Food Safety Lab

Food Truck Trouble!

Food Safety Lab:
Food Truck Trouble!
KT-1001-04

Version: 5.0
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At a glance

Lab overview

An outbreak of foodborne illness has been tracked to a food truck festival. Use DNA analysis to determine the source of the contaminated food.
Disclaimer: **None of the materials provided in this lab kit pose a pathogenic risk.** All DNA samples are synthetic and do not pose risks to human health

TECHNIQUES

Micropipetting
PCR
Restriction digestion
Gel electrophoresis

TOPICS

Microbiology
Infectious disease
Molecular diagnostics

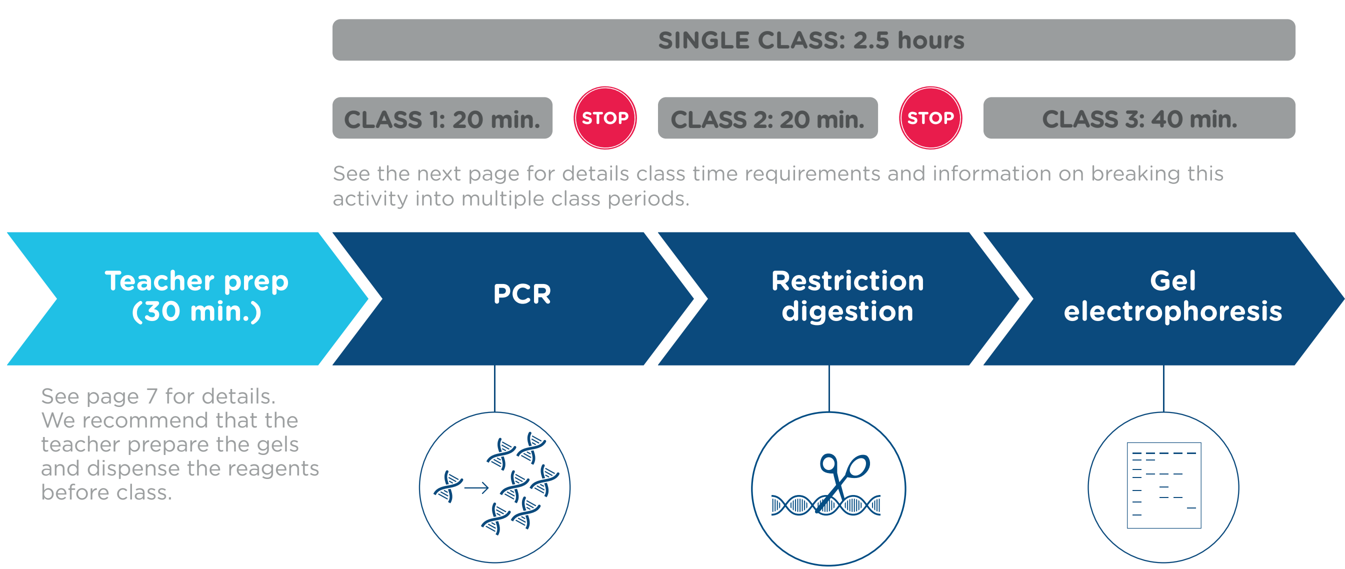
LEVEL

General high school
Advanced high school
College

Required lab skills

- Students must be proficient in accurately pipetting liquids in the 2-20 µl range.
- Instructional videos, worksheets, and free activities to help students build micropipetting skills can be found at https://www.minipcr.com/micropipetting/

Planning your time



Technical support

If you have any questions about implementing this activity, contact support@minipcr.com.

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Class time requirements

Steps	Time required
1. PCR	
A. Set up PCR samples and start the PCR program	20 minutes
B. Run PCR	45 minutes The PCR program can be started during class and left to run without being monitored.
STOP Optional stopping point: The PCR product is stable at room temperature for several days. For longer-term storage, place it in the freezer.	
2. Restriction digestion	
A. Set up reactions	10 minutes
B. 37 °C incubation	Recommended: overnight incubation
Note: This step can be shortened to a minimum of 15 minutes. Shorter incubations may lead to an incomplete digest, but the experimental results can still be interpreted conclusively. See page 37 for more information.	
STOP Optional stopping point: You can freeze the restriction reactions and run them on a gel at a later time.	
3. Gel electrophoresis	
Prep: Make gels	We recommend the teacher prepare the gels outside of class (see page 7). Allot 30 minutes of class time if you opt to have students prepare the gels.
A. Load gel	10 minutes
B. Run gel	15-25 minutes The gel does not need to be actively monitored during this time.
C. Interpret results	5 minutes

Materials needed

Supplied in kit (KT-1001-04)

- All components should be kept frozen for long-term storage.
- Reagents must be used within 12 months of shipment.
- Kit contains reagents for eight lab groups.
- Reagents for preparing gels, plastic tubes for distributing reagents to individual groups, plastic tubes for PCR, and pipette tips are sold separately. See below for details.

Contents	Provided	Required per group	Storage
Simulated DNA samples • Non-pathogenic *E. coli* Control DNA • Pathogenic *E. coli* Control DNA • Lettuce Sample DNA • Tomato Sample DNA	100 µl each	10 µl each per group	Freezer
2X EZ PCR Master Mix, Load Ready™	700 µl	75 µl per group	Freezer
Food Safety Lab Primer Mix	480 µl	50 µl per group	Freezer
Restriction Enzyme XmnI	50 µl	50 µl for an entire class	Freezer
Nuclease Free Water	100 µl	50 µl for an entire class	Freezer
Fast DNA Ladder 2	150 µl	15 µl per group	Freezer

Electrophoresis reagents and plastics sold separately

- This lab requires 2% agarose gels with a fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®).
- This lab requires plastic tubes for distributing reagents to individual groups and 0.2 ml PCR tubes for running PCR.
- The Learning Lab Companion Kit (KT-1510-01) provides appropriate reagents to make and run eight gels when using the blueGel™ or Bandit™ electrophoresis systems, as well as plastic tubes for distributing reagents to individual groups and plastic tubes for PCR.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

Required equipment

- This lab is compatible with any thermal cycler.
- This lab is compatible with any horizontal gel electrophoresis system in combination with:
  - A fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®)
  - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines equipment from miniPCR bio that meets these requirements:

AVAILABLE AT MINIPCR.COM

Item	Recommended quantity
miniPCR thermal cycler	Each group will have 4 PCR samples Groups can share machines
Gel electrophoresis and visualization system Option 1: blueGel™ OR GELATO™ electrophoresis systems with integrated blue light transilluminator	1 blueGel can be shared by two groups 1 GELATO can be shared by four groups
Option 2: Bandit™ STEM Electrophoresis Kit paired with the Viewit™ Illumination Kit	1 Bandit + 1 Viewit per group
Option 3: Bandit™ STEM Electrophoresis Kit paired with a blueBox™ blue light transilluminator	1 Bandit per group + 1 blueBox for the class to share
Micropipettes and tips 2-20 µl adjustable micropipette 20-200 µl adjustable micropipette	 1 pipette per group 1 pipette for teacher prep

Other materials supplied by user

- Distilled water
- Microwave or hot plate
- Heat-resistant flask or beaker
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

Teacher prep

Protective gloves and eyewear should be worn for the entirety of this experiment.

