	In PCR tube as “reference tube” for students to check their volume	50 µL

☐ Determine how many positive and negative control PCR reactions you will need to set up (see next page).

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Lab

Continued

Preparation

3	During the lab	5 min
	Prepare Positive and Negative PCR Controls:	
☐	Each PCR reaction with loading dye yields *55 µL* total.	
☐	At 15 µL per gel, there is enough PCR product for 3 gels in one reaction.	
☐	To determine number of control reactions needed, divide the number of gels by 3 and round up to the nearest whole number: — e.g., 8 groups ÷ 3 = 2.67 reactions → *3 PCR reactions* — *Teacher sets up 3 Positive Control and 3 Negative Control PCR reactions for the 8 gels in the class.* **Note** > *The positive control DNA includes both the* rbcL *gene (found in all plants) and* cry1Ab *DNA (found in Bt-corn). The negative control is water.*	

Continues next page >

Lab

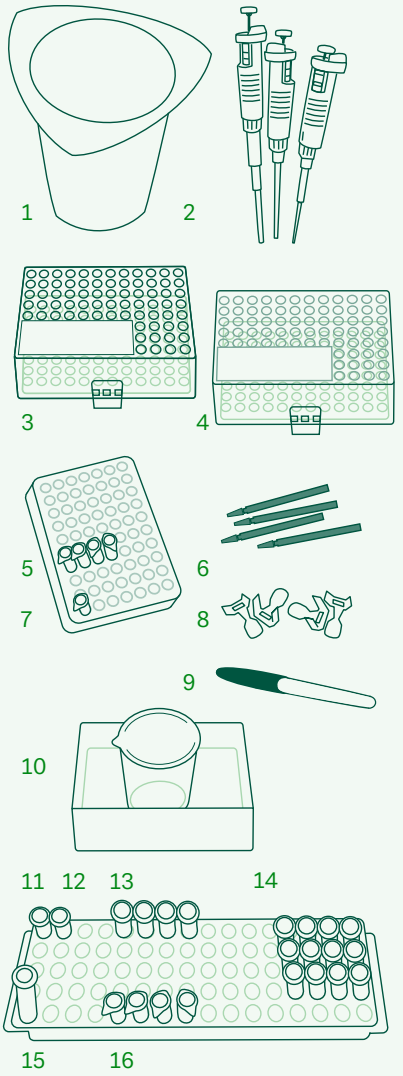
Continued

Preparation

4 | 1–2 days before the lab | 30 min

☐ Set up lab stations (1 per group of four):

1	Waste bucket
2	P20, P200, P1000
3	P1000 blue tips *for pipetting and making scoops*
4	P20 and P200 yellow tips
5	4 PCR Tubes and Rack
6	4 micropestles
7	50 µL reference tube
8	4 cap locks
9	Permanent marker
10	95% Isopropanol and 70% EtOH on ice
11	440 µL TE/RNAse
12	176 µL 5M NaCl
13	88 µL Primer Mix
14	12 empty tubes
15	4.4 mL Lysis Buffer
16	88 µL Master Mix



Note > *Cap locks prevent tubes from opening due to pressure and allow for easier handling. If you do not have cap locks available, you may cover all the tubes with a sheet of foil after all students have placed their tubes in the heat block.*

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Lab
Continued

Preparation

5	**Class Equipment Notes**
	Micropestles
☐	Clean the micropestles with soap and water, and soak in ethanol to sterilize. This will reduce cross-contamination when they are used by the next class.
	Centrifuge
☐	You will need one to complete this lab with your class, but we recommend having more than one in the room to prevent bottleneck situations.
☐	If you only have one, we recommend doing the DNA extraction part of the lab in lock step to allow for easier management of the centrifuge.
☐	Never start the centrifuge with an uneven number of tubes—every tube must be counterbalanced with another tube, otherwise it can damage the equipment.
	Heat block
☐	Allow to preheat prior to the start or at the beginning of class to 99˚C.
☐	We recommend leaving a note next to the heat block to caution students against touching the heated blocks because it may not be apparent that the unit is on.
☐	If you do not have a heat block available, set up a water bath at 99˚C—take similar precautions for safety.
	Thermal Cycler
☐	Place a Thermal Cycler Grid next to the thermal cycler for students to record PCR tube placement—*place all tubes as close to the center as possible*.
☐	Use the following program (it will take 1.5–2 hours to complete).

1	95˚C for 10 min
2	30 cycles of — 95˚C for 30 sec — 60˚C for 45 sec — 72˚C for 1 min
3	72˚C for 10 min
4	4˚C hold

Lab

Continued

Preparation

Preparation, Part 2: Gel Electrophoresis

1 — 1–2 days before the lab — 30 min

☐ Aliquot reagents for Gel Electrophoresis

Example for a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
Loading Dye	4 students X 5 µL X 1.1 (overage)	22 µL
100bp ladder	15 µL per gel	15 µL
Positive PCR Control	15 µL per gel	15 µL
Negative PCR Control	15 µL per gel	15 µL

Note > *Control reactions should have been set up during the PCR. Before aliquoting, add 5 µl loading dye per 50 µl reaction, then mix and flick or spin down the samples.*

2 — 1–2 days before the lab or day of lab — 30 min

☐ Prepare 1X TAE and 2% Agarose gels. See *Preparing 2% Agarose Gels* resource. (1 gel with at least 7 wells per group of four)

Options for pouring gels:

Students pour during lab	Give each group a 50 mL beaker with 20 mL* liquid agarose**. Gels typically take 15–20 minutes to solidify. *Amount of liquid agarose needed may vary between gel boxes. **Keep melted agarose in 65˚C water bath to prevent solidifying.
Teacher pours day before lab	Gels can be stored in the refrigerator wrapped in plastic with 1–2 mL of 1X TAE buffer to keep moist.
Teacher pours day of lab	You can reuse gels by remelting in the microwave.

Continues next page >

Lab

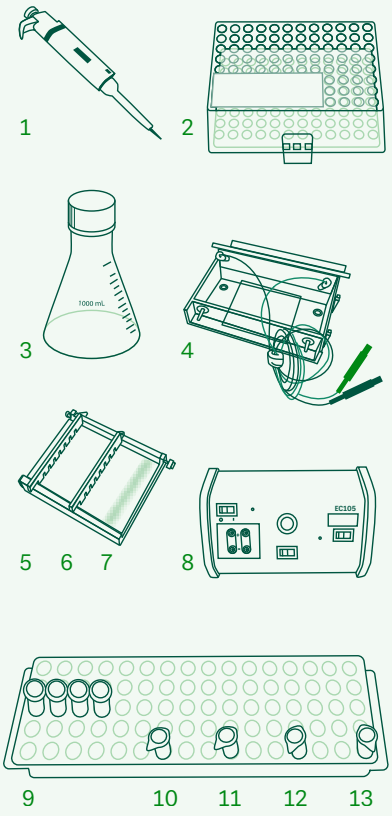
Continued

Preparation

3 | 1–2 days before the lab | 30 min

☐ Set up lab stations (1 per group of four):

1	P20 micropipette
2	P20 Micropipette tips
3	1X TAE Buffer (to fill gel box after gel is cast)
4	Gel box with lid
5	Gel casting tray and gates
6	Comb (add middle comb if two groups sharing one gel)
7	2% agarose in 1X TAE + GelGreen (pour here to cast gel)
8	Power supply
9	4 student PCR products
10	22 µL loading dye
11	15 µL 100 bp ladder
12	15 µL Positive Control PCR product
13	15 µL Negative Control PCR product



3 | After the lab | 15 min

☐ Properly dispose of lab supplies:

— Any excess solutions can go down the drain.
— Used micropipette tips and microtubes can go in the trash.
— Agarose Gels can also be discarded in the regular waste. GelGreen® DNA stain is not hazardous.
— Agarose gels can be reused. For best quality, do not reuse the agarose gel more than 5 times. To reuse gel, simply stuff the gel back into a bottle or a beaker. Make sure to keep different percentage gels in separate containers. Microwave gel until liquid to recast. You will need to re-add DNA stain if viewing DNA.

Lab

Continued

Preparation

Preparation, Part 3: Lateral Flow

1 Before the lab — 30 min

☐ Aliquot reagents.

For a group of four students:

Reagent	Factor in # of students per group	Final volume for each group
Lysis Buffer	4 students X 0.5 µL X 1.1 (overage)	2.2 µL

☐ Prepare Controls

For a group of four students:

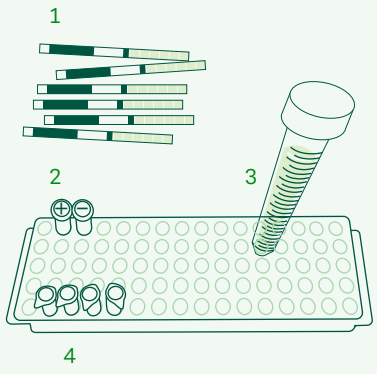
Negative Control (Lysis Buffer)	200 µL
Positive Control (cornmeal)	50 µL

Note > *One student in each group of four will need to prepare the cornmeal sample for the positive control using the same lateral flow protocol as they are using for their own food product.*

2 Before the lab — 30 min

☐ Set up lab stations (1 per group of four):

1	6 lateral flow strips
2	Positive and negative control
3	2.2 mL lysis buffer
4	1.5 mL tubes



Lab

Continued

Virtual Learning Options

1	Anytime	30 min
☐	Students click through and answer the questions embedded in this *DNA Extraction Simulation* from Learn Genetics at the University of Utah.	
2	Anytime	30 min
☐	Students click through this *PCR animation* from DNALC and answer the following questions: — Why are samples heated to 94°–96˚C at the start of PCR? — Describe “anneal” in your own words. — What molecule builds the new strand of DNA? — What do you notice after 5 cycles? — How many copies of the target DNA sequence do you have after 30 PCR cycles	
3	Anytime	30 min
☐	Students click through and answer the questions embedded in one of the following Gel Electrophoresis simulations: — *DNALC* — *University of Utah* — *LabXchange*	

