



Food Safety Lab

Food Truck Trouble!



Student guide



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Background information

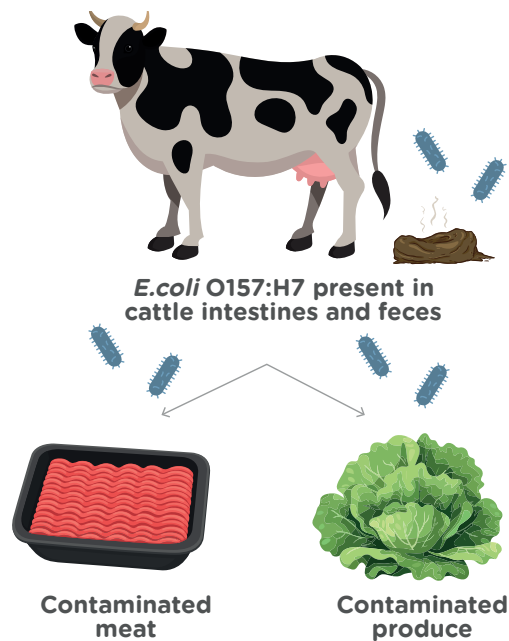
Foodborne illness

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

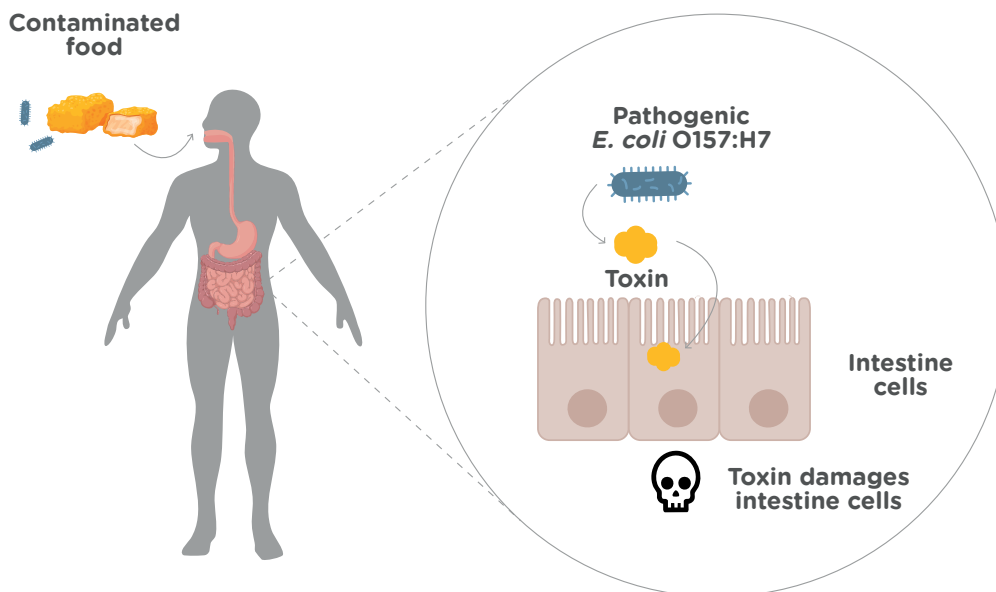
- Crops can be exposed to feces through improperly processed manure-based fertilizer or tainted irrigation water.
- Thoroughly washing fresh produce is important to help rinse away pathogens that may be present.



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.



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.



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?



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

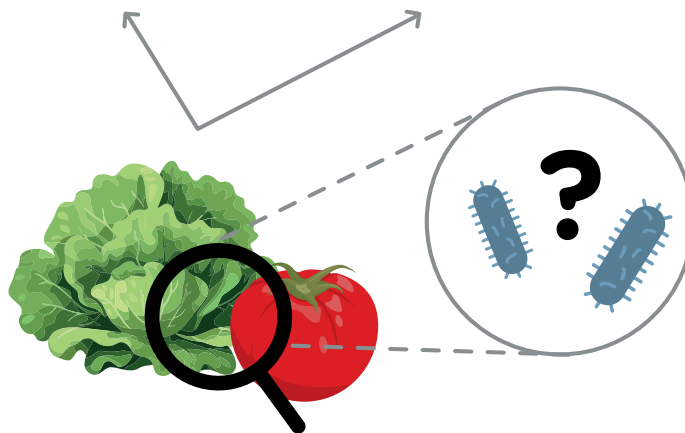
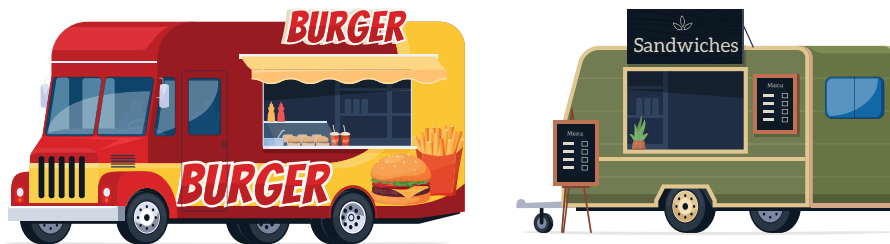
D. Based on this limited information, which food truck(s) are most likely to be the source of the pathogenic *E. coli* O157:H7?



