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

Instructor's Guide Contents

1. Synopsis

Students will help the International Space Program control an outbreak of pathogenic bacteria affecting space food in transit to Mars. They will do so by deploying essential molecular biology techniques such as PCR (polymerase chain reaction), restriction digest, and gel electrophoresis. This lab depicts real-world biotechnology applications in public health and surveillance.

- ***Techniques utilized***: PCR, DNA restriction endonuclease analysis, electrophoresis
- ***Time required***: One 110-min. period or two 55-min. periods
- ***Reagents needed***: miniPCR Food Safety Lab kit, gel electrophoresis reagents (See Sections 5 and 9)
- ***Suggested skill level***: Familiarity with DNA amplification and restriction analysis concepts, basic familiarity with micropipetting techniques

2. Learning goals and skills developed

Student Learning Goals:

- Understand the basic structure of DNA and its role in genetic inheritance
- Understand that PCR is a technique for amplifying specific parts of the genome
- Distinguish between pathogenic vs non-pathogenic bacterial strains
- Learn the role of certain *E. coli* strains in enterohemorrhagic diarrhea outbreaks
- Learn about bacterial diversity and use of genetic markers in serotype detection
- Discuss the use of DNA analysis in food safety and in public health surveillance

Scientific Inquiry Skills:

- Students will create hypotheses and predict results
- Students will compare their results to their predictions
- Students will generate tables to present their results
- Students will use experimental results to make conclusions based on hypotheses
- Students will follow laboratory safety protocols

Molecular Biology Skills:

- Principles and practice of PCR
- Preparation of agarose gels
- Agarose gel DNA electrophoresis
- Staining, visualization, and molecular weight analysis of DNA fragments
- Restriction digest of DNA fragments
- Analysis of restriction fragment length polymorphisms (RFLP)

Disclaimer: no pathogenic materials used

This experimental protocol engages students in a simulated investigation of *E. coli* contamination in the food supply chain. It uses the same scientific principles and experimental techniques as real-world food safety investigations, but does not require handling of potentially harmful or pathogenic biological samples. *None of the materials provided in the Food Safety laboratory kit pose a health or food safety hazard.* At no point are pathogenic bacteria used; references to "pathogenic" and "non-pathogenic" strains or DNA samples are used only to recreate a simulated Food Safety investigation scenario.

3. Standards alignment

Next Generation Science Standards - Students will be able to…

HS-LS1-1 Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

HS-LS2-7 Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.

HS-LS3-1 Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

HS-LS3-2 Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

HS-LS4-1 Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.

HS-LS4-4 Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

Common Core English Language Arts Standards - Students will be able to…

WHST.9-12.2 Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.

WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.

SL.11-12.5 Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest.

4. Scenario overview

An *E. coli* outbreak affects astronaut food aboard the International Space Station. DNA samples from two food racks are analyzed to determine which one might be responsible for the outbreak

The DNA Times

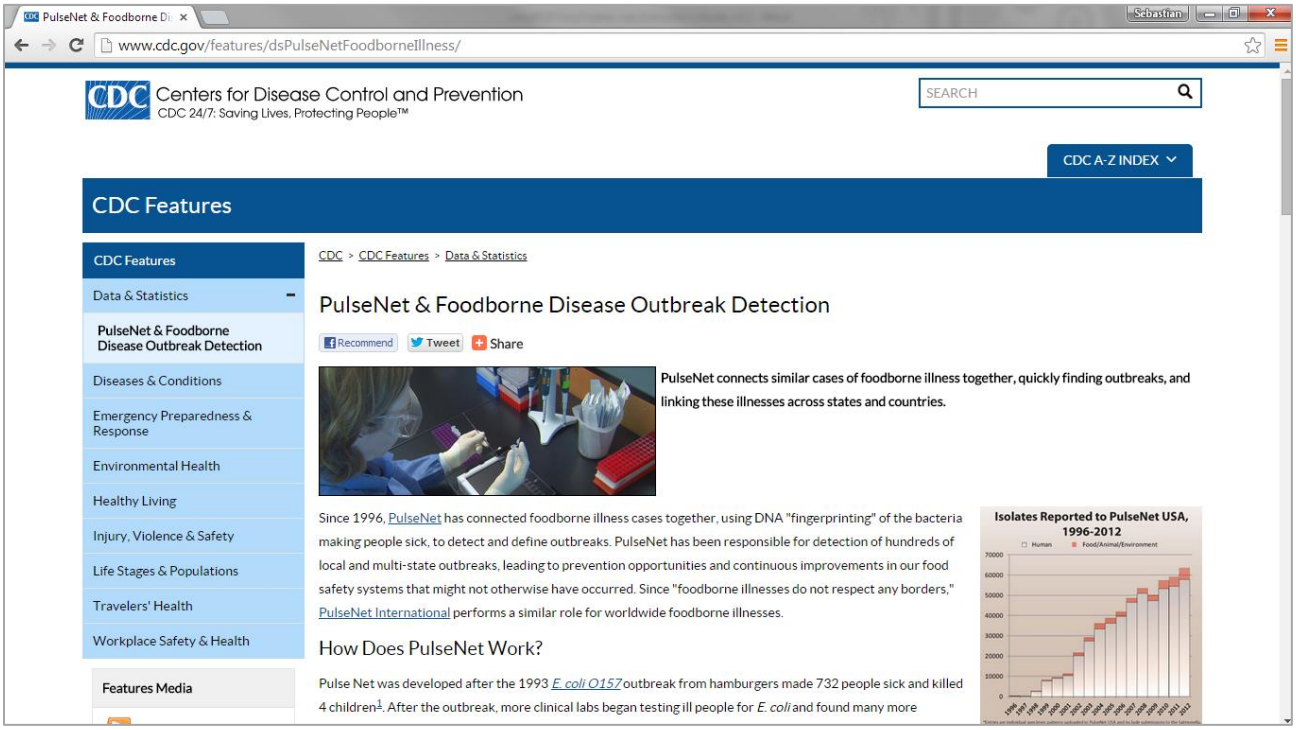
Tainted food aboard Space Station Mars colony at risk!