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Lab

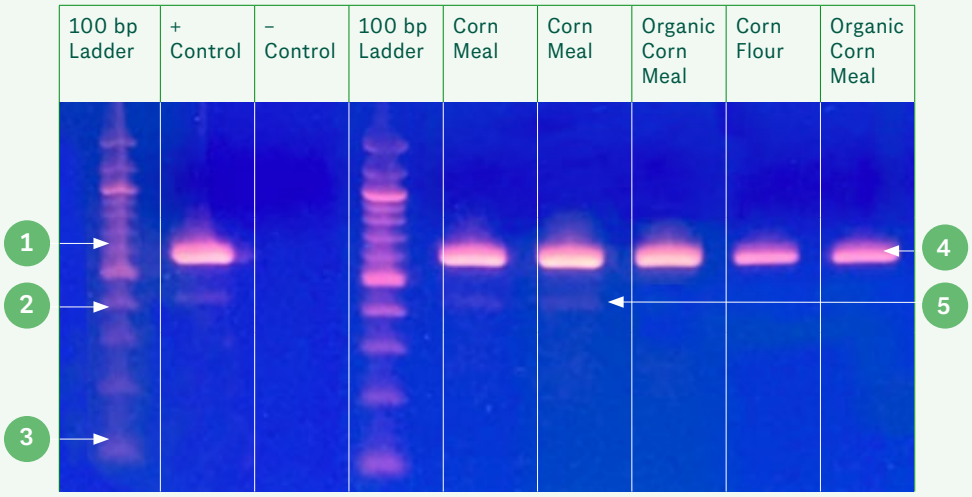
Continued

Virtual Learning Options

4 | Anytime | 30 min

☐ Give students one the following example gels and have them complete the *Student Guide.*

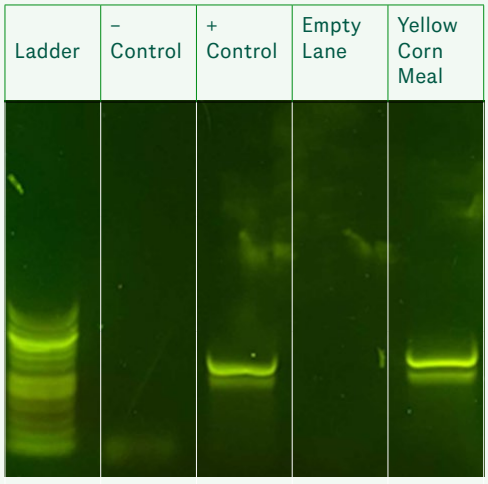
☐ Example 1



1	600 bp
2	400 bp
3	100 bp

4	*rbcL*, 599 bp
5	*cry1Ab*, 412 bp

☐ Example 2



Skills

Preparing 2% Agarose Gels

Teacher Note > *Watch this video from the University of Leicester for an overview, however, be sure to follow the instructions below: Making an Agarose Gel—University of Leicester.*

Procedure	
1	Prepare 1X TAE by adding 20 mL 50X TAE to 980 mL of distilled water.
2	Add 6 g agarose powder to a 500 mL or larger glass bottle, flask, or beaker.
3	Add 1X TAE to the agarose powder to a final volume of 300 mL.

Note > *For volumes of agarose less than 300 mL, make sure to pour the amount of liquefied agarose you need to a clean beaker and use the corresponding amount of GelGreen® for the volume, e.g., for 100 mL of agarose, use 100 µL of 1000X GelGreen®.*

4	Microwave on a low power setting (such as 50% or on "defrost") until liquid is translucent. Check every 5 minutes by removing the bottle with an oven mitt and swirling until melted. • *Caution:* Agarose can be superheated and let off steam explosively. Microwaving at a low power setting for longer reduces this possibility. • After making sure there are no visible lumps, microwave at full power for 20 seconds to dissolve any remaining solute.
5	Let the agarose cool slightly on the benchtop for 5–10 minutes (until you can touch the bottle without burning your hand and the agarose is still liquid or 50–60˚C).
6	While the agarose is cooling, prepare 10 gel trays with combs (this lab requires at least 7 wells).
7	To the 300 mL of cooling agarose, add 300 µL of GelGreen® DNA stain (provided at a 1,000X concentration). Swirl thoroughly to mix.
8	Immediately, pour approximately 15 mL (if using the miniPCR system) of agarose with GelGreen® and 25–30 mL (if using another system such as Fotodyne) to each of the prepared gel trays (work quickly to avoid agarose solidifying).

Note > *If the agarose has solidified after adding the GelGreen®, you can still microwave the gel to liquefy. The GelGreen® will lose optimal activity after microwaving, however.*

9	Do not move the tray until the gel has completely cooled and solidified.
10	Carefully pull the combs out to create the wells (pull straight up).

Bt-maize and Organic Crops Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions below after closely reading the background material.

1. What is *Bacillus thuringiensis* (Bt)?

It is a bacterium found in the soil that produces proteins that are toxic to some insect species.

2. How do delta endotoxins or Cry proteins function?

They kill insects by destroying the cells of their gut lining.

3. Make a claim about how Bt-maize is different from organic corn. Use the Organic Label Explained image above as evidence to support your claim.

Bt-maize is different from organic corn because it has been genetically modified to include a *cry1Ab* gene from Bt bacteria. This means that it will be toxic to pests, including the corn borer. Organic corn is grown from seeds that have not been genetically engineered so they are susceptible to pests.

What is Polymerase Chain Reaction (PCR)? Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions after closely reading the background material.

1. Why is PCR a useful tool for analyzing DNA?

Often, the starting amount of DNA in a sample is too small to be analyzed or used as it is. PCR can then be used to make a large enough amount of the particular DNA sequence of interest so that it can be studied.

2. What might happen if you do not include a buffer or magnesium chloride in your PCR master mix?

Both a buffer and magnesium chloride are essential for *Taq* polymerase activity. Without them, the enzyme could not function and new strands of DNA would not be built.

3. Describe the structure and function of a PCR primer.

Structure: A single strand of DNA about 20 nucleotides long

Function: Binds to a sequence of DNA flanking the target sequence to be amplified

4. Describe each of the three steps in one PCR cycle using non-scientific language.

a. The two DNA strands separate under high temperature (denaturation).
b. The primers stick to the separated DNA strands on either side of the target sequence (annealing).
c. The *Taq* molecule reads the target DNA sequence starting at the primer and builds a new DNA strand (extension).

Analyzing PCR and Lateral Flow Results Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions after closely reading the background material.

1. Will the fragment of plant DNA appear as the top or bottom band of the PCR product in the gel? Explain how you know.

The fragment of plant DNA will appear as the top band in the gel because it moves more slowly through the agar than the Bt gene fragment. This is because it is longer than the Bt fragment by 168 base pairs.

2. In each case (one band, two bands, or no bands), determine whether or not the food product contains Bt-corn. Fill in this information in the "What it means" column of the data table. Then provide your reasoning in the "How you know" column of the data table.

# Bands	What it means	How you know
1	The food product does NOT contain Bt-corn.	It is most likely the *rbcL* band, indicating the DNA was successfully amplified from the food product but Bt DNA was not present.
2	The food product DOES contain Bt-corn.	These are most likely the *rbcL* and *cry1Ab* bands, indicating the DNA was successfully amplified from the food product and Bt DNA was also present.
0	DNA was not successfully extracted and/or amplified from the food product—you cannot determine whether or not it contains Bt-corn.	If the ladder and/or other DNA samples are visible on the gel, then the DNA extraction and/or PCR were likely unsuccessful.

Continues next page >

Analyzing PCR and Lateral Flow Results Questions

ANSWER KEY **Do not share with students**

Continued

3. Do you think it is more likely that you will see a positive result for Bt-corn in the PCR or in the Lateral Flow? Explain your thinking.

Possible answers include:

— More likely to see a positive result in the lateral flow because it is detecting three endotoxin proteins whereas the PCR is detecting only one endotoxin-coding gene.

— More likely to see a positive result in the lateral flow because the DNA extraction process is long and complex, introducing more opportunity for error.

— More likely to see a positive result in the PCR because, if the food product contains heavily processed corn, the endotoxin proteins may be denatured in the processing.

— More likely to see a positive result in the PCR because it is a more sensitive test than lateral flow (it amplifies the DNA that is present to millions of copies vs. lateral flow which detects the proteins that are present in their existing amount).

Student Guide, Part 1: Pre-Lab

ANSWER KEY — **Do not share with students**

Directions

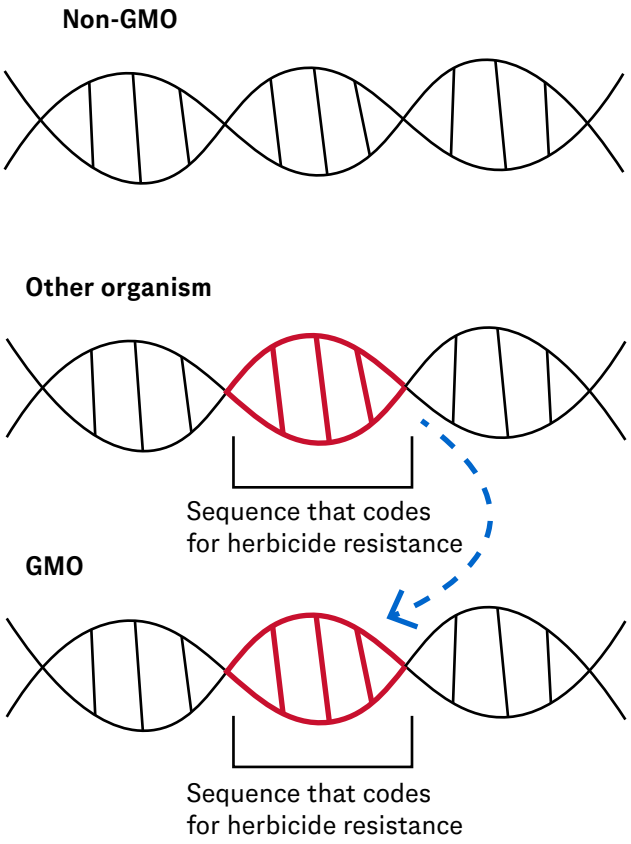
In this lab, you will play the role of a plant geneticist exploring the use of genetically engineered corn in food products. To begin, carefully examine the foods labeled "A" an "B" and answer the questions below.

1. Make a prediction for which food(s) (A, B, both, neither) contains a genetically engineered crop and record a reason for why you think that.

Prediction	Product A likely contains a genetically engineered crop as there is no labeling regarding whether or not it is organic (i.e., made with non-GE crops).
Reasoning	Any answer supported with appropriate rationale may be accepted.