Overview

The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

Prep	Time required	Timeline
Dispense reagents	10 minutes	Can be completed up to one week before performing PCR
Dilute restriction enzyme	5 minutes	Can be completed up to one week before use
Prepare electrophoresis buffer and agarose gels	20 minutes	Varies - If using gel reagents from miniPCR, gels can be prepared and stored for up to five days before performing gel electrophoresis

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Dispense reagents

- Reagents (except for the restriction enzyme) can be dispensed up to one week in advance and stored in the refrigerator until use.
- The lab kit provides sufficient reagents for eight lab groups.

Materials needed

From the lab kit (stored in the freezer):

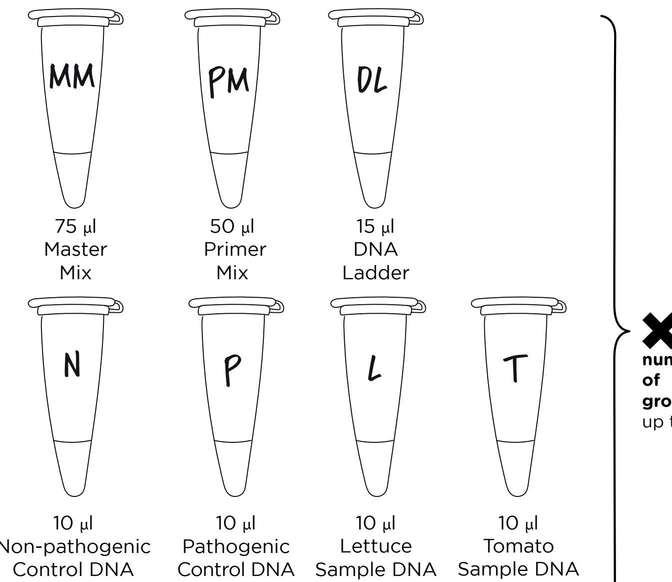
- 2X EZ PCR Master Mix
- Food Safety Lab Primer Mix
- Fast DNA Ladder 2
- Non-pathogenic Control DNA
- Pathogenic Control DNA
- Lettuce Sample DNA
- Tomato Sample DNA

Supplied by user:

- Plastic tubes for dispensing reagents (1.5 ml or 0.2 ml tubes can be used)
- 2-20 µl and 20-200 µl micropipettes and tips
- Fine-tipped permanent marker

Note: Leave the restriction enzyme XmnI in the freezer

1. Thaw reagents by placing tubes at room temperature.
2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.
3. When you open each tube, check for liquid stuck inside the lid. If necessary, put the lid back on and repeat step 2.
4. For each lab group, dispense the following reagents into labeled plastic tubes. 1.5 ml or 0.2 ml plastic tubes can be used.
   - 2X EZ PCR Master Mix (label tube as "MM") 75 µl
   - Food Safety Lab Primer Mix (tube PM) 50 µl
   - Fast DNA Ladder 2 (tube DL) 15 µl
   - Non-pathogenic Control DNA (tube N) 10 µl
   - Pathogenic Control DNA (tube P) 10 µl
   - Lettuce Sample DNA (tube L) 10 µl
   - Tomato Sample DNA (tube T) 10 µl
5. If you are dispensing the reagents more than 24 hours before class, store the tubes in the refrigerator until use. Dispensed reagents can be stored in the refrigerator for up to one week.



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Dilute restriction enzyme

- Because of the small volumes involved, we recommend that the teacher add the restriction enzyme directly to each student's sample.
- We recommend diluting the restriction enzyme to avoid the need to pipette 1 µl volumes. When diluted, 2 µl of restriction enzyme will be added to each sample.
- Diluted restriction enzyme can be stored in the freezer for up to seven days before use.
- If you have access to a 0.5-10 µl micropipette and you are very experienced with pipetting 1 µl volumes, you can skip this step and add 1 µl of undiluted restriction enzyme to each sample in Part 2 of the experiment.

Materials needed

From the lab kit (stored in the freezer):

- Restriction Enzyme XmnI
- Nuclease Free Water

Supplied by user:

- 20-200 µl micropipettes and tips
- Recommended: microcentrifuge

1. Remove the tube of Restriction Enzyme XmnI from the freezer. The solution remains liquid in the freezer, so there is no need to thaw the tube.

2. Do NOT uncap the tube of restriction enzyme until you collect the liquid at the bottom of the tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.

3. Carefully open the Restriction Enzyme tube, making sure no liquid remains trapped inside the screw-top cap. Repeat step 2 if necessary to collect all the liquid in the bottom of the tube.

4. Add 50 µl of Nuclease Free Water to the tube of restriction enzyme.

5. Pipette up and down 10 times to mix very well.

6. If diluting the restriction enzyme the day your students will use it, place the tube on ice until use. If diluting the restriction enzyme in advance, you can store the tube in the freezer for up to seven days before use.

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Prepare gel electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - Follow the manufacturer's instructions to prepare buffer solution.
   - The volume of buffer needed varies depending on the gel electrophoresis system.
     - For the blueGel and Bandit electrophoresis systems, 600 ml of TBE buffer is sufficient for at least eight gel runs.
     - For other systems, refer to the manufacturer's instructions for:
       (1) The buffer volume needed to prepare agarose gels.
       (2) The buffer volume needed for use as a running buffer.

2. Prepare 2% agarose gels with fluorescent DNA stain.
   - You will need four lanes plus one lane for DNA ladder per group. If groups are sharing gels, a single lane for ladder per gel is sufficient.
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using. Refer to the manufacturer's instructions.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

Detailed instructions for preparing buffer and gels for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/gelpouring

Bandit
https://links.minipcr.com/BanditDNAgel

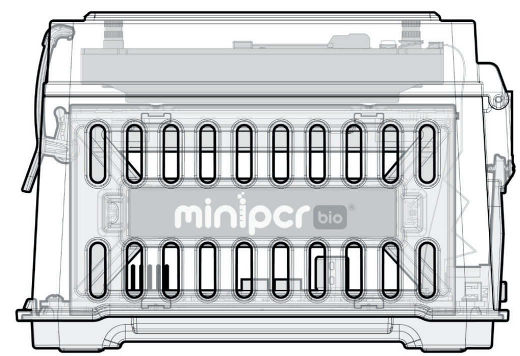
Student workstation setup

Part 1: PCR

At the start of this experiment, every lab group should have:	
2X EZ PCR Master Mix, Load Ready™ (tube MM)	75 µl
Food Safety Lab Primer Mix (tube PM)	50 µl
Simulated DNA samples • Non-pathogenic Control DNA (tube N) • Pathogenic Control DNA (tube P) • Lettuce Sample DNA (tube L) • Tomato Sample DNA (tube T)	10 µl each
0.2 ml PCR tubes	4
2-20 µl micropipette and tips	
Space in a thermal cycler for 4 samples	

If using miniPCR thermal cyclers:

- Groups will need a miniPCR thermal cycler and power supply.
- Download the miniPCR app from the app store or at www.minipcr.com/downloads.
- Machines can be programmed ahead of time by the teacher or during class by the students.
- If you want to monitor the reaction in real time during the run, groups will need their miniPCR thermal cycler to remain connected to a computer or a compatible phone or tablet.