An *E. coli* outbreak threatens the Space Colonization Program. Armed with food samples in transit to Mars, students will use DNA technology to find the source of this interstellar foodborne illness outbreak

Houston, October 30th, 2021. A case of enterohemorrhagic *E. coli* infection has been detected in an astronaut aboard the International Space Station. The suspect is food cargo in transit to the International Mars Colonies. Until the source of the outbreak can be identified, the International Space Agency has halted its mission to resupply Mars, threatening the future of humans on the red planet.

Science students volunteer with high-tech DNA analysis equipment to identify the source of this unsavory outbreak.

Foodborne illness outbreaks are a public health concern on Earth too



Source: Centers for Disease Control and Prevention PulseNet & Foodborne Disease Outbreak Detection (http://www.cdc.gov/features/dsPulseNetFoodborneIllness/)

- **Outbreaks of *E. coli* O157:H7 and other foodborne pathogens occur every year**
- **A single outbreak can last several months**
    - O157:H7 infection results in 2,100 hospitalizations annually
    - Recalls of millions of pounds of food
- **Molecular DNA analysis can help detect and stop the spread of infection**
    - Fast and specific detection of low concentrations of microorganisms
- **The CDC PulseNet's national laboratory network of 87 laboratories has the capacity to match up bacteria from sick people using DNA fingerprinting**
    - Learn more: http://www.cdc.gov/pulsenet/

5. Laboratory set-up manual

Reagent	Volume needed per lab group	Storage	Teacher's checklist
2X EZ PCR Master Mix, Load Ready™ • 2x Mix with *Taq* DNA polymerase • dNTPs (included) • PCR buffer with Mg^{2+} (included) • Gel loading dye (included)	75 µL	-20°C freezer	
3X Food Safety Primer Mix • Pair of *'Food Safety'* primers	50 µL	-20°C freezer	
Template DNA • *Sample A DNA* • *Sample B DNA* • *Pathogenic Control DNA* (control P) • *Non-pathogenic DNA* (control NP)	10 µL each	-20°C freezer	
Restriction enzyme • *XmnI*	4 µL	-20°C freezer	
100 bp DNA Ladder, Load Ready™ • Molecular weight marker	10 µL	-20°C freezer	
DNA staining agent • e.g., GreenView Plus (for blue light illuminators)	Follow supplier instructions, usually 10,000x	Protect from light	
2% agarose gel • Electrophoresis grade agarose	9 gel lanes per Lab group	Room temp.	
Electrophoresis buffer • e.g., 1X TBE if using blueGel™	~50 ml if using blueGel™	Room temp.	

Supplied in Kit

Equipment and Supplies	Checklist
PCR Thermal cycler: e.g. miniPCR™ machine	
Micropipettes: • 0.5-10 µL: one for the whole class • 2-20 µL: one per lab group • 20-200 µL: one for the teacher	
Disposable micropipette tips	
PCR tubes: 8 x 200 µL microtubes per lab group	
Microtubes: 7 x 1.5 or 1.7 mL tubes per lab group to aliquot reagents	
Horizontal gel electrophoresis apparatus: e.g., blueGel™	
DC power supply for electrophoresis apparatus (included with blueGel™)	
Transilluminator: UV or Blue light (included with blueGel™)	
Scale for weighing agarose	
250ml flasks or beakers to dissolve agarose	
Microwave or hot plate to dissolve agarose	
Microcentrifuge (optional; only needed to collect liquid at tube bottom)	
Gel documentation system (optional, or use cell phone camera instead)	
Other supplies: • Heat block or water bath (not needed if using miniPCR) • UV safety goggles (only if using UV transilluminator) • Disposable laboratory gloves • Permanent marker	