2. In the space below, draw or describe what you think is happening inside a genetically engineered crop to explain how it is different from a standard crop.

Example



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Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Continued

3. Choose a food product you would like to test and make a prediction for whether or not your food product contains a GE crop.

Your food product	Answers vary.
Will it contain a GE crop?	Answers vary.
Reasoning	Any answer supported with appropriate rationale may be accepted.

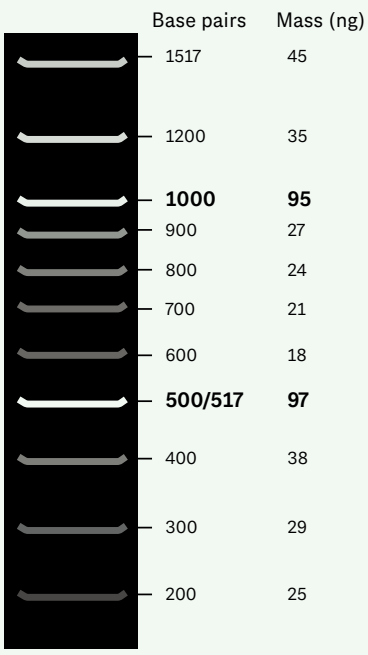
Student Guide, Part 2: Lab

ANSWER KEY — **Do not share with students**

Directions
Record your data below after completing the lab.

1. Draw or paste the picture of your gel below. Label each well and the DNA ladder (without writing directly on the picture).

The 100 bp ladder:
Lane 2 should be empty (negative PCR control).
Lane 3 should have two bands (positive PCR control).



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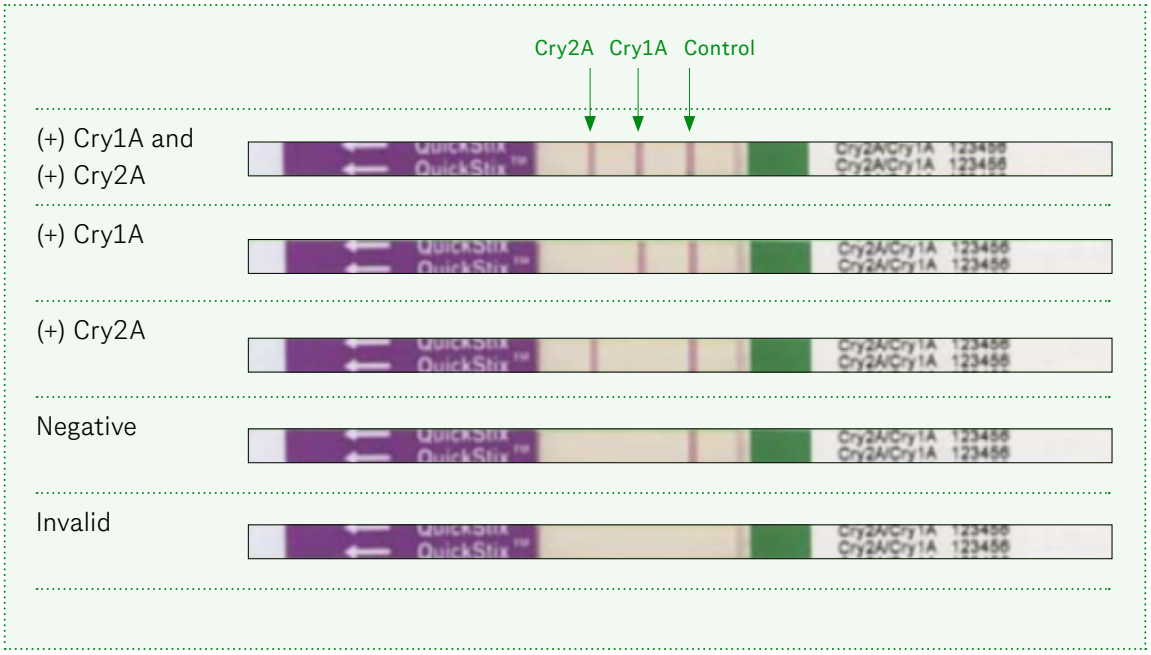
Student Guide, Part 2: Lab

ANSWER KEY **Do not share with students**

Continued

2. Draw or paste your lateral flow test strip below.
   Label each pink line (without writing directly on the strip).



Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Directions
Analyze your results from the lab by answering the questions below.

1. PCR Results: *(Example)*

   a. Were you able to successfully amplify DNA from your food product?

   Yes

   b. Does your food product contain the *cry1Ab* gene?

   Yes

   c. Explain how you know.
   The gel shows…which means…:

   The lane of the gel where I loaded my PCR reaction shows two bands—one around the 600 bp DNA fragment in the ladder and one in between 400 and 500 bp fragments in the ladder. This means the PCR reaction amplified the 599 bp segment of the *rbcL* gene found in all plants as well as the 431 bp segment of the *cry1Ab* gene found in Bt-corn.

2. Lateral Flow Results: *(Example)*

   a. Does your food product contain delta endotoxin proteins?

   Yes

   b. If yes, which protein(s)? (Cry1Ab/Cry1Ac and/or Cry2A)

   Cry1Ab/Cry1Ac and Cry2A

   c. Explain how you know.
   The gel shows…which means…:

   The strip shows three pink lines, which means both Cry1A and Cry2A delta endotoxin proteins were present in the sample. The third line is the control, meaning the strip is valid.

3. Use evidence from your PCR and lateral flow results to make a claim about whether or not your food product contains Bt-corn. Does this support or refute your prediction?

   Example:

   My food product contains Bt-corn because the PCR showed a positive result for the *cry1Ab* gene and the lateral flow showed a positive result for both Cry1A and Cry2A delta endotoxin proteins. This means there are likely more Bt genes in the corn used to make my food product than just *cry1Ab*. This supports my prediction because the corn chip I used did not have an "organic" label on the bag, meaning it likely contains genetically engineered crops.

Continues next page >

Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Continued

4. Collect class data to determine what proportion of food products contain the *cry1Ab* gene and delta endotoxin proteins. (Do not include data from unsuccessful DNA isolations in which no bands are present on the gel or data from invalid lateral flow test strips in which no control line was present.)

   Identify three to five patterns in the class data.

There is a higher/lower percent of food products containing the *cry1Ab* gene than delta endotoxin proteins.

Most food products tested contain/do not contain Bt-corn.

Food products of _____ type were more/less likely to contain Bt-corn.

Student Guide, Part 4: Constructing a Scientific Model

ANSWER KEY — **Do not share with students**

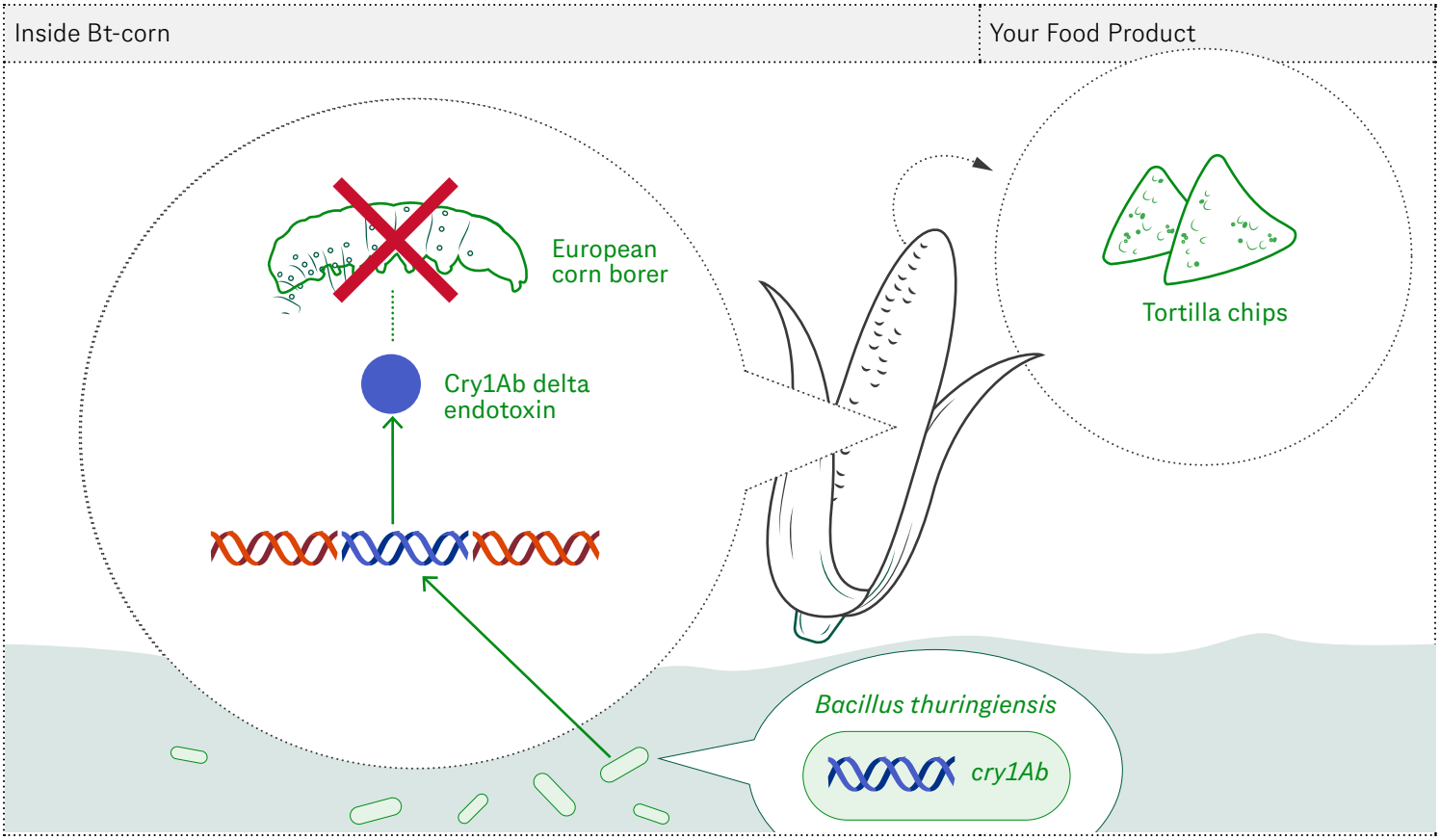
Directions

Review the scientific model you drew on Question #2 of the Student Guide, Part 1: Pre-Lab and consider what changes you could make based on what you have learned. Then, follow the steps below to create a new model.