Detailed instructions for using a miniPCR thermal cycler

https://links.minipcr.com/minipcrRUN

Part 2: Restriction digestion

At the start of this experiment, every lab group should have:	
PCR samples from the previous step	4
0.2 ml PCR tubes	4
Access to the diluted restriction enzyme XmnI (See page 9 for dilution instructions) We recommend that the teacher add restriction enzyme directly to the samples for the students	
2-20 µl micropipette and tips	
Access to a miniPCR thermocycler or other 37 °C heat source	

Part 3: Gel electrophoresis

At the start of this experiment, every lab group should have:	
Restriction digestion samples from the previous step	4
Fast DNA Ladder 2 (tube DL)	15 µl
Electrophoresis buffer *Volume depends on your electrophoresis system	30 ml TBE if using a blueGel or Bandit
2-20 µl micropipette and tips	
5 wells in a 2% agarose gel with fluorescent DNA stain	

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Student guide

Background information

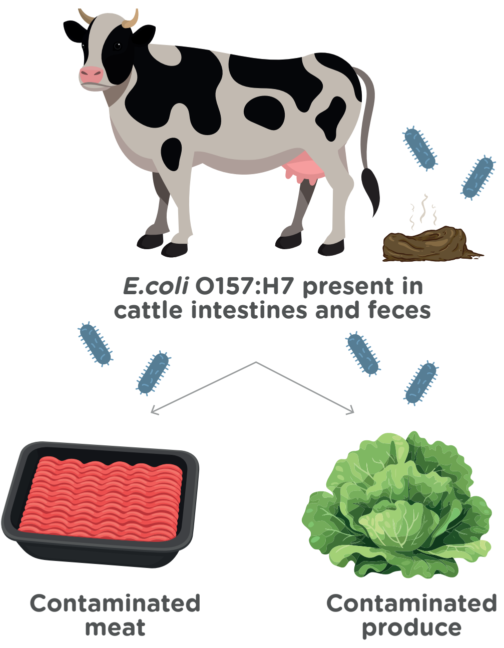
Foodborne illness

1

- Each year in the United States, 48 million people are sickened by **foodborne illness**, or food poisoning, after consuming tainted foods or beverages.
- The most common cause of foodborne illness is contamination with **pathogenic bacteria**.
- This activity will focus on ***E. coli* O157:H7**, a specific **strain** of bacteria that causes foodborne illness in humans.

2

- *E. coli* O157:H7 normally lives in the intestines of healthy livestock like cattle.
- Initial food contamination typically occurs through contact with cattle feces.



3

- Meat most often becomes contaminated with fecal bacteria during ground meat production.
- Cooking foods to safe temperatures can limit or eliminate bacterial growth in meat products.

4

- Crops can be exposed to feces through improperly processed manure-based fertilizer or tainted irrigation water.
- Thoroughly washing fresh produce is important to help rinse away pathogens that may be present.

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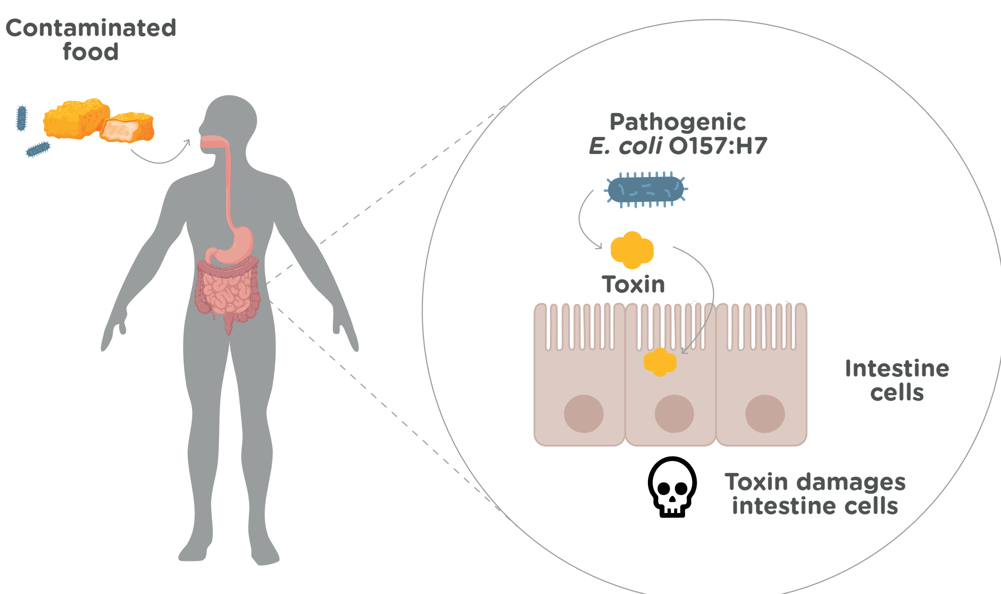
E. coli O157:H7 infection

1

- While most *E. coli* strains are **non-pathogenic** and part of a healthy gut, the *E. coli* O157:H7 strain produces a **toxin** that damages the lining of human intestines.
- Humans who ingest pathogenic *E. coli* O157:H7 can experience symptoms including vomiting, abdominal cramps, and bloody diarrhea.



2

- Not everyone exposed to pathogenic *E. coli* O157:H7 will become ill, and those who do typically recover from the symptoms.
- However, some people can experience serious complications, including kidney failure. Severe illness is more likely in vulnerable populations such as young children, older adults, and individuals with compromised immune systems.

Background: Stop and think

Circle the word that accurately completes the statement:

Q1. Several strains of *E. coli* can normally be found in the human digestive tract. These strains of *E. coli* are usually (pathogenic/non-pathogenic).

Q2. *E. coli* O157:H7 is not typically found in the human digestive tract. When ingested, *E. coli* O157:H7 are (pathogenic/non-pathogenic) to humans.

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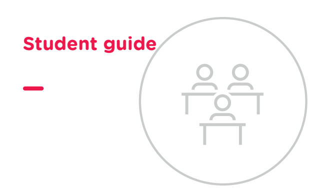

Today's lab

Scenario

- You work in a lab run by the city's Department of Public Health.
- Following a food truck festival, several attendees reported vomiting, abdominal pain, and bloody diarrhea.
- Because finding a group of patients with similar gastrointestinal symptoms may point to a shared foodborne illness, **stool testing** for pathogenic bacteria was performed.
- Testing detected pathogenic *E. coli* O157:H7 in the digestive tracts of all symptomatic patients.

Your goal

It is your job to identify the source of the pathogenic *E. coli* O157:H7!

1. First, you will analyze where people ate at the food festival to determine which food trucks require inspection.
2. Then, you will use DNA testing to detect the presence of pathogenic *E. coli* O157:H7 in food samples.

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Pre-lab activity: Attendee interview data

Ten attendees were surveyed about the food trucks they visited at the festival. Some of these individuals contracted foodborne illness, while others displayed no symptoms.

Answer the questions below to determine which food truck(s) are likely to be the source of the pathogenic *E. coli* O157:H7.