Planning your time

This experiment has 5 stages:

A. PCR reaction set up
B. PCR programming and monitoring
C. Restriction digest
D. Separation of PCR products and restriction fragments by DNA electrophoresis
E. Size determination of restriction fragments and interpretation

An overview of the 110-minute experimental plan is represented below:

Preparatory activity	Experimental stage
Dispense reagents and prepare equipment • *15 min*	**A PCR set up** • 15 min
	B PCR programming & monitoring • 5 min PCR programming • 40 min PCR monitoring
STOP *Possible stopping point (store PCR product in fridge or freezer)*	
	C Restriction digest • 10 min set-up • 15 min incubation
STOP *Possible stopping point (heat inactivate at 65°C, store in freezer)*	
Pour agarose gels (e.g. during stage B) • *5 min*	**D Gel electrophoresis** • 20 min
	E Size determination & interpretation • 5 min visualization, discussion

Quick guide: Preparatory activities

A. **PCR set up and aliquot reagents**

- Thaw tubes containing the primers and DNA samples by placing them on a rack or water bath at room temperature
- For each lab group conducting 4 PCR reactions label and dispense six tubes:
  - PCR Master Mix 75 µL
  - Primer Mix 50 µL
  - Sample A DNA 10 µL
  - Sample B DNA 10 µL
  - Control P DNA 10 µL
  - Control NP DNA 10 µL
- Each lab group will additionally need (at a minimum):
  - Micropipettes *(we recommend a 2-20µL micropipette for each lab group)*
  - Disposable micropipette tips and a small beaker or cup to dispose them
  - 8 PCR tubes (200 µL)
  - A permanent marker (fine-tipped)

B. **PCR programming and monitoring**

- Ensure each lab group's bench is set up with a miniPCR and power supply
- Ensure the miniPCR machines that are going to be monitored through the PCR reaction are connected to a computer or compatible tablet

C. **Restriction digest**

- Have a 0.5-10µL micropipette, which can be shared across lab groups

D. **Gel electrophoresis**

- Gels can be poured in advance of the class (as described below)
- Pre-poured gels can be stored in the fridge, in a sealed container or wrapped in plastic wrap, and protected from light
- If running the gel on a different day than the PCR, completed PCR tubes can be stored in the fridge for up to one week until they are used, or in the freezer for longer-term storage

E. **Size determination and interpretation**

- Have the banding pattern of the 100 bp DNA Ladder handy to help interpret the electrophoresis results (see page 16)

6. Instructor laboratory guide

A *PCR set up*

1. **Label 4 PCR tubes** (200 µL tubes) per lab group
   - 1 tube labeled "A": for Space Sushi DNA sample (sample A)
   - 1 tube labeled "B": for Space Burgers DNA sample (sample B)
   - 1 tube labeled "P": Control PCR for pathogenic *E. coli* DNA
   - 1 tube labeled "NP": Control PCR for non-pathogenic *E. coli* DNA

   ⚠ Also label each tube with the group's name on the side wall

2. **Add PCR reagents** to each 200 µL PCR tube

	Tube A	**Tube B**	**Tube P**	**Tube NP**
Template DNA	Sample A, 5 µL	Sample B, 5 µL	Control P, 5 µL	Control NP, 5 µL
Primer Mix	10 µL	10 µL	10 µL	10 µL
PCR Master Mix	15 µL	15 µL	15 µL	15 µL
TOTAL VOLUME	***30 µL***	***30 µL***	***30 µL***	***30 µL***