1. A scientific model represents the observable (visible) and unobservable (invisible) features of a phenomenon. Draw a model that explains what occurs inside Bt-corn in a food product to make it different from standard corn.

   Fill in the adjacent table to help plan your model. Then, draw your model below (or on a new sheet of paper or a whiteboard).

Organism being engineered	corn
Name of new gene	*cry1Ab*
Protein new gene codes for	Cry1Ab delta endotoxin
Trait the new genes lead to	pest-resistance (causes cell lysis in insect gut leading to death)
Organism new gene came from	Bt (*Bacillus thuringiensis*)—soil bacteria



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Student Guide, Part 4: Constructing a Scientific Model

ANSWER KEY **Do not share with students**

Continued

2. After viewing other models and receiving feedback on your model, describe three limitations of your model.

   Consider the following questions:

   a. What parts of the system are not shown in the model?

   b. What is an assumption in your model and how does it impact the reliability of the model?

   c. What is estimated, rather than observed directly, in your model?

Examples of limitations include:

Precise molecular structures of DNA, RNA, and proteins and how these structures relate to their function

A sense of quantity and scale (e.g., How many copies of the gene are present in each corn cell? How many proteins are made from one gene? How do the sizes of the molecules compare?)

Specific location/time of gene expression (e.g., Is the new gene in every cell or only certain cells? Is it only "on" during certain stages of development?)

There are likely other genes added to the corn to make it pest-resistant—e.g., additional cry genes.

Cannot directly observe the processes of transcription and translation

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Thermal Cycler Grid

Directions
Write your initials in the box that matches where you placed your PCR tube in the thermal cycler. Fill in the middle spots of the thermal cycler first.

Teacher:

_____

Period:

_____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Background Reading: Bt-maize and Organic Crops

Humans have been farming for thousands of years and simultaneously, trying to control crop pests. Pesticides are substances that fight off or kill unwanted weeds and insects. According to the Environmental Protection Agency (EPA), over 35 billion dollars are spent on about 5 billion pounds of pesticides every year worldwide (Niederhuber, 2015). Despite the high use of pesticides, the Food and Agricultural Organization of the United Nations (FAO) states that farmers still lose 20–40% of their crops to pests and diseases (Niederhuber, 2015).

One particularly useful tool to control pests is naturally found in a soil bacterium called *Bacillus thuringiensis* (Bt). These bacteria produce proteins that are toxic to some insects but safe for humans and other mammals. One family of proteins produced by Bt are Cry proteins, which are a type of delta-endotoxin. When eaten by particular insects, they destroy the cells that line their gut, leading to death. An insect that is susceptible to delta endotoxins is the European corn borer, which often causes significant problems for maize (corn) crops.

Though Bt-derived pesticides have been used since the early 20th century, genetic engineering has recently allowed scientists to make crops that contain DNA from the *Bacillus thuringiensis* bacteria. The crops are modified at the embryo stage to include DNA that codes for delta-endotoxin proteins, including the *cry1Ab* gene. As a result, the crops make their own Cry proteins and are now toxic to pests, such as the corn borer, that would otherwise kill them. The first Bt-maize (corn) seeds became available in 1996 and, as of 2020, makeup 82% of US corn crop acreage (USDA, 2020).

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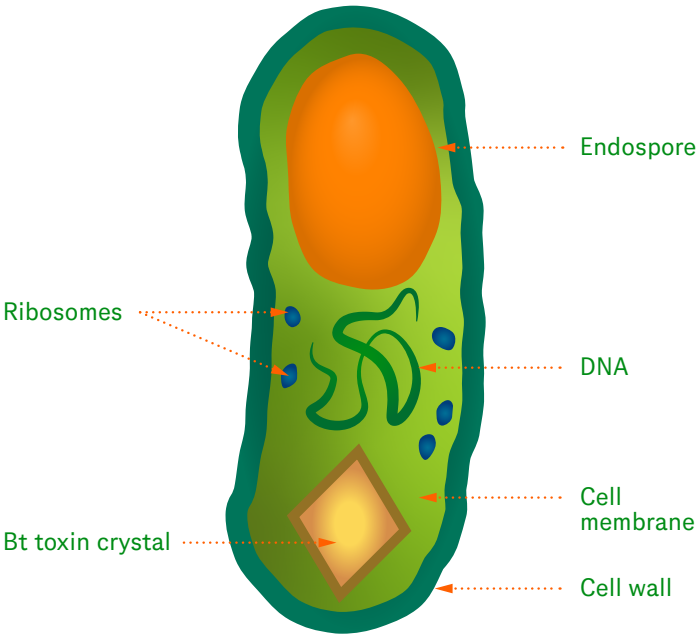
Bacillus thuringiensis (Bt)



Lepidopteran larvae eating non-GE corn

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Background Reading: Bt-maize and Organic Crops

Continued

What does *organic* mean?

For a food product to be labeled 'organic,' it must meet a set of requirements defined by the United States Department of Agriculture (USDA). Part of these standards stipulates that organic farmers cannot plant genetically engineered crops such as Bt-corn. However, since they are biologically derived, pesticides produced from the *Bacillus thuringiensis* bacteria are permitted. The requirements for a crop to be labeled 'organic' are:

- No prohibited substances, such as synthetic pesticides, are applied for at least 3 years to the land.
- Agricultural practices preserve natural resources and conserve biodiversity (i.e., crop rotation).
- Pests and diseases are controlled primarily through physical, mechanical, and biological controls.
- There is no use of genetic engineering.

The chart below shows the difference between organic labels. Any products labeled *100% Organic*, *Organic*, and *Made with Organic* cannot include genetically modified ingredients.

Sources

Bt Corn: Health and the Environment Extension

Delta Endotoxin—An Overview

Insecticidal Plants: The Tech and Safety of GM Bt Crops

Use and Impact of Bt Maize

Recent Trends in GE Adoption

About the Organic Standards

Organic Labels Explained

Organic products are labeled according to the percentage of organic ingredients they have. This chart shows what to expect from different labels.

Source: United States Department of Agriculture

100% Organic	Organic	Made with Organic	Organic Ingredients
✓ Organic seal allowed	✓ Organic seal allowed	X Organic seal NOT allowed; Must specify which ingredients are organic	X Organic seal NOT allowed; Product cannot be described as 'organic'
✓ 100% certified organic ingredients and processing aids	✓ 95% certified organic ingredients	✓ At least 70% certified organic ingredients	X No specific % certified organic
✓ No GMOs	✓ No GMOs	✓ No GMOs	X May contain GMOs
✓ All ingredients comply with National List of Allowed and Prohibited Substances	✓ Non-organic ingredients comply with National List	✓ Non-organic ingredients comply with National List	X Compliance with National List not required
✓ Certification required	✓ Certification required	✓ Certification required	X Certification NOT required

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Bt-maize Questions and Organic Crops Questions

Directions
Answer the questions below after closely reading the background material.

1. What is *Bacillus thuringiensis* (Bt)?

2. How do delta endotoxins or Cry proteins function?

3. Make a claim about how Bt-maize is different from organic corn. Use the Organic Label Explained image above as evidence to support your claim.

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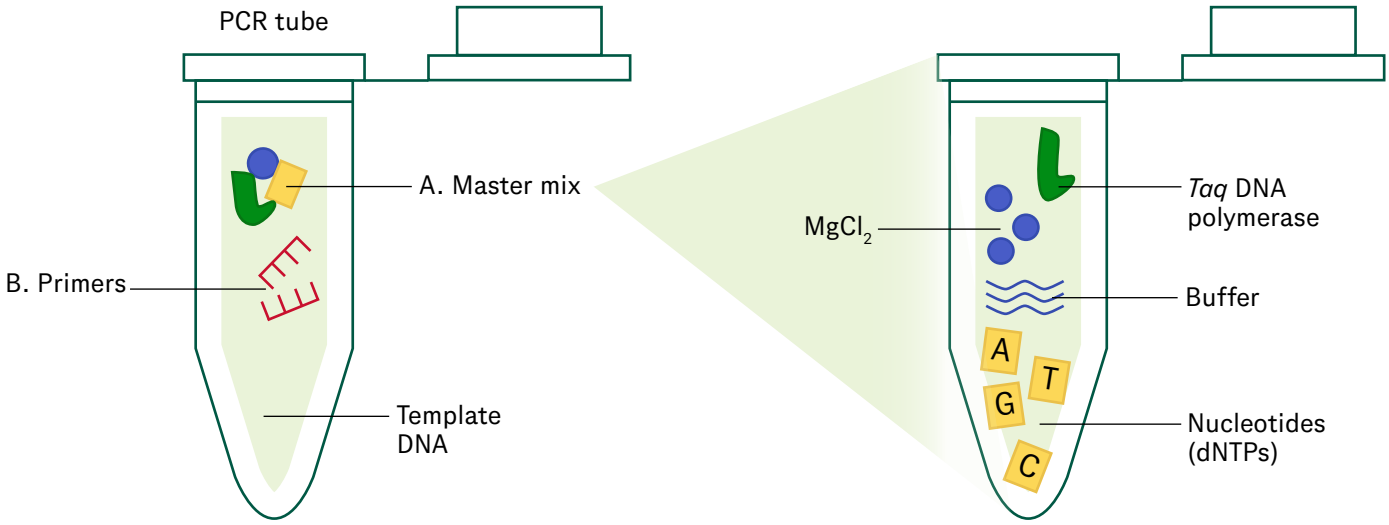
Background Reading:
What is Polymerase Chain Reaction (PCR)?

Polymerase Chain Reaction (PCR) is a technique used in biotechnology to make millions of copies of a small segment of DNA. Often, the starting amount of DNA in a sample is too small to be analyzed or used as it is. PCR can then be used to make a large enough amount of the particular DNA sequence of interest so that it can be studied. For example, PCR is used to detect whether someone is infected with SARS-CoV-2 (the virus that causes COVID-19). The amount of virus genetic material in the person's tissue sample is too small to be detected as is, so PCR is performed to amplify a segment of the virus' genome. This allows the lab to determine if the virus is present or absent in the patient sample.