	Person	Food Trucks Visited					
		Falafel	Burger	Gyro	Poke	Tacos	Sandwich
Foodborne illness symptoms present	1						x
	2		x				x
	3	x	x	x	x	x	x
	4		x	x			
NO Foodborne illness symptoms present	5	x			x		
	6			x	x		
	7			x		x	
	8	x			x	x	
	9			x			
	10						x

Critical thinking

A. Are there any people with symptoms of foodborne illness who only visited one food truck? What can you infer from this?

B. Is there a single food truck visited by every person with symptoms of foodborne illness? What can you infer from this?

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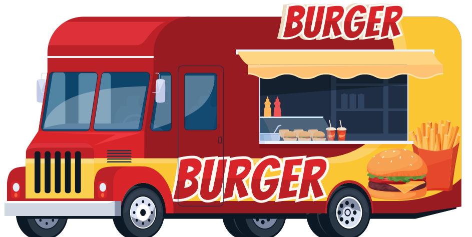
C. Are there any food trucks that none of the people without symptoms of foodborne illness visited? What can you infer from this?

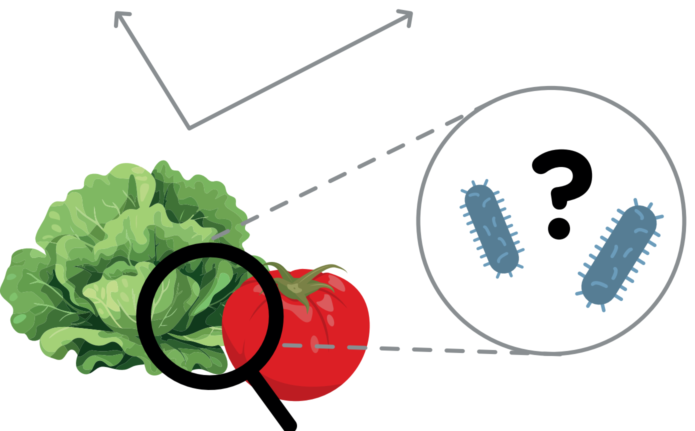
D. Based on this limited information, which food truck(s) are most likely to be the source of the pathogenic *E. coli* O157:H7?

Food truck inspection results

1

- The interview data suggest that people were exposed to *E. coli* O157:H7 at two possible sources: The Burger Barn and The Sandwich Shop.
- Health inspections of the two food trucks yielded no concerns with food storage practices, food handling techniques, or employee hygiene.
- Since there was no evidence of food safety issues at the food trucks, the contamination likely occurred before the ingredients reached the food trucks.





2

- You discover that both food trucks used two ingredients from the same suppliers: **lettuce from Field Fresh Farms and tomatoes from Red Ripe Ranch.**
- No other ingredients came from shared suppliers, so either the lettuce or the tomatoes are likely contaminated.

3

- To prevent more people from getting sick, it is essential to determine whether the lettuce or tomatoes carried the pathogenic *E. coli* O157:H7.
- Identifying the source of pathogenic *E. coli* O157:H7 will help the affected farm trace the contamination and issue recall notices.

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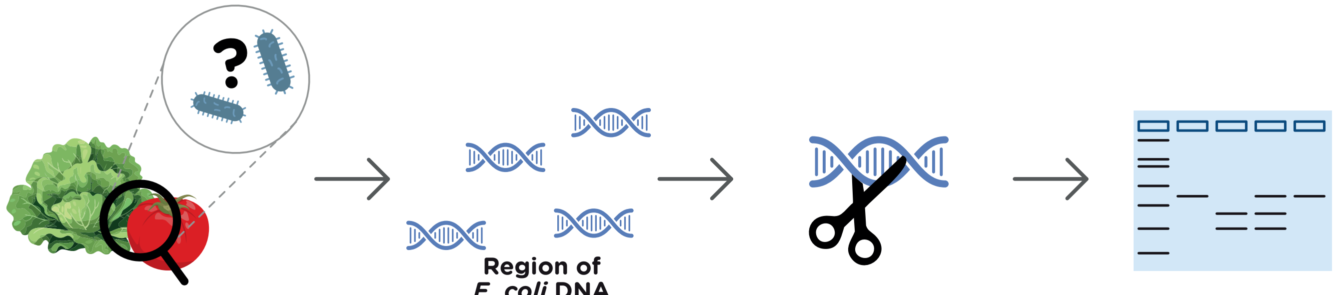
DNA testing

1

- You will identify the pathogenic bacteria by detecting the presence of a short **DNA sequence** that is specific to *E. coli* O157:H7.
- This test is highly sensitive and capable of detecting even low levels of *E. coli* O157:H7 contamination. This is important because only small amounts of the bacteria are needed to cause illness in humans.



DNA collection → **Polymerase chain reaction** → **Restriction digestion** → **Gel electrophoresis**

2

DNA was collected from lettuce from Field Fresh Farms and tomatoes from Red Ripe Ranch. If bacteria are present on either food, bacterial DNA will be present in these samples.

3

You will use the **polymerase chain reaction** to make billions of copies of a segment of *E. coli* DNA that differs between pathogenic O157:H7 and non-pathogenic strains.

4

Next, you will perform a restriction digestion to differentiate pathogenic *E. coli* O157:H7 DNA from non-pathogenic strains. This is explained in detail on the next page.

5

Finally, you will use gel electrophoresis to visualize the results and determine if the lettuce or the tomatoes were contaminated. This is explained in detail on page 22.

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Identifying pathogenic *E. coli* O157:H7 using a restriction enzyme

- In this lab, PCR is used to copy a 400 base pair (bp) segment of *E. coli* DNA that contains a sequence unique to the pathogenic strain O157:H7
- Then, a **restriction digestion** is performed on the PCR product to test for the presence of the pathogenic strain.

2

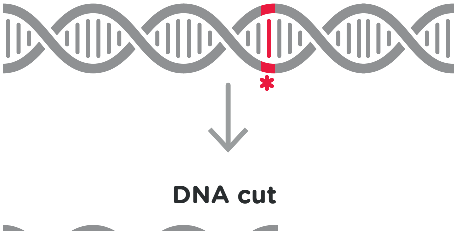
- **Restriction enzymes** recognize and cut specific short DNA sequences.
- This lab uses a restriction enzyme that cuts a DNA sequence found only in pathogenic *E. coli* O157:H7.

3

- Non-pathogenic *E. coli* lacks that DNA sequence, so the PCR product will remain uncut.
- A final step called gel electrophoresis is then used to visualize if the DNA was cut.

Pathogenic *E. coli* O157:H7		Non-Pathogenic *E. coli*

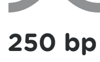
Restriction enzyme recognition site present



400 bp PCR product

DNA cut

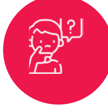
DNA not cut



250 bp

150 bp

400 bp



Background: Stop and think

Q3. What is the role of PCR in this experiment? To copy DNA from...
A. all strains of *E. coli*.
B. non-pathogenic strains of *E. coli*.
C. pathogenic stains of *E. coli*.

Q4. What is the role of restriction digestion in this experiment? To identify the presence of...
A. non-pathogenic strains of *E. coli*.
B. any pathogenic strain of *E. coli*.
C. only pathogenic *E. coli* O157:H7.