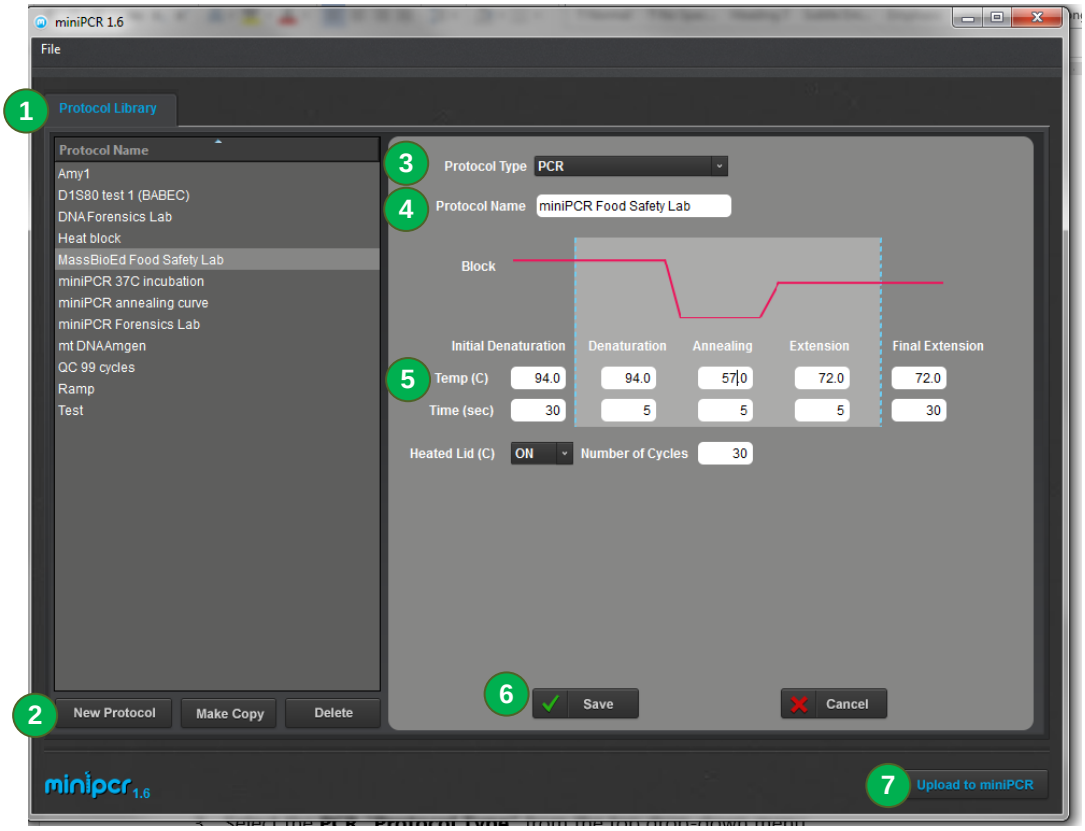
⚠ Use a micropipette to add each of the reagents.
Remember to change tips at each step!

3. **Gently mix the reagents by pipetting up and down 3-4 times, cap the tubes**
   - Make sure all the liquid volume collects at the bottom of the tube (if necessary, spin the tubes briefly using a microcentrifuge.)

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

B *PCR programming and monitoring (illustrated using miniPCR™ software)*

1. Open the miniPCR™ software app and remain on the **"Protocol Library"** tab



2. Click on the **"New Protocol"** button on the lower left corner
   - *Optional*: select existing protocol programmed in advance, skip to step 7

3. Select the **PCR "Protocol Type"** from the top drop-down menu

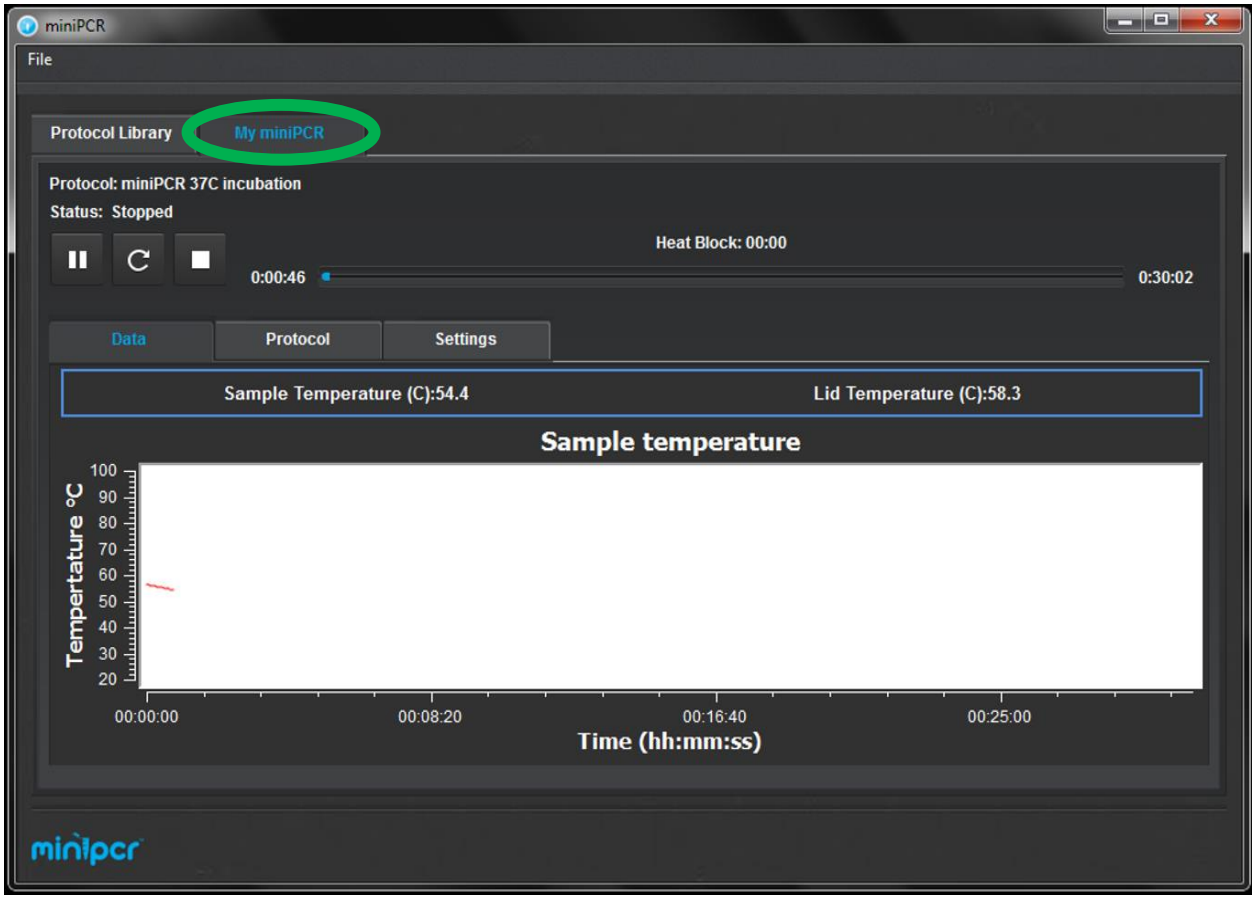
4. Enter the Protocol Name; for example **"Group 1 – Food Safety Lab"**

