miniPCR™ Genes in Space Food Safety Lab: Mars Colony at Risk!

Student's guide

4. Scenario overview

Astronauts aboard the International Space Station (ISS) have come down with severe cases of food poisoning. A doctor believes the culprit is a pathogenic (disease-causing) strain of *E. coli* bacteria that may have contaminated the food supply. But is it the space burgers or the space sushi that are harboring the dangerous bacteria? In this experiment, you will use DNA analysis tools to figure out which food source is contaminated with pathogenic *E. coli* and which is carrying its non-pathogenic (harmless) relative

Watch this video:

https://links.minipcr.com/GiS-biotech-videos

5. Glossary

- **Agarose** – A polysaccharide that is the main component of Agar. It is used primarily to make gels for gel electrophoresis.
- **Annealing** – The 2nd Step in a PCR. Slowly cooling the material undergoing PCR in order to allow primers to bind to the correct DNA sequence.
- **Buffer** – A solution (usually of a specific pH) that resists a change in pH. Electrophoresis buffer is used in Gel Electrophoresis to carry a current through the gel and to maintain a pH at which DNA is best protected from hydrolysis (it won't break down).
- **Denaturation** – The 3rd step in a PCR. The process by which a molecule loses its natural 3-dimensional folded structure, usually due to high heat or extreme pH, this causes hydrogen bonds in the molecule to break. In this lab heat is used to denature the DNA double-helix, causing the two strands to separate from one another.
- **DNA Ladder** – A collection of DNA strands of specific known lengths that can be used to estimate the size of other DNA strands when the two are run next to each other in a gel.
- **Enterohemorrhagic** – causing hemorrhages, or ruptured blood vessels that cause rapid blood loss, within the intestines.
- **Extension** – The PCR step in which the enzyme Taq polymerase adds complimentary base pairs to the template DNA starting at the location of the primer, the results of this step are complete, double-stranded DNA molecules of which one strand is brand new.
- **Gel Electrophoresis** – The process of moving DNA (which is negatively charged) through a gel by creating an electric field that pushes them away from the negative terminal and towards the positive terminal. This allows you to see the differences in lengths of DNA fragments as shorter segments will travel farther in the gel than longer segments.
- **Incubation** – Storing something at the best environmental conditions (i.e. temperature) for the process you want it to undergo.
- **Loading Dye** – A mixture added to PCR Product before running in a gel that dyes the DNA so that it can be viewed within the gel (usually under light of a specific wavelength). Loading dye also increases the density of the PCR product to be added to the gel so that it will sink into the gel wells within the electrophoresis buffer.
- **Microcentrifuge** – A lab device that rapidly spins small tubes to concentrate liquid at the bottom of the tube or separate different materials.
- **Pathogenic** – causing disease.

- **PCR** – Polymerase Chain Reaction, a method by which one can rapidly amplify a desired strand of DNA to billions of copies or more.
- **PCR Product** – The solution obtained after a PCR that contains the amplified DNA strands as well as any excess reactants that were added in previous steps.
- **Primer Mix** – A solution containing forward and reverse primers that bind to a complementary DNA sequence that serve as the bookends of the DNA segment you wish to amplify. This mix is added before you conduct a PCR and is essential to the process.
- **Restriction enzyme** – A protein that cuts DNA at a specific base-pair sequence (called a "restriction digest") that is used to discover differences in DNA lengths between different samples when a gel electrophoresis is conducted.
- **Template DNA** – The existing strand of DNA which primers bind to create a complementary strand and thus a second copy of that DNA segment.

Laboratory guide

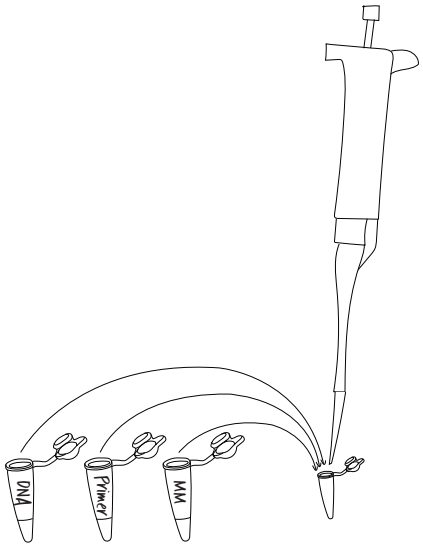
Protective gloves and eyewear should be worn for the entirety of this experiment.