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Student guide

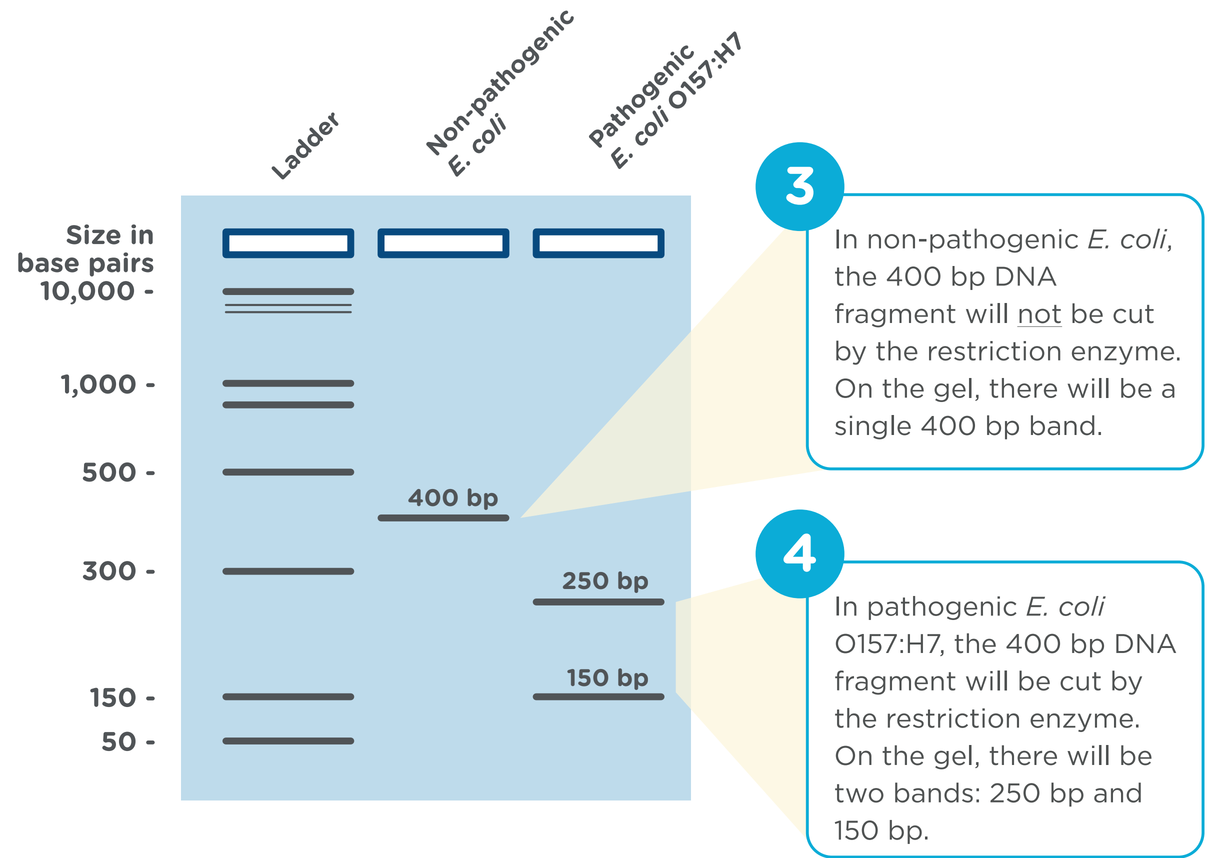
Interpreting gel electrophoresis results

1

- As the final step in this lab, you will use gel electrophoresis to visualize the restriction digestion results and determine whether the pathogenic *E. coli* O157:H7 strain was present in any of the food samples.
- **Gel electrophoresis** separates DNA fragments by size. At the end of a gel electrophoresis experiment, smaller pieces of DNA will have traveled farther through an **agarose gel** than larger pieces of DNA.

2

- Your testing will include two important **control samples**:
  - DNA from non-pathogenic *E. coli* grown in a lab
  - DNA from pathogenic *E. coli* O157:H7 grown in a lab
- These controls will show you how it looks when non-pathogenic *E. coli* vs. pathogenic *E. coli* O157:H7 are present in a sample.



By comparing the results from the lettuce and tomato samples to the control samples, you can determine whether one of these foods was the source of the pathogenic *E. coli* O157:H7!

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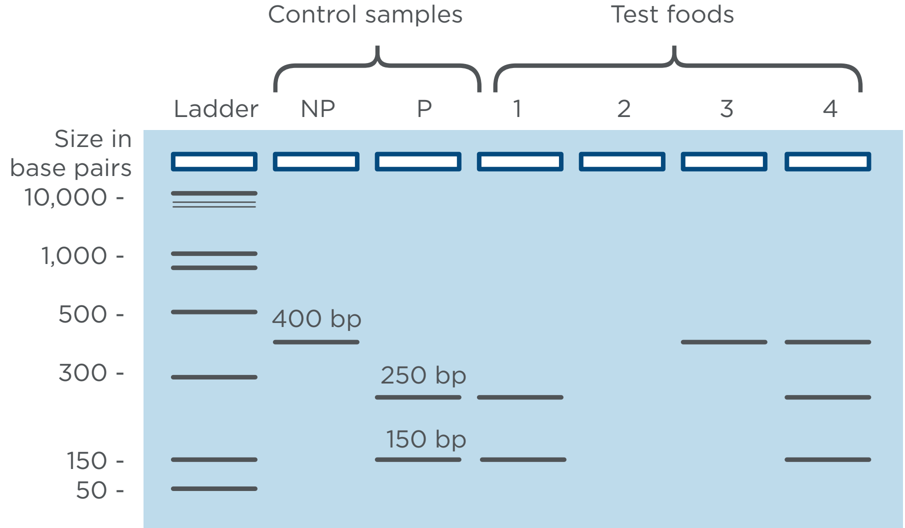
Imagine someone used the same experimental design to test four different foods for contamination with pathogenic *E. coli* O157:H7. Compare the test food results to the two control samples to interpret the results shown below.



Q5. Which test food shows <u>only</u> non-pathogenic *E. coli* present?

A. Test food 1
B. Test food 2
C. Test food 3
D. Test food 4

Q6. Which test food shows <u>only</u> pathogenic *E. coli* O157:H7 present?

A. Test food 1
B. Test food 2
C. Test food 3
D. Test food 4

Q7. Which test food shows <u>both</u> non-pathogenic *E. coli* and pathogenic *E. coli* O157:H7 present?

A. Test food 1
B. Test food 2
C. Test food 3
D. Test food 4

Q8. Which test food shows no *E. coli* present?

A. Test food 1
B. Test food 2
C. Test food 3
D. Test food 4

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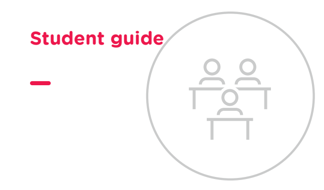

Glossary

Foodborne illness: Sickness caused by consuming foods or beverages contaminated with pathogens.

Pathogenic bacteria: Bacteria that can cause disease or illness. Pathogenic bacteria are the most common source of foodborne illness in humans.