5. 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**: 25 or 30 cycles (25: faster, 30: stronger bands)
   - **Final Extension**: 72°C, 30 sec
   - **Heated Lid**: ON

6. Click **"Save"** to store the protocol

7. Click **"Upload to miniPCR"** (and select the name of your miniPCR™ machine in the dialogue window) to finish programming the thermal cycler. Make sure that the power switch is in the ON position.

8. Click on **"miniPCR [machine name]"** tab to monitor the PCR reaction



The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, discussing the PCR process, and to export the reaction data for analysis.

Once the PCR run is completed (approximately 30 min), the screen will show "**Status: Completed" and all LEDs on your miniPCR machine will light up.**

You can now open the miniPCR lid and remove your PCR tubes.

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

The PCR product can be stored for up to 1 week in the fridge, one year in the freezer

C ***Restriction digest*** *(this step will use half of the volume in the PCR tubes)*

1. **Before the end of the PCR run, label 4 new PCR tubes** (200 µL) per lab group
   - 1 tube labeled "AX": PCR product from Space Sushi DNA (A)
   - 1 tube labeled "BX": PCR product from Space Burgers DNA (B)
   - 1 tube labeled "PX": PCR product from Pathogenic *E. coli* control
   - 1 tube labeled "NPX": PCR product from Non-Pathogenic *E. coli*

2. At the end of your PCR run, **carefully remove 15 µL of PCR product from each tube and transfer it into the new tubes** as follows:

	Tube AX	Tube BX	Tube PX	Tube NPX
PCR Product	PCR A	PCR B	PCR P	PCR NP
Volume transferred	15 µL	15 µL	15 µL	15 µL

You will be transferring half of the PCR volume into each tube. Save the other half inside the tube for later analysis!

3. **Add 1 µL of Restriction Enzyme (XmnI) into each of the four new tubes**
   - Tubes **AX, BX, PX, NPX**
   - Pipette the restriction enzyme **directly into the** PCR product solution
   - Pipette up and down several times to mix well
   - Cap the tubes ensuring the liquid remains at the bottom

4. Program PCR machine (or a water bath) for a **37°C incubation for 15 minutes**
   - If using miniPCR™:
     - Select "Heat Block" Protocol Type (drop-down menu)
     - Enter the Protocol Name (e.g. 'XmnI digest')
     - Input the incubation Temperature and Time: 37°C, 15 min
     - Save and upload to miniPCR

5. **Insert the 4 tubes** containing Restriction Enzyme and PCR product in the miniPCR machine or water bath at 37°C
   - AX, BX, PX, NPX tubes

6. **Remove the tubes** after completing the 15-minute incubation

Storage (optional stopping point): If the restriction digest will not be used immediately for gel electrophoresis, it can be stored frozen for one week, but first the XmnI enzyme must be heat inactivated by incubating at 65°C for 20 min.

D *Gel electrophoresis – Pouring agarose gels (Preparatory activity)*

TIME MANAGEMENT TIP

If the lab is going to be completed in a single time block, agarose gels should be prepared during the PCR run to allow the gels to settle.

If the lab is going to be performed over two periods, gels can be prepared up to one day ahead of the second period and stored in a refrigerator, covered in plastic wrap and protected from light.