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

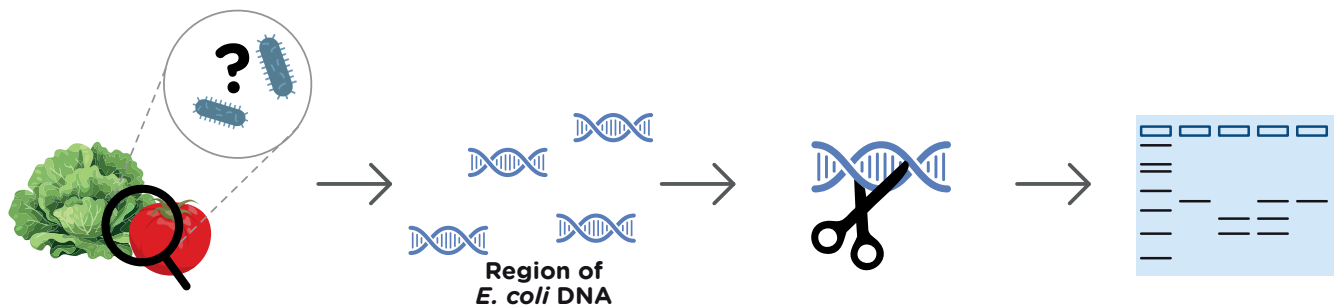
- To prevent more people from getting sick, it is essential to determine whether the lettuce or tomatoes carried the pathogenic *E. coli* O157:H7.
- Identifying the source of pathogenic *E. coli* O157:H7 will help the affected farm trace the contamination and issue recall notices.



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.



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

1

- 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

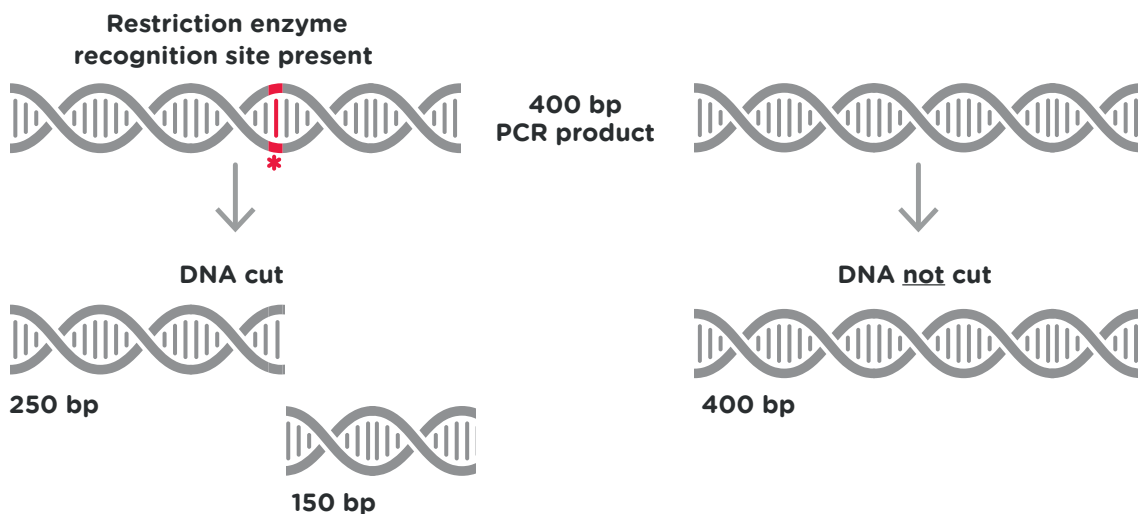
- **Restriction enzymes** recognize and cut specific short DNA sequences.
- This lab uses a restriction enzyme that cuts a DNA sequence found only in pathogenic *E. coli* O157:H7.

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***



Background: Stop and think

Q3. What is the role of PCR in this experiment?

To copy DNA from...

- all strains of *E. coli*.
- non-pathogenic strains of *E. coli*.
- pathogenic strains of *E. coli*.

Q4. What is the role of restriction digestion in this experiment? To identify the presence of...

- non-pathogenic strains of *E. coli*.
- any pathogenic strain of *E. coli*.
- only pathogenic *E. coli* O157:H7.