PCR setup

1. **Label 4 PCR tubes** (200 µl tubes)
   - Note: Label tubes on the upper side wall, as writing on the cap or lower side wall may rub off during PCR.
   - Label each tube with the name of the sample:
     - A (food sample A DNA, space sushi)
     - B (food sample B DNA, space burger)
     - P (pathogenic control DNA)
     - NP (non-pathogenic control DNA)

2. **Add PCR reagents to each PCR tube**

	Tube A	Tube B	Tube P	Tube NP
2X EZ PCR Master Mix	15 µl	15 µl	15 µl	15 µl
Primer Mix	10 µl	10 µl	10 µl	10 µl
DNA Sample	Food sample A	Food sample B	Pathogenic control	Non-pathogenic control
	5 µl	5 µl	5 µl	5 µl
TOTAL VOLUME	30 µl	30 µl	30 µl	30 µl

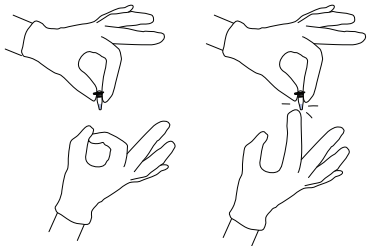


Use a micropipette to add each of the reagents. Remember to change tips at each step!

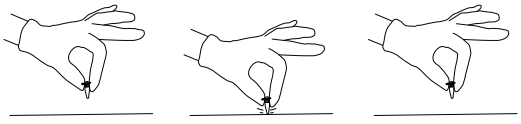
Note: EZ PCR Master Mix™ contains:
- Taq DNA polymerase • dNTPs
- PCR buffer with Mg^{2+} • Gel loading dye

3. **Cap the tubes and ensure the reagents mix well**
   - You may flick each tube with your fingers to ensure proper mixing.
   - Gently tap tubes on your bench to collect liquid at the bottom.

Flick to mix



Tap to collect liquid at bottom



4. **Place the tubes inside the miniPCR® machine**
   - Press firmly on the tube caps to ensure a tight fit.
   - Close the PCR machine lid and tighten it gently.

PCR programming

These instructions are illustrated using miniPCR® software on a Windows PC. Software interfaces vary slightly by operating system. See the miniPCR® User's Guide for more details.

If using a different thermal cycler, PCR protocol parameters should remain the same (step 7).

1. **Open the miniPCR® app and remain on the "Library" window**

2. **Connect your miniPCR® thermal cycler to your device using the supplied USB cable or via Bluetooth®**
   - Note: Bluetooth® is only available on certain models. To connect via Bluetooth®, select the (m) icon, located by "Devices" at the left of the desktop app or at the top of the mobile app.

3. **Make sure your miniPCR® thermal cycler is plugged in and that the power switch is turned on**
   - Note: If your machine begins running a previously loaded protocol, you may stop it by clicking or tapping the "X" symbol in the top left box of the "Monitor" window.

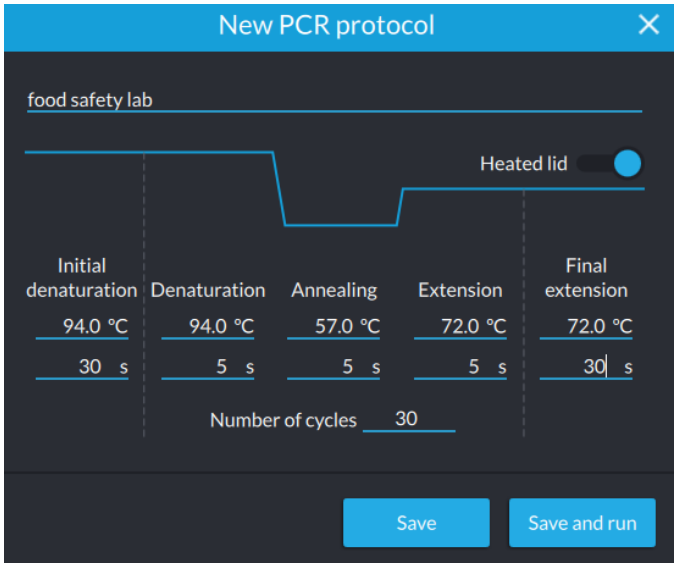
4. **While in the "Library" window, click the ⊕ button to create a new protocol**
   - Button is located in the upper right hand corner of the window.