1. Prepare a clean and dry agarose gel casting tray
   - Place a well-forming comb at the top of the gel (9 lanes per group)

2. For each lab group, prepare a 2% agarose gel using electrophoresis buffer
   - Work with 1X electrophoresis buffer. *If needed, dilute 20X buffer to 1X working concentration in distilled H_2O*
   - Adjust volumes and weights according to the size of your gel tray *e.g., add 0.4 g of agarose to 20 ml 1X TBE buffer for blueGel™*
   - Mix reagents in glass flask or beaker and swirl

3. Heat the mixture using a microwave or hot plate (blueGel: 30-40 seconds)
   - Until agarose powder is dissolved and the solution becomes clear
   - Use caution, as the mix tends to bubble over the top and is very hot

4. Cool the agarose solution for about 2-3 min at room temperature
   - Swirling the flask intermittently

5. Add gel staining dye (e.g. GreenView Plus)
   - Follow dye manufacturer instructions
   - Typically, 1.0 µL of staining dye per 10 mL of agarose solution

   Note: Follow manufacturer's recommendations and state guidelines if handling and disposing of ethidium bromide

6. Pour the cooled agarose solution into the gel-casting tray

7. Allow gel to completely solidify (until firm to the touch) and remove the comb
   - Typically, 10-15 minutes

8. Place the gel into the electrophoresis chamber and cover it with buffer

D *Gel electrophoresis – Running the gel*

1. Make sure the gel is completely submerged in electrophoresis buffer.
    - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).
    - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to cover the gel and wells.

2. Load DNA samples onto the wells in the following sequence
    - Lane 1: **12 µL** of 100 bp DNA ladder
    - Lane 2: **12 µL** of PCR **Tube A** (product from 'Space Sushi')
    - Lane 3: **12 µL** of PCR **Tube B** (product from 'Space Burgers')
    - Lane 4: **12 µL** of PCR **Tube P** (product from 'Control P' DNA)
    - Lane 5: **12 µL** of PCR **Tube NP** (product from 'Control NP' DNA)
    - Lane 6: **12 µL** of Restriction Digest **Tube AX**
    - Lane 7: **12 µL** of Restriction Digest **Tube BX**
    - Lane 8: **12 µL** of Restriction Digest **Tube PX**
    - Lane 9: **12 µL** of Restriction Digest **Tube NPX**

    Note: there is no need to add gel loading dye to your samples. *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* are Load-Ready™!

3. Place the cover on the gel electrophoresis box

4. Insert the terminal leads into the power supply (not needed if using blueGel™)

5. If using blueGel™, simply press the "Run" button. In other systems, set the voltage at 100V. Conduct electrophoresis for 15-20 minutes, or until the colored dye has progressed to about half the length of the gel
    - Check that small bubbles are forming near the terminals in the box
    - Longer electrophoresis times will result in better size resolution

6. Once electrophoresis is completed, turn the power off and remove the gel from the box (not needed if using blueGel™ which has a built-in illuminator)

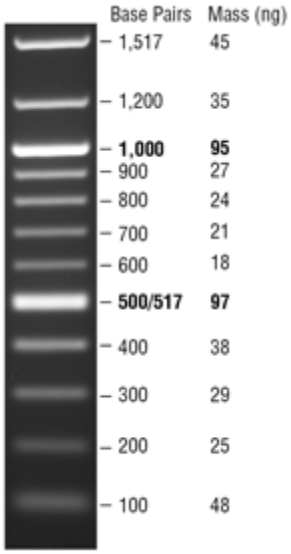
E *Size determination and interpretation*

1. Place the gel on the transilluminator (or turn on the blueGel™ illuminator)
   - *Wear UV-protective goggles if using UV light (not needed for blueGel™)*