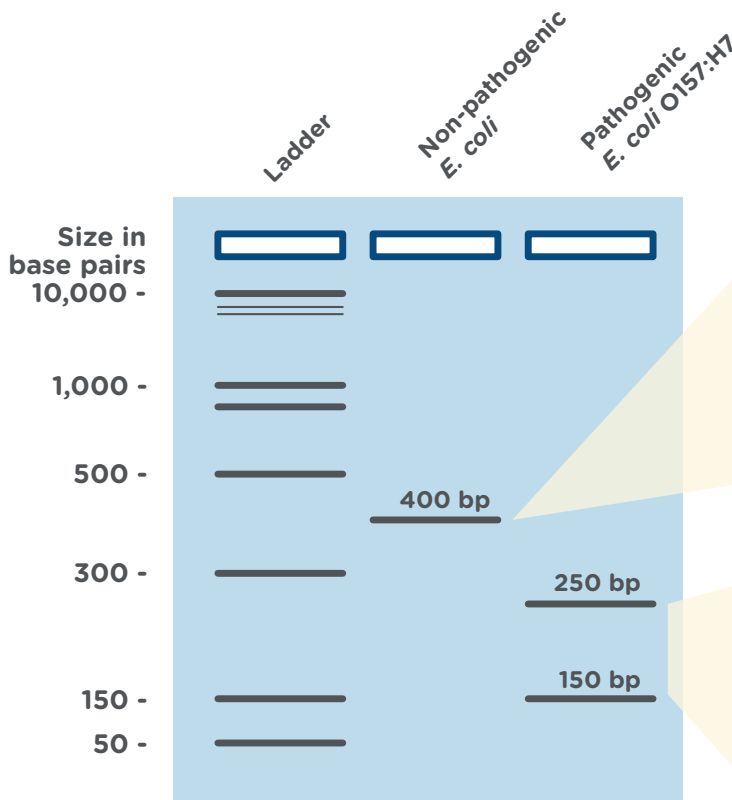
Interpreting gel electrophoresis results

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.



3

In non-pathogenic *E. coli*, the 400 bp DNA fragment will not be cut by the restriction enzyme. On the gel, there will be a single 400 bp band.

4

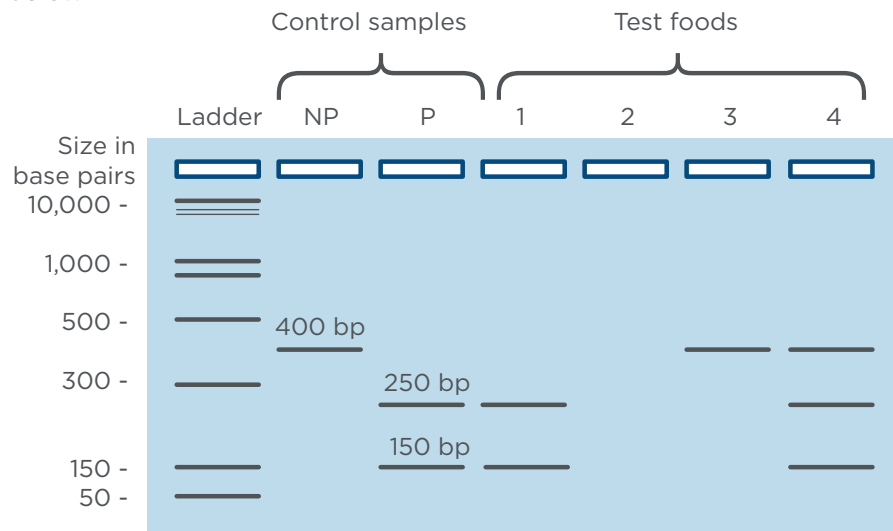
In pathogenic *E. coli* O157:H7, the 400 bp DNA fragment will be cut by the restriction enzyme. On the gel, there will be two bands: 250 bp and 150 bp.

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!



Background: Stop and think

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 only 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 only 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 both 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



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/>.



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

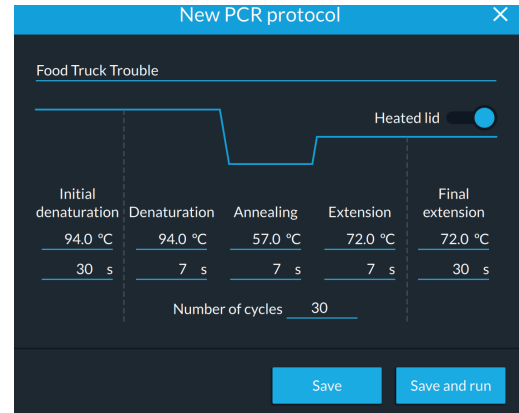
3. Close the caps on the tubes. When they are closed correctly, you should feel the caps snap into place.
4. Flick each tube to mix the contents.
5. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tubes, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.