5. **Select "PCR" from the drop-down menu**

6. **Enter a name for the protocol; for example: "GMO Lab"**

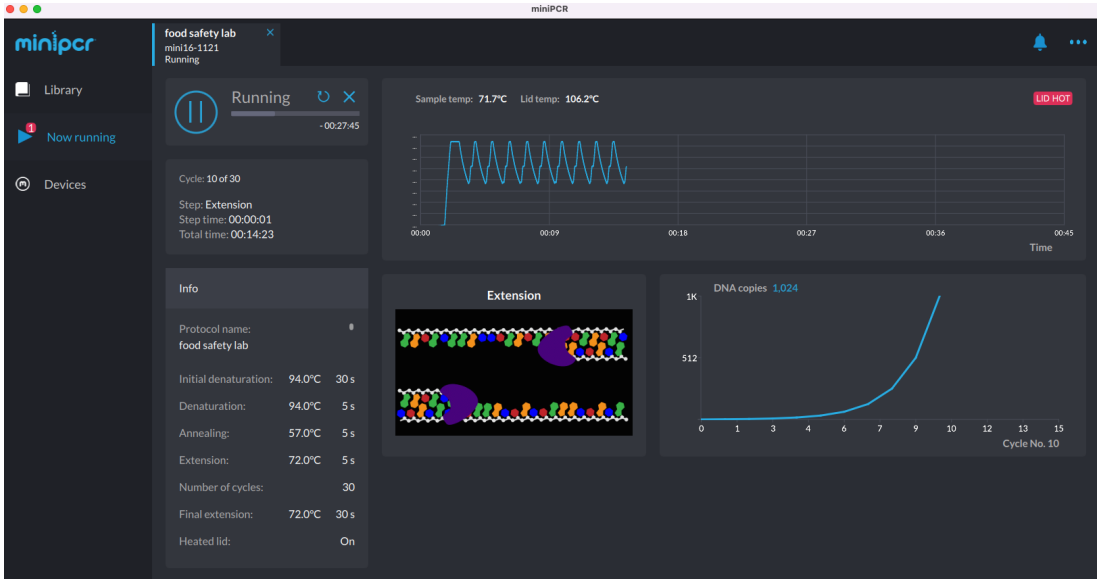
7. **Enter the PCR protocol parameters:**
   - Initial denaturation 94°C, 30 sec
   - Denaturation 94°C, 5 sec
   - Annealing 57°C, 5 sec
   - Extension 72°C, 5 sec
   - Number of cycles 30
   - Final extension 72°C, 30 sec

   Note: The "Heated lid" slider should be in the on position.



8. **Click "Save and run" to start the protocol**
    - If connected to more than one machine, choose the serial number of the miniPCR® thermal cycler you are using. If asked "Do you want to stop the current protocol…?", click "Yes".
    - The lights on the front of the miniPCR® thermal cycler will blink 3 times to indicate that the protocol has been loaded.
    - Note: If needed, you may unplug the USB cable or disconnect Bluetooth® once the protocol has been loaded. Even if disconnected from your device, the protocol will continue to completion as normal.

9. **Choose "Monitor" window**
    - The "Monitor" window can be selected on the left column in the desktop app and at the top in mobile app.
    - If more than one miniPCR® thermal cycler is connected to the same device, choose which machine you would like to monitor using the tabs at the top of the window (desktop app) or bottom of the Library (mobile app).