2. Verify the presence of PCR product (lanes 2 through 5)

3. Verify the efficiency of the restriction digest (lanes 6 through 9)

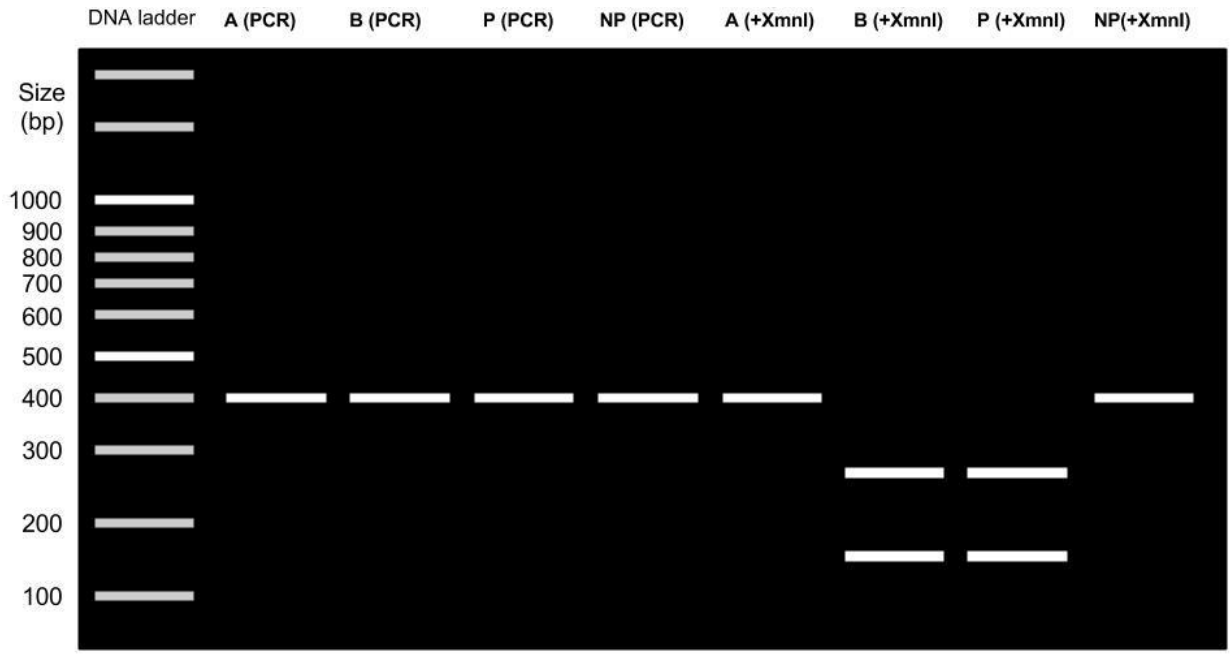
4. Ensure there is sufficient DNA band resolution in the 100-400 bp range of the 100bp DNA ladder
   - Run the gel longer if needed to increase resolution
   - You DNA ladder run should look approximately as follows:



5. Document the size of the PCR amplified DNA fragments
   - Capture an image with a smartphone camera (blueGel™)
   - If available, use a Gel Documentation system

Expected experiment results

Expected results:



This shows the idealized experimental results

- Intensity of the bands will depend on
  - the efficiency of the PCR reaction
  - the efficiency of gel-loading
  - the quality of the detection reagents and system
- The migration patterns of the DNA bands will vary with
  - the length of electrophoresis
  - the electrophoresis voltage
  - the efficiency of the restriction digest (for lanes 6 through 9 – the digest can be partial or complete
    - How many DNA bands would you see in a partial digest?

7. Study questions

1. ***Questions before experimental set-up***

   - What is a foodborne disease outbreak, and why do they occur?
   - How are outbreaks of foodborne disease detected?
   - What foods are most commonly associated with foodborne illness?
   - How can the food become contaminated?
   - What can be done to prevent and control foodborne illness?

   More information about foodborne illness outbreaks is available at the CDC website: http://www.cdc.gov/foodsafety/facts.html

- What is the *E. coli* O157:H7 strain? What human disease can it be responsible for?
  - It is the strain most frequently isolated from humans affected by enterohemorrhagic diarrhea caused by *E. coli* infection
  - O157:H7 infection is the predominant cause of hemolytic-uremic syndrome (HUS), which can lead to death

- What type of protein does the *E. coli* H antigen gene encode? How can this gene be useful in food safety biotechnology?
  - The H antigen gene encodes a subunit of the bacterial flagellum, a structure involved in bacterial motility
  - There are at least 56 types of flagellar H antigen proteins encoded by the bacterial *fliC* locus
  - The H7 antigen is associated with shiga-producing, enterohemorrhagic *E. coli* strains like the one studied in this lab
  - Molecular analysis of the H antigen serotype, in combination with the O antigen serotype, can help food safety professionals evaluate the potential pathogenicity of a bacterial strain

2. ***Questions during PCR run***

- What is happening to DNA molecules at each step?
  - Denaturation
  - Annealing
  - Extension

- Why do we need to add an enzyme (*Taq* DNA polymerase) to the PCR mix?

- What temperature do you think is optimal for most enzymes?

- What makes *Taq* DNA polymerase unique?

- How many more molecules of DNA will we have at the end of each cycle?
  - And at the end of the entire PCR reaction?

- How will we know whether the PCR reaction has worked?
  - And how can we recover the product?

3. ***Questions after gel electrophoresis and visualization***

- Looking at lanes 2-5, was the PCR reaction successful?
  - How can we tell?