Components of PCR

A. Master Mix



The new DNA molecules are built by a DNA polymerase that has been isolated from a bacteria called *Thermus aquaticus (Taq)*. This bacteria is a thermophile, meaning it thrives in extremely hot temperatures (it was discovered in hot springs in Yellowstone National Park). This means its polymerase remains functional at the high temperatures necessary for PCR.

The buffer keeps the pH in an optimal range and magnesium chloride increases enzyme activity for *Taq* polymerase. An equal mix of adenine, guanine, cystine, and thymine are also included in the reaction as building blocks for the new DNA molecules.

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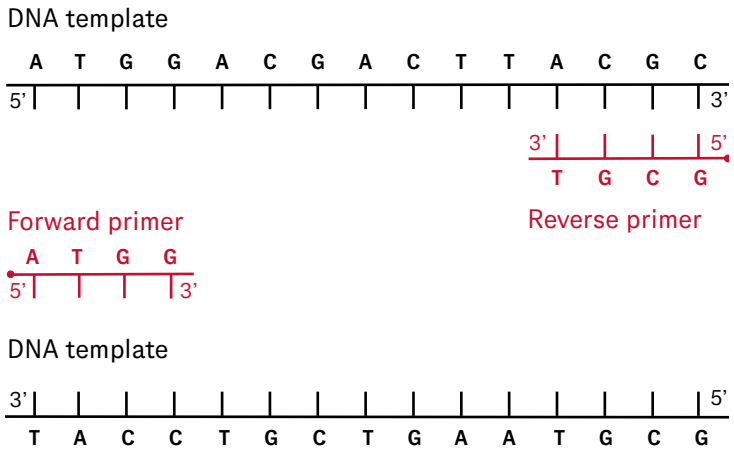
Background Reading: What is Polymerase Chain Reaction (PCR)?

Continued

B. Primers



Primers allow the PCR reaction to amplify (make lots of copies of) a very specific region of DNA. Each primer is a small single-stranded DNA (~20 nucleotides) that is complementary to the DNA sequence. One PCR reaction requires two primers (one forward and one reverse) so that only the DNA segment in which you are interested is copied. *Taq* DNA Polymerase binds to the primers and begins building a new DNA strand that is complementary to the template in the 5' to 3' direction.

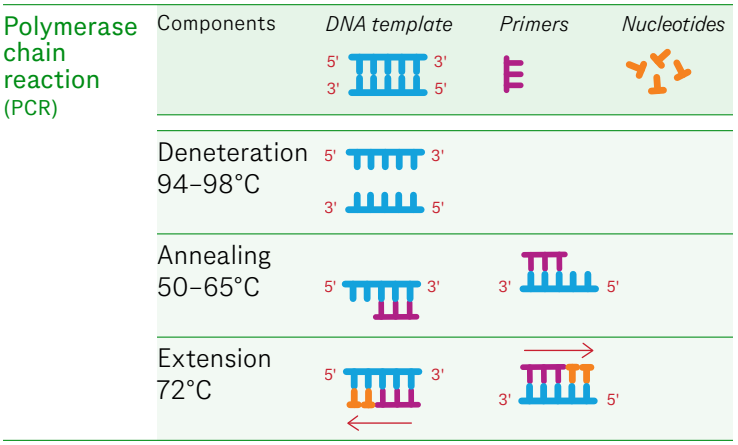
Sources

Polymerase Chain Reaction (PCR) Fact Sheet

Function of Taq DNA Polymerase in PCR

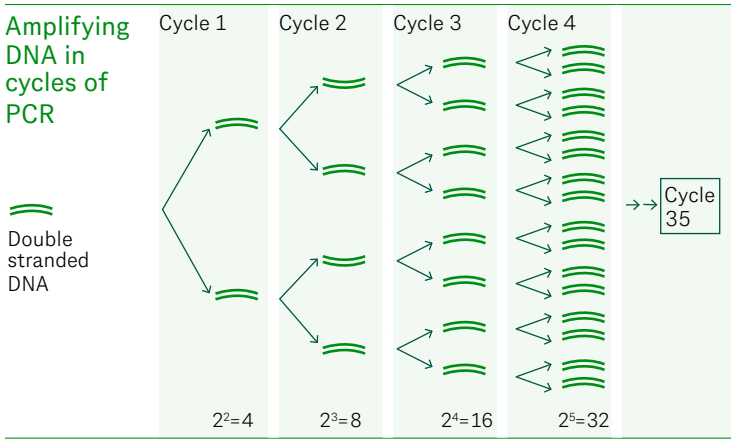
Polymerase Chain Reaction

Temperature Cycle



The first step of PCR is to heat the reaction to 95°C. This high temperature breaks the hydrogen bonds between the base pairs, allowing the primers and *Taq* polymerase access to the nucleotides. Therefore, it is called the *Denaturation* step. Next, the temperature is cooled to around 60°C, which causes the primers to bind to their complementary sequences in the DNA template. This process is called *Annealing*. After the primers are bound, the temperature rises to 72°C, which is the optimal temperature for *Taq* polymerase to build a new DNA molecule. The polymerase "reads" the target DNA sequence and adds nucleotides to the primer that are complementary to the sequence. This is the *Extension* step. When the cycle is complete, there are now two copies of the original DNA.

This process repeats to complete 25–40 total cycles, resulting in exponential growth in the number of copies of the original DNA template. At the end of the cycles, there is sufficient DNA to be analyzed.



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What is Polymerase Chain Reaction (PCR)? Questions

Directions
Answer the questions after closely reading the background material.

1. Why is PCR a useful tool for analyzing DNA?

2. What might happen if you do not include a buffer or magnesium chloride in your PCR master mix?

3. Describe the structure and function of a PCR primer.

4. Describe each of the three steps in one PCR cycle using non-scientific language.

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Background Reading: Analyzing PCR and Lateral Flow Results

To determine whether or not your food product contains Bt-corn, you will conduct two tests:

1. PCR to amplify the *cry1Ab* gene from Bt bacteria (*Bacillus thuringiensis*) and gel electrophoresis to visualize the results.
2. Lateral flow to detect the Cry1Ab delta endotoxin protein encoded by the *cry1Ab* gene.

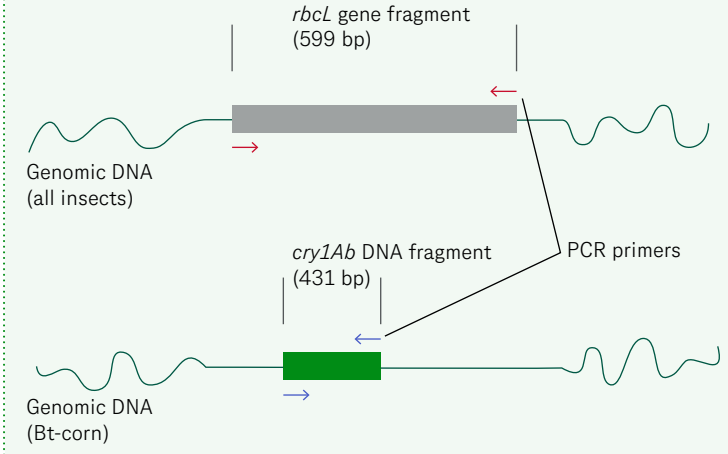
PCR Reactions

The primer mix you used when setting up your PCR contained two sets of primers. One set amplified the *rbcL* (RuBisCO) gene found in all plants. This gene codes for an enzyme that plays a role in the Calvin Cycle, which is essential to photosynthesis. If the DNA from your food product was isolated successfully, PCR amplification of this gene from the corn genome will produce a DNA fragment *599 bp* in length. This PCR reaction is used to verify that you had a successful DNA extraction and the fragment should be present in all samples, regardless of whether or not the food product contains Bt-corn. In other words, it serves as an internal control, or reference gene.

The second set of primers amplifies the gene *cry1Ab*, which is naturally found in Bt bacteria (*Bacillus thuringiensis*) and codes for a protein that, when ingested by insects, leads to cell lysis in their gut and eventually death. It has been added to Bt-corn to make it insect-resistant. Corn that has not been genetically engineered to include *cry1Ab* will not contain this gene. These primers will produce a DNA fragment *431 bp* in length.

Below is a diagram of the genes that we are amplifying through PCR.

PCR Amplification



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Background Reading:
Analyzing PCR and Lateral Flow Results

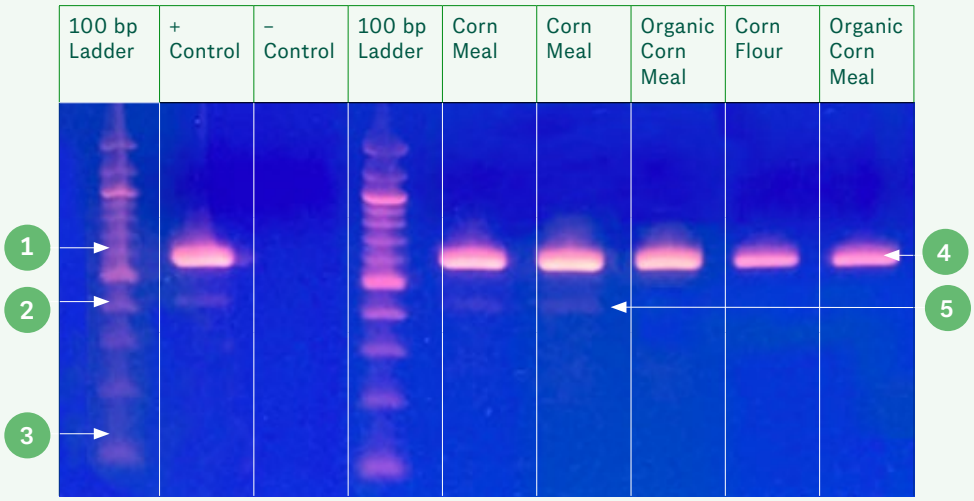
Continued

Gel Electrophoresis Results

Because DNA is negatively charged, it will migrate toward the positive electrode of a gel and shorter fragments of DNA move at a faster rate than longer ones. This means the *rbcL* gene fragment will migrate more slowly during electrophoresis and produce a band closer to the top of the gel. Because the *cry1Ab* DNA fragment is smaller, it will move more quickly through the gel and appear closer to the bottom. Therefore, if the food product contains Bt-corn, you will see two bands in the same lane on the gel—one at 599 bp and one at 431 bp.