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>

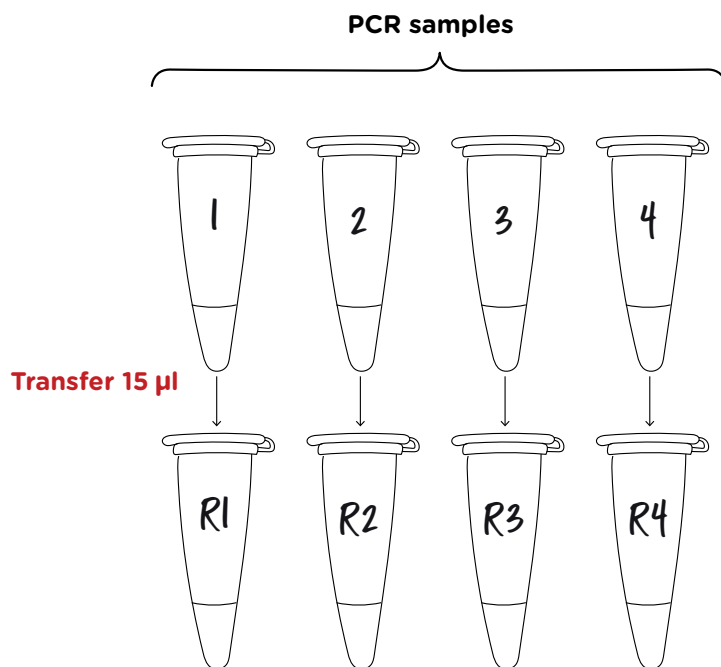


Restriction digestion



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 μ l 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 μ l of each PCR product to its corresponding newly labeled tube.



3. Ask your instructor to add 2 μ l 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 $^{\circ}$ C overnight. You can use a miniPCR in heat block mode or any water bath or incubator set to 37 $^{\circ}$ C.



Gel electrophoresis



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

- Place the prepared gel into the electrophoresis chamber.
- 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.
- 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
- 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
- To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.
- 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.
- If desired, take a photo to document the gel electrophoresis results.
- Compare the bands from the DNA samples to the DNA ladder to obtain size estimates.

Detailed operating instructions for miniPCR electrophoresis systems



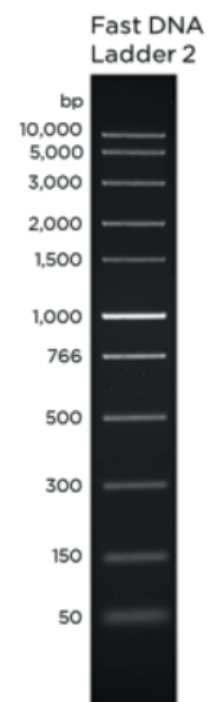
blueGel

<https://links.minipcr.com/blueGelRun>



Bandit

<https://links.minipcr.com/BanditViewit>

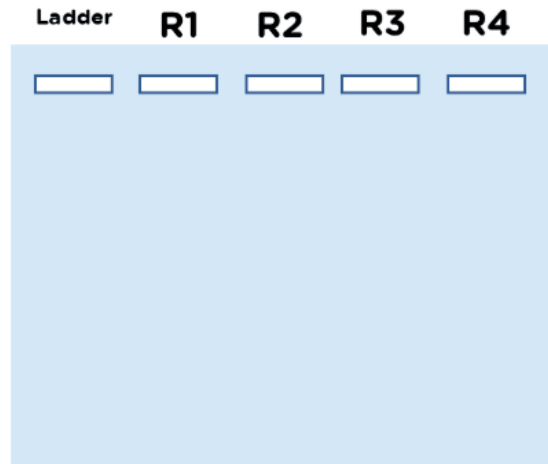




Post-lab questions

Interpreting results

- Use the image on the right to draw what your gel looks like. For each sample, draw the bands you see on your gel.
- 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.
- Use your gel electrophoresis results to complete the table below.
 - Use checkmarks to record the gel electrophoresis results in the first two rows of the table.
 - 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.



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

Critical thinking

- What would be your next step to prevent other people from getting sick from the contaminated produce?
- 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 Explain your reasoning:
 - B. Experiment II
 - C. Experiment III
 - D. Experiment IV

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 Explain your reasoning:
 - B. Experiment II
 - C. Experiment III
 - D. Experiment IV

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 Explain your reasoning:
 - B. Experiment II
 - C. Experiment III
 - D. Experiment IV



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	HindIII	Ddel
recognition site:	recognition site:	recognition site:
5'...GAATTC...3'	5'...AAGCTT...3'	5'...CTNAG...3'
3'...CTTAAG...5'	3'...TTCGAA...5'	3'...GANTC...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

5'...CTCCTGAGGAG...3'
3'...GAGGACTCCTC...5'

Sickle cell mutation

5'...CTCCTGTGGAG...3'
3'...GAGGACACCTC...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?



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.



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 highly 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