The miniPCR® software allows each lab group to monitor the reaction parameters in real time.

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10. **When the PCR run has completed (approximately 30 min), app status will show "Finished" and the red, yellow, and green LEDs on your miniPCR® thermal cycler will light up and stay on**

Be careful not to touch the metal lid which may still be hot.

11. **PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer**
    - Tubes may remain inside the miniPCR® thermal cycler for several days following protocol completion.

Set up restriction digest

1. **Label four new PCR tubes (200 µl tube)**
   - Note: Label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off.
   - Label the tubes:
     - "AX": This will contain the PCR product from food sample A as well as the restriction enzyme
     - "BX": This will contain the PCR product from food sample B as well as the restriction enzyme
     - "PX": This will contain the PCR product from the pathogenic control as well as the restriction enzyme
     - "NPX": This will contain the PCR product from the non-pathogenic control as well as the restriction enzyme

2. **Transfer 15 µl of the appropriate PCR reaction to each tube**
   - Note: Do not discard your leftover PCR product. You will use this in the next step (gel electrophoresis).

3. **Use a 0.5-10 µl micropipette to add 1 µl restriction enzyme to each tube**
   - Eject the restriction enzyme directly into the liquid already in your tube and pipette up and down several times to mix. Proper mixing is essential.
   - Note: Your teacher might perform this step for you.

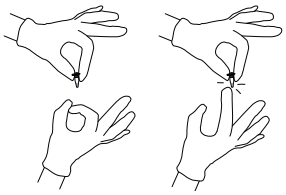
	Tube AX	Tube BX	Tube PX	Tube NPX
PCR product	Food sample A 15 µl	Food sample B 15 µl	Pathogenic control 15 µl	Non-pathogenic control 15 µl
Restriction enzyme	1 µl	1 µl	1 µl	1 µl
TOTAL VOLUME	16 µl	16 µl	16 µl	16 µl

4. **Cap the tube then flick to ensure the reagents are well mixed**

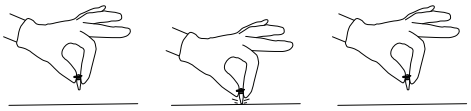
5. **Gently tap the tubes on your bench to ensure that the liquid volume collects at the bottom**

6. **Incubate at 37°C for 15 minutes**
   - You can use a miniPCR® thermocycler in Heat Block mode, or a water bath or dry block incubator.

Flick to mix



Tap to collect liquid at bottom

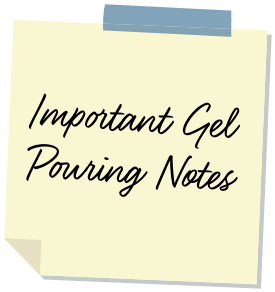


Optional stopping point

If the restriction digest will not be used immediately for gel electrophoresis:

- Incubate at 65°C for 20 minutes.
- You can use a miniPCR® thermocycler in Heat Block mode, or a water bath or dry block incubator.
- Store samples in the freezer for up to one week.

Gel electrophoresis - Pouring gels (before or during class period)



Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

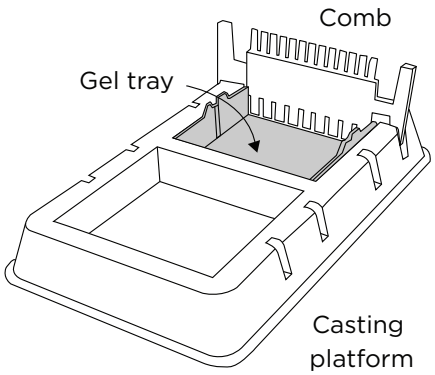
You will need eight lanes plus one lane for ladder per group. Groups can share gels if you use two combs.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

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

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.