By examining your agarose gel, you will determine 1) whether or not you successfully extracted and amplified DNA from your food product and 2) whether or not it contains Bt-corn. In the example below, lane 1 is the DNA ladder, lanes 2 and 3 are the positive and negative controls, lane 4 is another ladder, and lanes 5–9 are various corn-based products.

Gel Electrophoresis Results of *RuBisCo* and BT PCR



1	600 bp
2	400 bp
3	100 bp

4	*rbcL*, 599 bp
5	*cry1Ab*, 412 bp

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Background Reading: Analyzing PCR and Lateral Flow Results

Continued

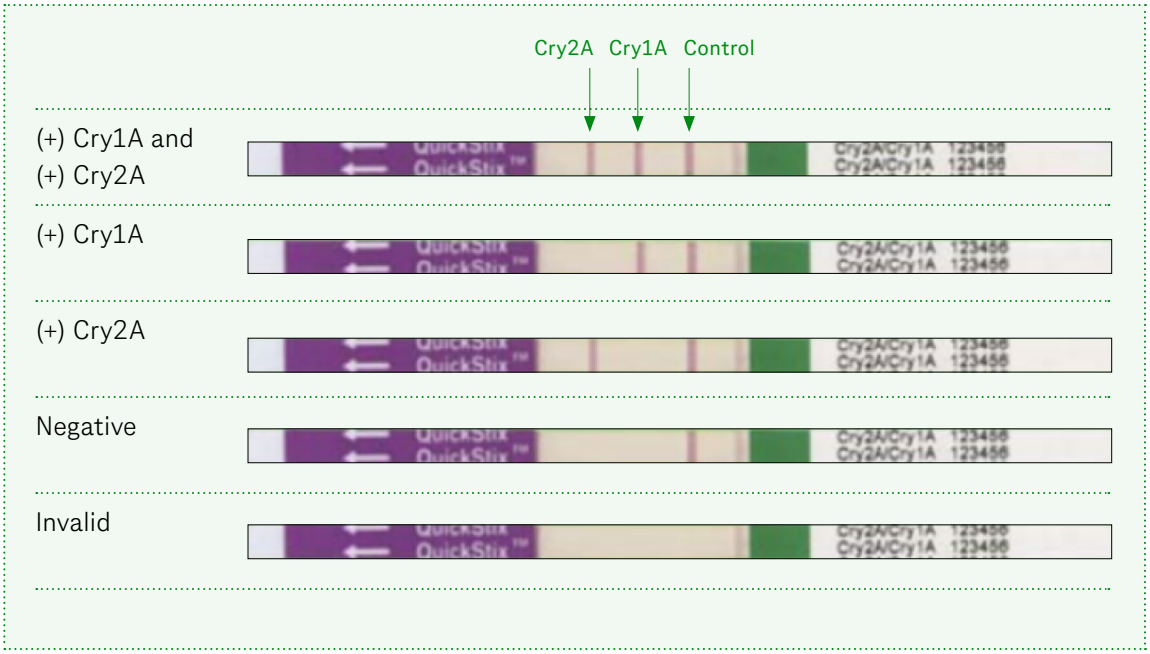
Lateral Flow

A lateral flow test can be used to quickly detect the presence of a particular protein in a sample. It uses similar principles to an ELISA, in that it contains an immobilized antibody to the protein of interest and causes a visible positive result, usually a pink line, if the applied liquid contains the protein. Pregnancy tests (which detect a pregnancy-specific hormone in urine) and COVID-19 rapid tests (which detect coronavirus proteins in blood or saliva) are examples of lateral flow tests.

You will use a lateral flow "strip" test (named because it uses strips that look similar to pH strips) to detect the presence or absence of Cry1Ab/Cry1Ac, and/or Cry2A delta endotoxin proteins. These proteins are encoded by the genes from Bt bacteria that have been introduced to Bt-corn (the *cry1Ab* gene you are detecting using PCR encodes the Cry1Ab protein). For each protein present in the sample, you will see a pink line. You will also see a pink "control" line if the strip is properly functioning. If one or more delta endotoxin proteins are present, this indicates that Bt-corn was used to make the food product.

The example below shows all possible results from the lateral flow test strips.

Lateral Flow Possible Results



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Analyzing PCR and Lateral Flow Results Questions

Directions
Answer the questions after closely reading the background material.

1. Will the fragment of plant DNA appear as the top or bottom band of the PCR product in the gel? Explain how you know.

2. In each case (one band, two bands, or no bands), determine whether or not the food product contains Bt-corn. Fill in this information in the "What it means" column of the data table. Then provide your reasoning in the "How you know" column of the data table.

# Bands	What it means	How you know
1		
2		
0		

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Analyzing PCR and Lateral Flow Results Questions

Continued

3. Do you think it is more likely that you will see a positive result for Bt-corn in the PCR or in the Lateral Flow? Explain your thinking.

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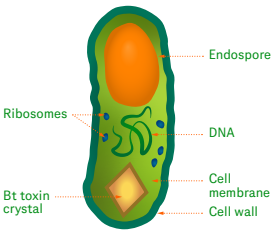
Vocabulary Tool

Directions
For each vocabulary word, write a new sentence that helps you practice using it.

Word	Image	Definition	Example Sentence	My Sentence
***Bacillus thuringiensis* (Bt)**		A type of bacteria that lives in the soil and produces proteins that are toxic to some insect species	*Bacillus thuringiensis* (Bt) has been exploited in farming for its pesticide properties for almost a century.	
GE crop *Genetically Engineered crop*		A crop whose genetic material has been changed using tools of biotechnology	Pest-resistant crops, such as Bt corn, are *GE crops*.	
Delta Endotoxin *Cry proteins*		Proteins produced by *Bacillus thuringiensis* bacteria that are toxic to insects	Bt-corn is pest-resistant because it makes *delta endotoxins*, which kill insects that eat it.	
Organic	USDA ORGANIC	Food produced with the use of feed or fertilizer of plant or animal origin without employment of chemically formulated fertilizers, genetic engineering, growth stimulants, antibiotics, or pesticides	The term *organic* refers to the process of how certain foods are produced.	

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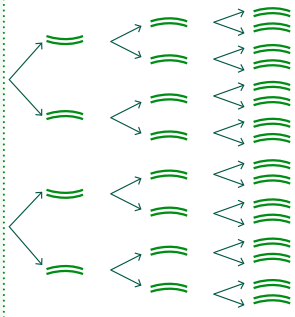
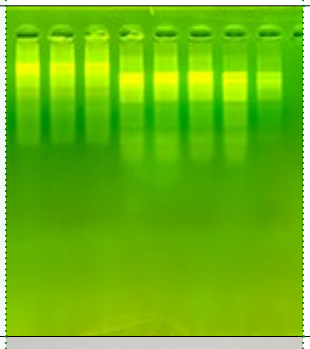
Vocabulary Tool

Continued

Word	Image	Definition	Example Sentence	My Sentence
PCR *Polymerase Chain Reaction*		A technique used in biotechnology to make millions of copies of a small segment of DNA	In order to detect Bt-corn in your food product, you will amplify its DNA using *PCR*.	
Agarose Gel Electrophoresis		A technique used to separate molecules based on mass and charge	After performing PCR, you will visualize the DNA using *gel electrophoresis*.	
Lateral Flow Test		A test that uses antibodies to detect the presence of a protein	The COVID-19 rapid test is a *lateral flow test* that detects coronavirus proteins in bodily fluids.	

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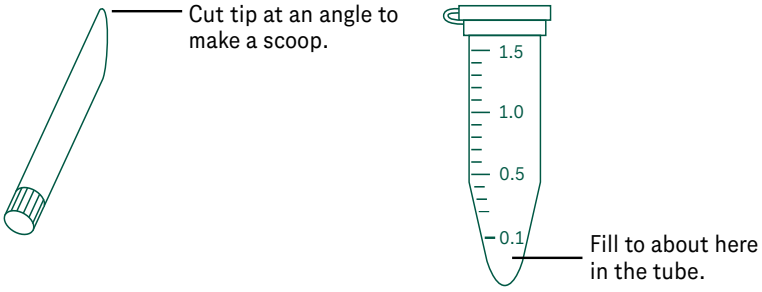
Student Protocol

Part 1: DNA Extraction and PCR

A		Lyse the Food Product
☐	1	Label a clean 1.5 mL microtube with your initials and group # on the lid and side of the tube. 
☐	2	Prepare your food product by finely crushing a very small amount in between two pieces of clean paper towel. — Use a small spatula or cut the end of a P1000 tip at an angle to make a scoop. — Scoop up a small amount of food product to the labeled tube—enough to fill up halfway to the 100 µL mark (~50 µL). 
☐	3	Add 200 µL of *Lysis Buffer* to the tube.

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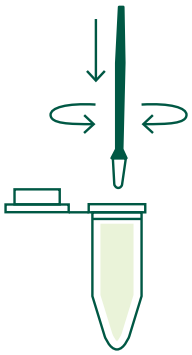
Student Protocol

Part 1: DNA Extraction and PCR

Continued

☐ 4 Use a micropestle to macerate the food product—twist down and rotate with force to *crush it as much as possible* (at least 1 minute). Avoid pushing down the micropestle too fast as this may cause the lysis buffer and possibly some food product to splash out of the tube.

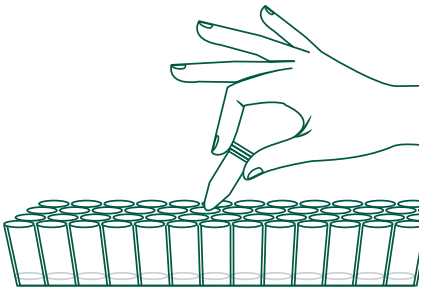
Note > *Periodically close and flick the tube to re-suspend the food product and make sure it is thoroughly crushed. (This helps break up the cell to release the DNA into the solution.)*



☐ 5 Add 800 µL of *Lysis Buffer* and close the lid tightly.

Mix by vortexing or "racking" (keep a finger on the top of the tube to prevent it from opening).