***E. coli* O157:H7:** A strain of *E. coli* that can cause foodborne illness in humans. It is commonly found in the intestines of healthy livestock, such as cattle. However, *E. coli* O157:H7 produces Shiga toxin, which damages the intestinal lining in humans. Ingesting this strain of bacteria can lead to gastrointestinal symptoms, including abdominal pain, vomiting, and bloody diarrhea. *E. coli* O157:H7 infections can also cause kidney failure. People under the age of 5 and over 65 are more likely to experience severe illness.

Strain: A subgroup within a species that shares certain genetic traits not found in other members of the same species. There are many different laboratory tests that can be used to identify different strains of bacteria, including DNA testing.

Non-pathogenic: Microorganisms that do not cause illness or disease.

Toxin: A poisonous substance naturally produced by living organisms. *E. coli* O157:H7 produces Shiga toxin, which damages the intestinal lining of humans.

Stool testing: A medical examination of a fecal sample used to identify the causes of illnesses that result in gastrointestinal symptoms. When foodborne illness is suspected, stool can be tested to identify pathogens using various methods, including DNA analysis.

DNA sequence: The order of nucleotides, or bases, in a DNA molecule. DNA sequences can be used to classify organisms, including strains of bacteria.

Polymerase Chain Reaction (PCR): A technique used to make multiple copies of a specific DNA segment for further study. In this activity, PCR is used to copy a 400 bp segment of bacterial DNA. For more detailed information on PCR, refer to https://www.minipcr.com/polymerase-chain-reaction/.

Restriction digestion: The use of a restriction enzyme to cut, or digest, a DNA sample.

Restriction enzyme: An enzyme that recognizes a specific, short DNA sequence (typically 4-8 base pairs long) and cuts the DNA at that location. In this activity, you will use a restriction enzyme that allows you to identify pathogenic *E. coli* O157:H7.

Gel electrophoresis: A method that separates pieces of DNA by length. For more detailed information on electrophoresis, refer to https://www.minipcr.com/gel-electrophoresis/.

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Agarose gel: A type of gel commonly used in gel electrophoresis to separate DNA molecules based on size. At the microscopic level, the inside of an agarose gel resembles a web or fine mesh. Small molecules can move through the pores with relative ease, while larger molecules are slowed down.

Control samples: Samples used to ensure that your experiment is working properly and to help you interpret your results. In this activity, there are two control samples. (1) DNA from non-pathogenic *E. coli* and (2) DNA from pathogenic *E. coli* O157:H7.

Student lab protocol

Set up PCR samples

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Number four 0.2 ml PCR tubes 1, 2, 3, 4. Write on the upper sidewall of the tube.

2. Add PCR reagents to the labeled tubes according to the table below. To prevent contamination, use a new tip for each addition.

	Tube 1	Tube 2	Tube 3	Tube 4
Master Mix (tube MM)	15 µl	15 µl	15 µl	15 µl
Primer Mix (tube PM)	10 µl	10 µl	10 µl	10 µl
DNA Sample	Non-pathogenic Control DNA (tube N) 5 µl	Pathogenic Control DNA (tube P) 5 µl	Lettuce Sample DNA (tube L) 5 µl	Tomato Sample DNA (tube T) 5 µl
Total volume	30 µl	30 µl	30 µl	30 µl

3. Close the caps on the tubes. When they are closed correctly, you should feel the caps snap into place.

4. Flick each tube to mix the contents.

5. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tubes, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.

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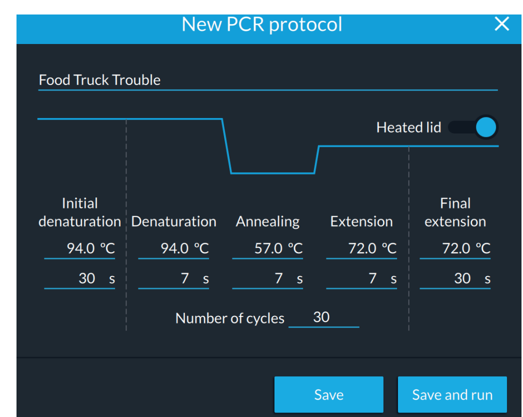
Run PCR



- Program your thermal cycler with the following parameters:

Initial denaturation	94°C, 30 sec
Denaturation	94°C, 7 sec
Annealing	57°C, 7 sec
Extension	72°C, 7 sec
Number of cycles	30
Final extension	72°C, 30 sec

- The PCR takes approximately 45 minutes when using a miniPCR® thermal cycler.

- PCR product is stable at room temperature for several days. For longer-term storage, move tubes to a freezer.

Detailed instructions for using a miniPCR thermal cycler

https://links.minipcr.com/minipcrRUN

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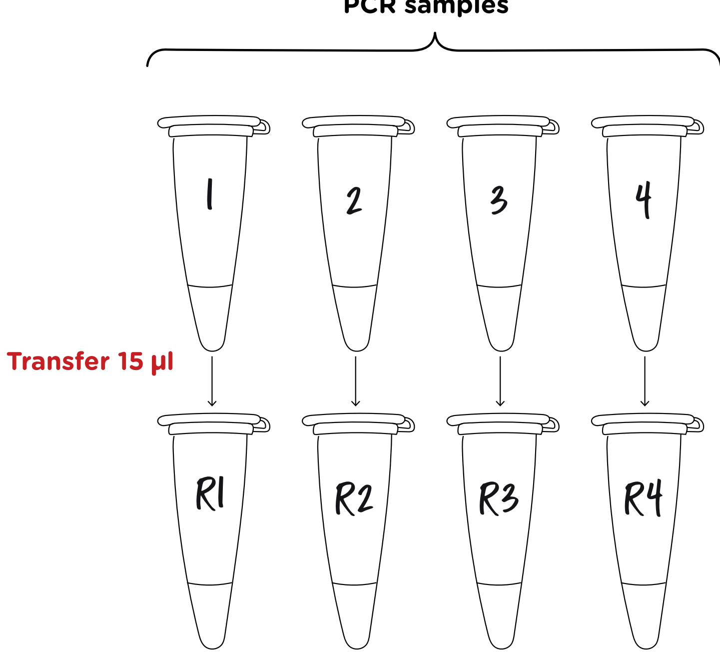
Restriction digestion

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Label four 0.2 ml PCR tubes R1, R2, R3, R4. Write on the upper sidewall of the tube.
2. Transfer 15 µl of PCR product from tube 1 to tube R1. Using a new tip for each sample, repeat for the remaining PCR products. You will transfer 15 µl of each PCR product to its corresponding newly labeled tube.



3. Ask you instructor to add 2 µl of diluted restriction enzyme to each one of your samples.
4. Close the caps on the tubes. When they are closed correctly, you should feel the caps snap into place.
5. Flick each tube to mix the contents.
6. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tubes, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.
7. Incubate at 37 °C overnight. You can use a miniPCR in heat block mode or any water bath or incubator set to 37 °C.