3. **Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

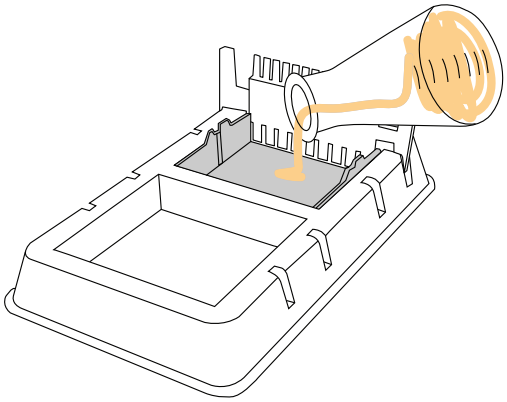
IMPORTANT NOTE: There are several ways to prepare agarose gels

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

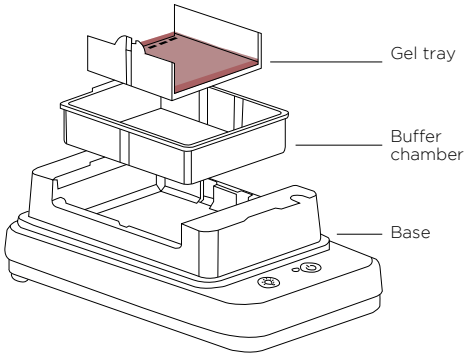
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



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

Gel electrophoresis - Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

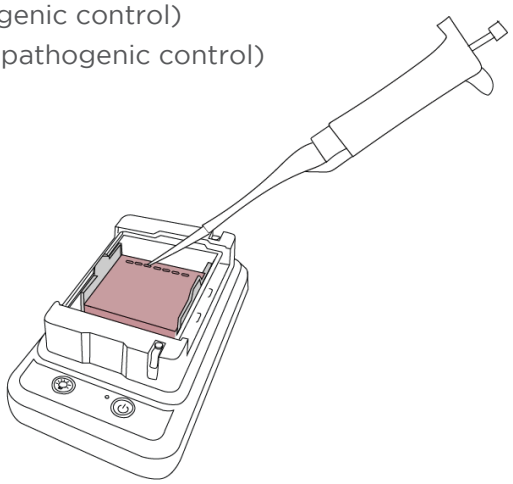


1. **Place the gel tray containing your gel in the buffer chamber**
   - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
   - The wells of the gel should be on the same side as the negative electrode, away from the power button.

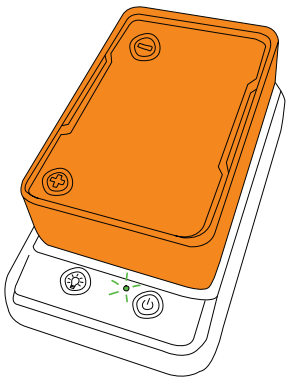
2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

3. **Load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl DNA Ladder
   - **Lane 2:** 12 µl Tube A (PCR product for food sample A, space sushi)
   - **Lane 3:** 12 µl Tube B (PCR product for food sample B, space burger)
   - **Lane 4:** 12 µl Tube P (PCR product for pathogenic control)
   - **Lane 5:** 12 µl Tube NP (PCR product for non-pathogenic control)
   - **Lane 6:** 12 µl Tube AX (restriction digest for food sample A, space sushi)
   - **Lane 7:** 12 µl Tube BX (restriction digest for food sample B, space burger)
   - **Lane 8:** 12 µl Tube PX (restriction digest for pathogenic control)
   - **Lane 9:** 12 µl Tube NPX (restriction digest for non-pathogenic control)

   Note: Change pipette tips between samples to prevent contamination.

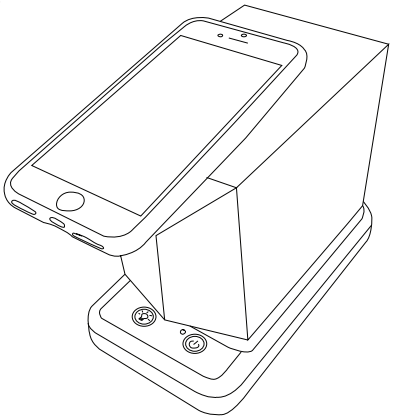


4. **Place the orange cover on the blueGel™ electrophoresis system**
    - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
    - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
    - The electrodes of the lid should be aligned with the metal leads on the base.
    - The orange lid should sit flush with the blue base using little force.