Note > *Press down firmly and drag the tube across the rack to mix the contents.*



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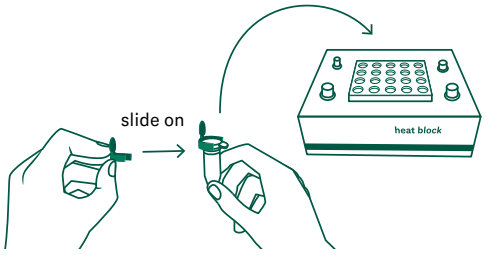
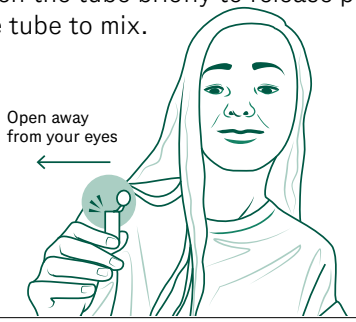
Student Protocol

Part 1: DNA Extraction and PCR

Continued

☐	6	Slide a cap lock onto your tube (opposite the tube hinge) to prevent it from popping open when heated. Place it in the 99˚C heat block or water bath for 5 minutes. 
☐	7	Open the tube briefly to release pressure, then close. Flick or "rack" the tube to mix. 
☐	8	In a centrifuge, spin your tube for 5 minutes at the highest speed, 12,000–14,000 rpm (at least 10,000 x g). Get your tube from the centrifuge and *carefully* place it in the rack without shaking it. You should see: — A pellet at the *bottom* of the tube (we do not want this) — Maybe an oily layer at the *top* of the tube (we do not want this)
☐	9	Get another clean 1.5 mL microtube and label it with your initials and group # on the lid.

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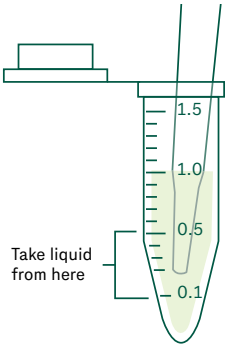
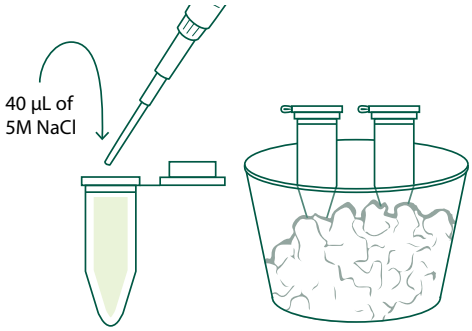
Student Protocol

Part 1: DNA Extraction and PCR

Continued

☐	10	Hold the tube you just centrifuged at eye level and use a P1000 to transfer about 400 µL of liquid *from the middle* of the food lysate tube to the clean tube you just labeled in Step 9. Be careful not to release the plunger of the micropipette when inserting the tip into the middle layer. Doing so may disturb/draw in some of the unwanted oily layer from the top. **Note >** *Do this without disturbing the pellet and without getting a lot of the oily layer. If you do disturb the pellet, re-centrifuge the sample.* 
B		Remove Impurities from the Sample
☐	11	Add 40 µL of 5M NaCl to the tube with the clear lysate (the result of breaking apart the food product). Shake the tube a few times to mix and incubate *on ice* for 5–10 minutes. The solution may become cloudy. 
☐	12	Place the tube with NaCl into a balanced microfuge and spin again for 5 minutes at the highest rpm. After spinning, *there may or may not be a noticeable pellet* at the bottom of the tube.

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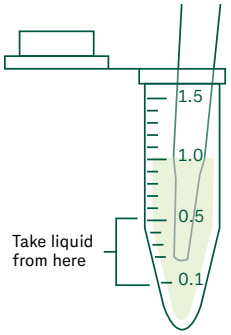
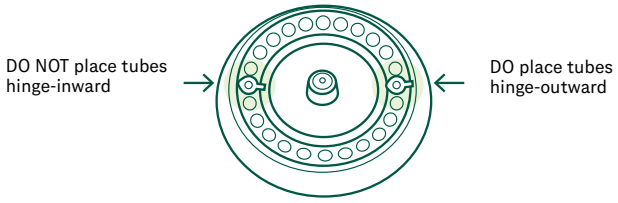
Student Protocol

Part 1: DNA Extraction and PCR

Continued

C		Isolate the DNA
☐	13	Get another clean 1.5 mL microtube. Label the tube with "DNA" and your initials and group number.
☐	14	Hold the tube at eye level and use a P1000 to transfer 600 µL of liquid from the *top* of the insect lysate/NaCl tube to the clean tube you just labeled "DNA." 
☐	15	Add 400 µL ice-cold isopropanol to your "DNA" tube. Mix contents by inverting your tube several times and then incubate on ice for at least 10 minutes or store in the freezer.

Note > *Potential Stopping Point: If there is not enough time to complete the DNA isolation, store samples in the freezer for up to 1 week.*

☐	16	Centrifuge the tube at top speed (12,000 x g) for 5 minutes. **VERY IMPORTANT >** *Orient the hinge of the tube to point outward and away from the middle of the microfuge. Nucleic acids (DNA) will pellet at the bottom-side of the tube under the hinge.* 

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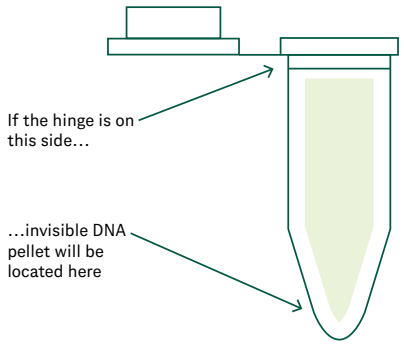
Student Protocol

Part 1: DNA Extraction and PCR

Continued

☐	17	Carefully pour the liquid out of the tube (we only want the pellet) and tap the mouth of the tube hard, onto a clean paper towel to remove the liquid on the lip of the tube. The pellet should be stuck to the bottom of the tube as a teardrop-shaped mark or may appear as minute speckles on the hinge-side of the tube. *Do not worry if there is no visible pellet.* 
☐	18	Add 400 µL of 70% ethanol to the tube and gently swirl the tube to wash the pellet. *Do not disturb the pellet.*
☐	19	*Optional (if pellet was disturbed):* Place the tube into a balanced microfuge with the hinge pointing out and spin at highest rpm for 5 minutes.
☐	20	Pour off the ethanol into a waste beaker. Tap the mouth of the tube hard onto a clean paper towel to remove the liquid on the lip of the tube.
☐	21	Open the cap and air-dry the pellet for about 5–10 minutes to evaporate all remaining isopropanol (residual alcohol will interfere with the PCR reaction). **Note >** *To speed up the evaporation process, place tubes on a heat block set at about 50-70°C. Keep caps open and monitor for evaporation. If most of the liquid has been removed from the tubes beforehand, this should take less than 5 minutes.*

Continues on next page >

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Student Protocol

Part 1: DNA Extraction and PCR

Continued

☐	22	Add 100 µL of TE/RNase buffer to your tube. Scrape the side of the tube where the pellet is (or should be) with the micropipette tip to facilitate re-suspension. Pipette up and down gently to collect DNA accumulated on the area underneath the hinge of the tube.
☐	23	Centrifuge the tubes for 1 minute to pellet any particulates that did not dissolve in solution. This is your isolated DNA!

Note > *Potential Stopping Point: If there is not enough time to set up the PCR, store samples in the freezer.*

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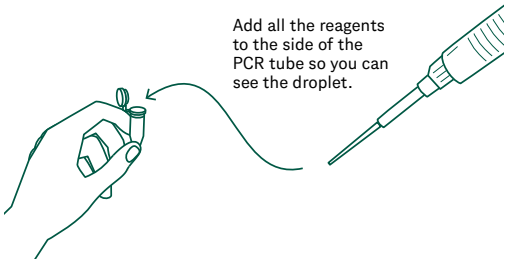
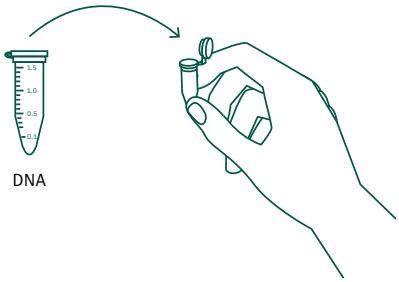
Student Protocol

Part 1: DNA Extraction and PCR

Continued

D	Set Up the PCR
☐ 24	Label a PCR tube with your initials on the side and top.
☐ 25	Pipette 20 µL of Master Mix into the PCR tube. 
☐ 26	Add 20 µL of Primer Mix into the PCR tube (use a new tip).
☐ 27	Add 10 µL of your extracted DNA into your PCR tube (use a new tip). Your final volume should be 50 µL. 
☐ 28	Make sure the cap is closed tight and *flick* the tube gently to *mix* the contents. Then *fling* the tube to move the liquid to the bottom. **Note >** *To fling: Hold the top of the tube firmly between your fingers and fling the tube in a wide downward arc motion with force.*

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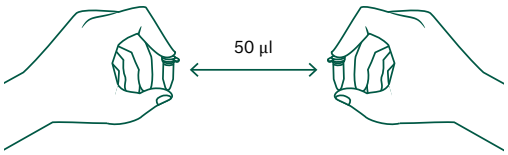
Student Protocol

Part 1: DNA Extraction and PCR

Continued

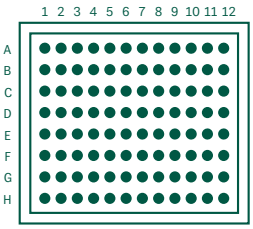
☐ 29 Compare the volume of your PCR tube with a reference PCR tube that has 50 µL in it.

Note > *If the volume of your tube does not match, set up a new PCR reaction.*



☐ 30 Place your PCR tube into the thermal cycler. Make sure to record the location of your tube on the Thermal Cycler Grid provided by your teacher (fill in the center first).