Gel electrophoresis

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load the restriction digestion samples in the following order. To prevent contamination, use a new tip for each sample.
   - Lane 1: 10 µl Fast DNA Ladder 2
   - Lane 2: 10 µl sample R1
   - Lane 3: 10 µl sample R2
   - Lane 4: 10 µl sample R3
   - Lane 5: 10 µl sample R4

4. Run the gel for 20 minutes.
   - The blueGel™ and Bandit™ electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range

5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the bands in the 150-500 bp range to interpret the results.

7. If desired, take a photo to document the gel electrophoresis results.

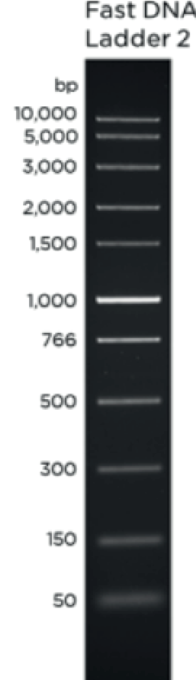
8. Compare the bands from the DNA samples to the DNA ladder to obtain size estimates.

Detailed operating instructions for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/blueGelRun

Bandit
https://links.minipcr.com/BanditViewit

Fast DNA Ladder 2



Student guide

Post-lab questions

Interpreting results

1. Use the image on the right to draw what your gel looks like. For each sample, draw the bands you see on your gel.

2. Next to each band, write approximately how long (in base pairs) the DNA in that band is. Use the image of the ladder on the previous page to help you.

3. Use your gel electrophoresis results to complete the table below.
   A. Use checkmarks to record the gel electrophoresis results in the first two rows of the table.
   B. Use the results to determine whether the two food samples were contaminated with pathogenic *E. coli* O157:H7, and record the result in the third row.

Ladder R1 R2 R3 R4

	Tube R1: Non-pathogenic *E. coli* control	Tube R2: Pathogenic *E. coli* O157:H7	Tube R3: Lettuce sample	Tube R4: Tomato sample
Non-pathogenic *E. coli* (400 bp)				
Pathogenic *E. coli* O157:H7 (250 + 150 bp)				
Pathogenic *E. coli* O157:H7 detected? (YES or NO)				

Critical thinking

4. What would be your next step to prevent other people from getting sick from the contaminated produce?

5. In this activity, you used DNA testing to detect a specific strain of bacteria. What is another situation where DNA testing could provide useful health information?

Advanced questions

Below are examples of unexpected experimental results. Match the gels for experiments I-IV to the scenarios described in questions 6-8.

6. Recall that during the PCR step of our experiment, we copied a 400 bp section of *E. coli* DNA. Which gel represents a failure of the PCR step?
   A. Experiment I
   B. Experiment II
   C. Experiment III
   D. Experiment IV

   Explain your reasoning:

7. Recall that during the restriction digestion, the PCR-amplified DNA pieces were cut by the restriction enzyme only if they contained a sequence unique to *E. coli* O157-H7. Which gel represents a failure of the restriction digestion step?
   A. Experiment I
   B. Experiment II
   C. Experiment III
   D. Experiment IV

   Explain your reasoning:

8. During the restriction digestion in this activity, the restriction enzyme molecules need to cut billions of copies of the PCR product. Depending on how long the samples are incubated, not all of the DNA may be cut. Which gel represents an incomplete restriction digestion?
   A. Experiment I
   B. Experiment II
   C. Experiment III
   D. Experiment IV

   Explain your reasoning:

In this activity, you identified the presence of a specific strain of bacteria using a restriction enzyme. Restriction enzymes are only useful diagnostic tools if the enzyme's recognition site is unique to the DNA sequence you want to detect. In today's lab, a restriction enzyme recognition site was present in the pathogenic *E. coli* DNA, but you can also use a restriction enzyme whose recognition site is absent only in your target DNA sequence.

For the following questions, assume you have access to these three restriction enzymes:

Available restriction enzymes		
EcoRI recognition site: 5'...G A A T T C ... 3' 3'...C T T A A G ... 5'	**HindIII recognition site:** 5'...A A G C T T ...3' 3'... T T C G A A ...5'	**DdeI recognition site:** 5'...C T N A G ...3' 3'...G A N T C ...5'
Note: "N" means any nucleotide can be in that position		

9. Sickle cell disease is caused by a single nucleotide mutation in the *β-globin* gene. The mutation is highlighted in red below.

Typical *β-globin* gene	**Sickle cell mutation**
5'...C T C C T G A G G A G ...3'	5'...C T C C T G T G G A G ...3'
3'...G A G G A C T C C T C ...5'	3'...G A G G A C A C C T C ...5'

   A. Which restriction enzyme would you use to differentiate between the typical *β-globin* allele and the sickle cell allele?

   B. Which allele of the *β-globin* gene would be cut by the restriction enzyme you selected?

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CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page.

Question:
Were either the tomatoes or lettuce contaminated with pathogenic *E. coli* O157:H7?

Claim Make a clear statement that answers the above question	
Evidence Provide data from the lab that supports your claim	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

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Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is hightly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100

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Instructor guide

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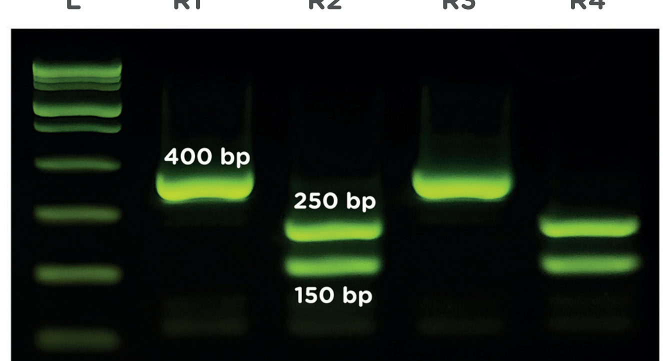
Expected results

- Overnight restriction digestion should produce gel electrophoresis results that resemble the photo to the right.
- A 15-minute incubation may lead to incomplete digestion, but the results can still be interpreted conclusively. Refer to the next page for more information.



This image represents results obtained after a 20 minute run using a blueGel electrophoresis system. Note that it is likely that the top of the Fast DNA Ladder 2 will not fully resolve with a short gel run.

	Tube R1: Non-pathogenic *E. coli* control	Tube R2: Pathogenic *E. coli* O157:H7	Tube R3: Lettuce sample	Tube R4: Tomato sample
Non-pathogenic *E. coli* (400 bp)	✓		✓	
Pathogenic *E. coli* O157:H7 (250 + 150 bp)		✓		✓
Pathogenic *E. coli* O157:H7 detected? (YES or NO)			NO	YES

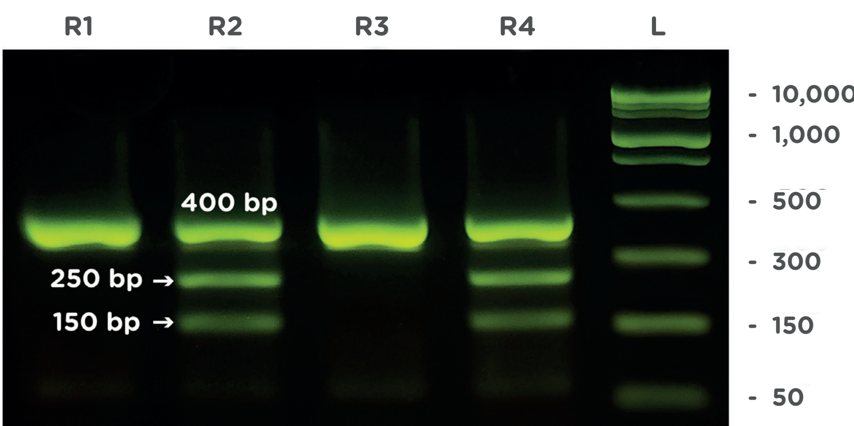
- This type of DNA testing is very sensitive, so it is not surprising that both raw foods showed the presence of *E. coli*.
- The lettuce sample tested positive only for non-pathogenic *E. coli,* which suggests that it is not the source of the pathogenic *E. coli* O157:H7 contamination.
- The tomato sample tested positive for pathogenic *E. coli* O157:H7. We can conclude that the tomatoes were a likely source of pathogenic *E. coli* O157:H7 contamination.