5. **Press the "Run" button**
    - Check that the green light beside the power button remains illuminated.

6. **Conduct electrophoresis for 15-25 minutes**
    - Note: Check the gel every 10 minutes to monitor sample migration.
    - Longer electrophoresis times will result in better size resolution. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

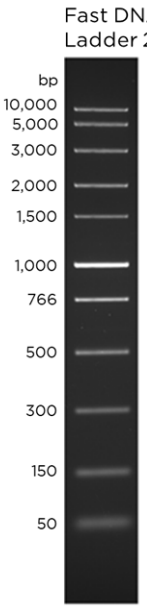


Gel electrophoresis - Visualizing results

1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
    - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
    - Gels may be viewed at the end of the run or periodically throughout the run.
    - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient band separation in the 150-500 bp range**
    - Run the gel longer if needed to increase resolutionPlace

3. **Document your results**
    - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
    - Compare the bands from the DNA samples to the ladder to obtain size estimates.



7. Study questions

Questions before experimental set-up

1. What is a foodborne disease outbreak, and why do they occur?

2. How are outbreaks of foodborne disease detected?

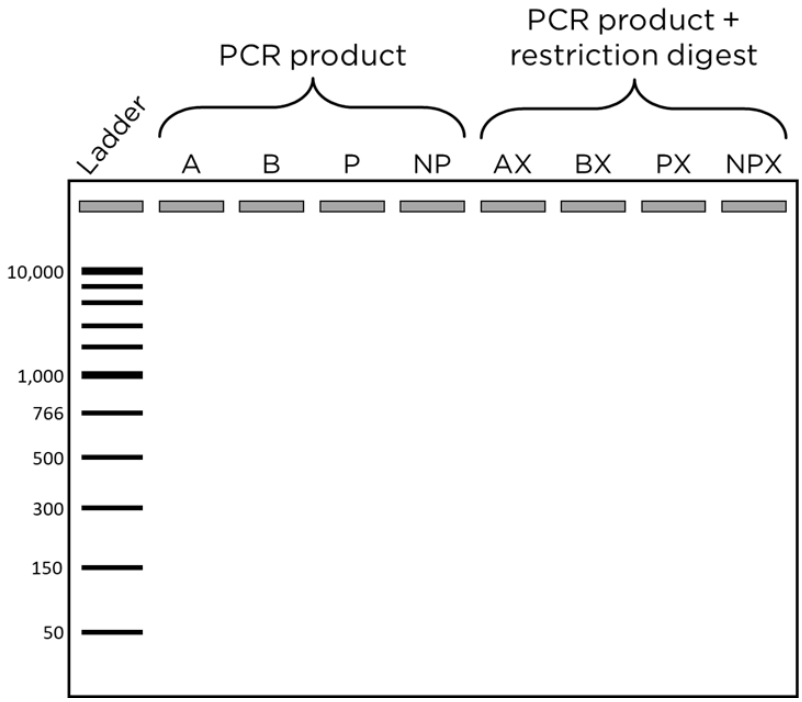
3. What is the *E. coli* O157:H7 strain? What human disease can it be responsible for?

4. What type of protein does the *E. coli* H antigen gene encode? How can this gene be useful in food safety biotechnology?

5. Why do we need to use PCR to detect a bacterial contamination in the food supply?

6. Following PCR amplification of the gene encoding the flagellar H antigen, how can we determine whether it is of the pathogenic or non-pathogenic kind?

Questions after gel electrophoresis and visualization



1. Sketch your experimental results on the image to the right.

2. What conclusions can you draw from lanes 2-5 (the PCR products)?

3. What conclusions can you draw from lanes 6-9 (the restriction digested PCR products)?

4. Based on your results, which food is contaminated? Explain how you can tell.

Discussion questions

1. How can biotechnology help prevent outbreaks of foodborne illness?

2. How can biotechnology help after an outbreak of foodborne illness is detected?