	Tube Location
1	Record your PCR tube location! Example: A11, A12



	PCR Thermal Cycler Parameters
1	95°C hold for 10 minutes
2	30 cycles of:
	95°C hold for 30 seconds
	60°C hold for 45 seconds
	72°C hold for 1 minute
3	72°C hold for 2 minutes
4	4°C hold for infinity

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Student Protocol
Part 2: Gel Electrophoresis

A		Prepare Agarose Gel
☐	1	Make sure you have all the materials needed to run your DNA on an agarose gel.
☐	2	Add 5 µL of loading dye to your 50 µL PCR reaction.
☐	3	Gently flick the tube to mix.
☐	4	Obtain a 2% agarose gel (teacher, see *Lab Preparation* for options on pouring gels). Make sure the gel is: a. Placed into the gel box with the wells oriented toward the negative (black) electrode. b. Covered in enough 1X TAE buffer to just cover the gel entirely.
☐	5	Use the P20 micropipette to load 15 µL of each sample into each of the following wells taking care not to puncture the gel. Make sure you load the gel from left to right with wells at the top of the gel box. a. Well 1 (far left): 100 bp ladder b. Well 2: Negative PCR control (water template) c. Well 3: Positive PCR control (*rbcL* and *cry1Ab*) d. Wells 4–7: Student PCR samples
B		Electrophoresis
☐	6	Plug your gel electrophoresis system into the power supply, cover with the lid, and run the gel at 150 volts for 10–20 minutes. **Note** > *Option to run on a lower voltage for a longer time to see better separation between bands. However, this may result in the DNA bands appearing dimmer.*
☐	7	Check that the gel is running by looking for small bubbles streaming off the electrodes.
☐	8	Turn the power supply off and take a picture of the gel through the hood (Minione equipment) or carefully remove the gel from the tray and visualize it on a UV transilluminator.
☐	9	Clean up by placing gel along with used tubes and tips in the trash. Gently rinse the gel electrophoresis system with water and air dry.

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Student Protocol
Part 3: Lateral Flow

A		Extract Proteins
☐	1	Label a 1.5 mL tube with your initials and group #.
☐	2	Prepare your food product by finely crushing a very small amount in between two pieces of clean paper towel. — Use a small spatula or cut the end of a P1000 tip at an angle to make a scoop. — Scoop up a small amount of food product to the labeled tube—enough to fill up halfway to the 100 µL mark (~50 µL).
☐	3	Add 200 µL of Lysis Buffer to the tube.
☐	4	Let the tube sit for 5–10 minutes. Occasionally, gently flick the tube to mix.
☐	5	Using a micropestle, crush the corn product in the Lysis Buffer.
☐	6	Add an additional 200 µL of Lysis Buffer.
☐	7	Let the tube sit for 1 minute.
☐	8	Label another 1.5 mL tube with your initials, group #, and “protein.”
☐	9	Transfer 200 µL of the supernatant (liquid at the top of the tube) to the new tube.
B		Lateral Flow Strip Test
☐	10	Dip the end of the lateral flow strip into the tube. Make sure the strips are oriented correctly with the arrow pointing down.
☐	11	Let the strip sit in the tube for 1–2 minutes. You will see the liquid sample travel up the strip. Make sure the sample travels all the way up to the top.
☐	12	Allow 10 minutes for the lateral flow strips to fully develop with results. You have the option to leave the strips to develop while in the tube or remove the strips and place on a piece of paper/paper towel to develop.

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Student Guide, Part 1: Pre-Lab

Directions

In this lab, you will play the role of a plant geneticist exploring the use of genetically engineered corn in food products. To begin, carefully examine the foods labeled "A" an "B" and answer the questions below.

1. Make a prediction for which food(s) (A, B, both, neither) contains a genetically engineered crop and record a reason for why you think that.

Prediction	
Reasoning	

2. In the space below, draw or describe what you think is happening inside a genetically engineered crop to explain how it is different from a standard crop.

Continues next page >

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Student Guide, Part 1: Pre-Lab

Continued

3. Choose a food product you would like to test and make a prediction for whether or not your food product contains a GE crop.

Your food product	
Will it contain a GE crop?	
Reasoning	

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Student Guide, Part 2: Lab

Directions
Record your data below after completing the lab.

1. Draw or paste the picture of your gel below.
   Label each well and the DNA ladder (without writing directly on the picture).

Continues next page >

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Student Guide, Part 2: Lab

Continued

2. Draw or paste your lateral flow test strip below.
   Label each pink line (without writing directly on the strip).

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Student Guide, Part 3: Data Analysis

Directions
Analyze your results from the lab by answering the questions below.

1. PCR Results:

   a. Were you able to successfully amplify DNA from your food product?

   b. Does your food product contain the *cry1Ab* gene?

   c. Explain how you know.
   The gel shows…which means…:

2. Lateral Flow Results:

   a. Does your food product contain delta endotoxin proteins?

   b. If yes, which protein(s)?
   (Cry1Ab/Cry1Ac and/or Cry2A)

   c. Explain how you know.
   The strip shows…which means…:

3. Use evidence from your PCR and lateral flow results to make a claim about whether or not your food product contains Bt-corn. Does this support or refute your prediction?

Continues next page >

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Student Guide, Part 3: Data Analysis

Continued

4. Collect class data to determine what proportion of food products contain the *cry1Ab* gene and delta endotoxin proteins. (Do not include data from unsuccessful DNA isolations in which no bands are present on the gel or data from invalid lateral flow test strips in which no control line was present.)

   Identify three to five patterns in the class data.

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Student Guide, Part 4: Constructing a Scientific Model

Directions

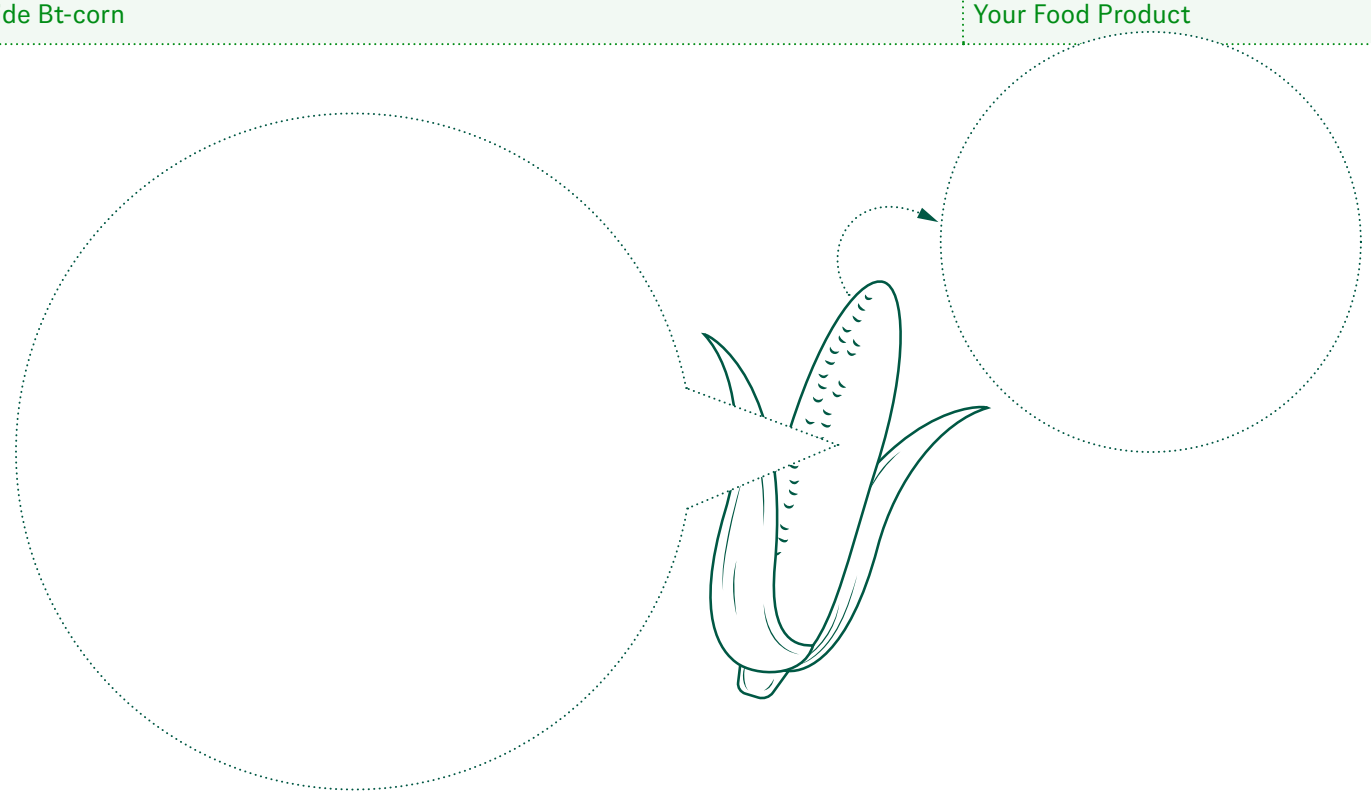
Review the scientific model you drew on Question #2 of the Student Guide, Part 1: Pre-Lab and consider what changes you could make based on what you have learned. Then, follow the steps below to create a new model.

1. A scientific model represents the observable (visible) and unobservable (invisible) features of a phenomenon. Draw a model that explains what occurs inside Bt-corn in a food product to make it different from standard corn.

   Fill in the adjacent table to help plan your model. Then, draw your model below (or on a new sheet of paper or a whiteboard).

Organism being engineered	
Name of new gene	
Protein new gene codes for	
Trait the new genes lead to	
Organism new gene came from	

Inside Bt-corn	Your Food Product



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FUTURELAB+

Student Guide, Part 4: Constructing a Scientific Model

Continued

2. After viewing other models and receiving feedback on your model, describe three limitations of your model.

   Consider the following questions:

   a. What parts of the system are not shown in the model?

   b. What is an assumption in your model and how does it impact the reliability of the model?

   c. What is estimated, rather than observed directly, in your model?

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Constructing a Scientific Model Rubric

Score	4	3	2	1
Content	Develops a model that accurately shows all *Key Features* of a phenomenon or process and their relationships.	Develops a model that accurately shows most key features of a phenomenon or process and their relationships.	Develops a model that shows some key features of a phenomenon or process and their relationships.	Develops a model that shows few features of a phenomenon or process and attempts to show relationships.
Presentation	Model is clear, organized, and concise.	Model is clear and organized.	Model is somewhat organized and somewhat clear.	Model is unclear and disorganized.
Limitations	Clearly and thoroughly describes at least three limitations of the model.	Describes three limitations of the model.	Describes more than one limitation of the model.	Describes one limitation or irrelevant limitations of the model.
Final Score				

Key Features

DNA containing *cry1Ab* gene

Protein the gene codes for

Relationship between the DNA the protein

Relationship between the protein and its trait/function

Origin of the new gene