For technical support, contact support@minipcr.com

For answers to the student questions, email answers@minipcr.com
Please include in the body of the email:
• The name of the lab
• Your name, school, and job title

Interpreting an incompete digestion

- Below are the results after a 15-minute restriction digestion.
- Note that the pathogenic control sample (R2) shows a 400 bp DNA fragment, in addition to the expected 250 and 150 bp fragments. This indicates that the restriction enzyme did not have enough time to cut all of the DNA.
- By comparing the food samples with the control samples, the experimental results can still be used to draw a clear conclusion that the tomatoes (tube R4) were a likely source of pathogenic *E. coli* O157:H7 contamination. If any of the 400 bp DNA was cut into 250 and 150 bp fragments, it follows that the sample is positive for pathogenic *E. coli* O157:H7.



Unexpected results and troubleshooting

If fluorescent DNA bands are faint or entirely absent from some experimental samples, the following may have occurred:

- Suboptimal PCR amplification: Pipetting errors during PCR setup can lead to suboptimal amplification of student-prepared samples.
- Failure to load the DNA samples on the gel: Loading DNA samples for gel electrophoresis takes practice. The bands will appear faint if students do not successfully deposit the full sample volume into the well. For gel loading tips, refer to https://www.minipcr.com/how-to-load-a-gel-electrophoresis/

If fluorescent DNA bands are not visible on the gel, even for the DNA ladder, the following may have occurred:

- Failure to use a fluorescent DNA stain: This lab requires agarose gels made with a fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®). DNA stains that reveal DNA with a visible blue compound are less sensitive and are not compatible with this lab kit.
- Incorrect visualization conditions: Fluorescent DNA stains (*e.g.*, SeeGreen™ or GelGreen®) must be viewed using a blue light or UV transilluminator. The blueGel system has an integrated blue light transilluminator. For DNA visualization, ensure that you have turned on the blueGel's blue light by pressing the light bulb button.
- Samples ran off the gel: If you run the gel too long, DNA samples may migrate off the gel. Monitor progress by occasionally checking the DNA samples in a transilluminator.
- Reagents were stored improperly and/or are expired: The lab kit can be stored in the freezer for up to twelve months after receipt. Storage under different conditions or in excess of this guidance may impair performance.

If all sample lanes have <u>only</u> a 400 bp DNA fragment, the restriction digestion failed, and the following may have occurred:

- Insufficient restriction enzyme added: To set up the restriction digestion, you must add 2 µl of the diluted restriction enzyme. We recommend the teacher complete this step as students struggle to pipette small volumes.
- Restriction enzyme was stored improperly and/or is expired: The restriction enzyme can be stored in the freezer for up to twelve months after receipt. Storage under different conditions or in excess of this guidance may impair performance.

For tips on picture-perfect gels, see https://www.minipcr.com/gel-electrophoresis-troubleshooting/

For additional technical support, contact support@minipcr.com

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Notes on lab design

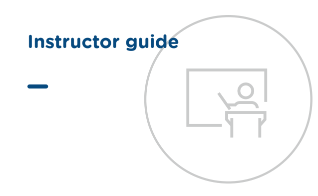
This lab serves as an introduction to using DNA testing to identify bacterial contamination. We believe this approach provides the right balance between intellectual engagement, inquiry, and accessibility. The design of this lab has simplified certain elements to achieve these goals. Some of these elements include:

- None of the materials provided in this lab kit pose a pathogenic risk. All DNA samples are synthetic and do not pose risks to human health.
- Clinical laboratories have access to a variety of different methods to detect O157:H7 *E. coli*, including:
  - Microbiology: selective culture on specialized media
  - Immunology: detection of the O157 antigen using specific antibodies or ELISA
  - Molecular: amplification of strain-specific genes using PCR or qPCR
  - Other DNA and protein-based methods are also available
  - The use of a diagnostic restriction digestion, such as the one done in this activity, is uncommon in clinical settings

Citations

Machado, J., Grimont, F., and Grimont, P.A. (2000). Identification of *Escherichia coli* flagellar types by restriction of the amplified *fliC* gene. Res Microbiol 151, 535–546. https://doi.org/10.1016/s0923-2508(00)00223-0

Wang, L., Rothemund, D., Curd, H., and Reeves, P.R. (2000). Sequence diversity of the *Escherichia coli* H7 *fliC* genes: Implication for a DNA-based typing scheme for *E. coli* O157:H7. J Clin Microbiol 38, 1786–1790. https://doi.org/10.1128/jcm.38.5.1786-1790.2000.



Additional student supports

E-worksheets: The student questions accompanying this lab are available for download here as editable text documents that you can customize and upload to your LMS. E-worksheets can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/minipcr-food-safety-lab/.

miniPCR tutorials: Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources most relevant to this lab are listed below.

- **Micropipetting**: Video, worksheet, and hands-on activity resources to train students in the basic use of a micropipette.
- **PCR**: Video and worksheet activity instructing students on the fundamentals and practice of PCR.
- **Gel electrophoresis**: Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.

miniPCR Digital: Interactive tools for experiment-based learning with or without hands-on lab kits. Visit https://digital.minipcr.com/

Learning goals and skills developed

Student learning goals

- Differentiate between pathogenic and nonpathogenic bacterial strains
- Understand the principles and practice of PCR
- Gain familiarity with restriction enzymes and their practical applications in biology
- Solve a real-world problem using DNA analysis

Scientific inquiry skills

- Identify or pose a testable question
- Identify dependent and independent variables and appropriate experimental controls
- Follow detailed experimental protocols
- Interpret data presented in a chart or table
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular biology skills

- Micropipetting
- PCR
- Restriction enzyme digestion
- Agarose gel electrophoresis

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Standards alignment

The standards alignment document for this activity is available for download here. This document can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/minipcr-food-safety-lab/.

This activity is aligned to the following standards:

- Next Generation Science Standards: High School Life Science
- Advanced Placement Biology
- Texas Essential Knowledge and Skills: Biology
- Texas Essential Knowledge and Skills: Biotechnology
- Biotechnician Assistant Credentialing Exam
- Common Core ELA/Literacy Standards (9-10)

For additional information on alignment to state standards, please contact support@minipcr.com