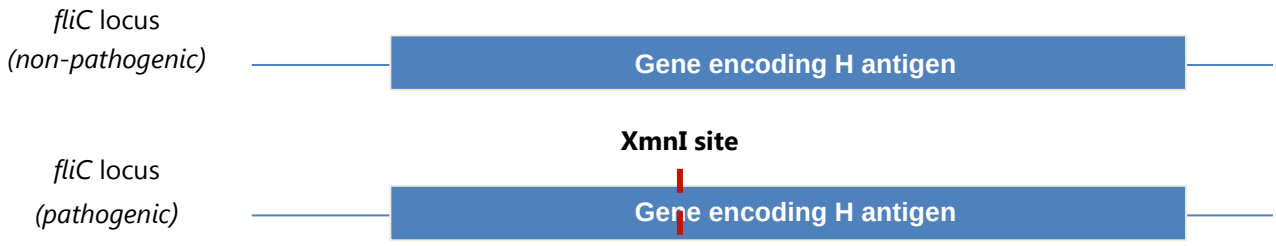
- Looking at lanes 6-9, was the restriction digest successful?
  - Was the restriction digest partial? How many bands would you see?
  - Was it a complete digest? How many bands would you see?
  - What could be the reasons for a partial digest? What experimental conditions would you adjust? (duration, enzyme concentration, other?)

- What organisms are restriction enzymes isolated from?
  - What have they been naturally selected for?
  - What other biotechnology uses exist for restriction enzymes?

- How are foodborne diseases diagnosed?
  - How can biotechnology help prevent outbreaks of foodborne illness?
  - How can biotechnology help after an outbreak of foodborne illness is detected?

- Why did we need to use PCR to detect a bacterial contamination in the food supply? Could we have used other detection methods?
  - The infective dose of O157:H7 pathogenic strains can be as low as 15 bacterial cells per gram of food, so very high sensitivity is needed
  - Such low bacterial count would be virtually impossible to detect without molecular DNA amplification (e.g. by antibody detection methods)

- 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?
  - We detect the presence of a restriction fragment length polymorphism (RFLP) in the gene associated with pathogenicity, schematically:



8. Student-centered investigations and extension activities

Student-Centered Investigations

1) Divide class into 2 sets of lab groups with 2 PCR tubes each

"Field inspectors" groups (2 PCR tubes each)

- 1 tube labeled "A": DNA sample from Food Sample A
- 1 tube labeled "B": DNA sample from Food Sample B

"NASA CONTROL Labs" groups (2 PCR tubes)

- 1 tube labeled "P": Control DNA P from a pathogenic strain
- 1 tube labeled "NP": Control DNA P from a non-pathogenic strain

Note: Use half the volumes specified in section 6 for each group

2) Analyze mixed-genotype samples

Set up an unknown sample consisting of mixed "A" and "B" DNA

- Result will be a mixed "pathogenic" and "non-pathogenic" sample
- Likely scenario in nature, as most samples contain multiple bacteria

Post-Lab Extension Activities

Lab Report – report on the findings of the written lab or further investigations

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

Student writing exercise – write a persuasive article about the use of biotechnology tools in public health and surveillance, and its applications in the food industry.

- Cite examples of real-world food poisoning outbreaks
- Investigate role of USDA in pathogen surveillance

Further resources:

- Outbreak Detection Since Jack in the Box: A Public Health Evolution
- CDC PulseNet Home
- www.genesinspace.org

9. Ordering information

To request miniPCR™ Food Safety Lab reagent kits, you can:

- Call (781)-990-8PCR
- email us at orders@minipcr.com
- visit www.minipcr.com

miniPCR™ Food Safety Lab kit (catalog no. KT-1001-03) contains:

- 2X EZ PCR Master Mix, Load-Ready™
  - including *Taq* DNA polymerase, dNTPs, PCR buffer, and gel-loading dye
- 3X Food Safety Lab Primer Mix
- Sample A DNA
- Sample B DNA
- Control P DNA ('pathogenic' control)
- Control NP DNA ('non-pathogenic' control)
- XmnI restriction endonuclease enzyme
- 100bp DNA ladder, Load-Ready™ (50µg/ml)

Materials are sufficient for 8 lab groups, or up to 32 students

All components should be kept frozen at -20°C for long-term storage

Reagents must be used within 12 months of shipment

Other reagents needed (not included in the kit):

- Agarose (electrophoresis grade)
- DNA staining dye (e.g. GreenView Plus)
- Gel electrophoresis buffer (e.g. TBE)
- Distilled or deionized H_2O

10. About miniPCR™ Learning Labs

This Learning Lab was developed jointly by **MassBioEd** and **miniPCR™** in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM.

We develop Learning Labs to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.
The guiding premise for this lab protocol is that a ~2-hour PCR-based lab plan developed around a real-life biotechnology application (in this case, a simulated investigation of a foodborne disease outbreak) provides the right balance between intellectual engagement, experimentation, inquiry, and discussion. The design of this lab has simplified certain elements to achieve these goals, but it is based on real-world science applications: the detection of pathogenic *E. coli* using PCR and restriction fragment length polymorphism analysis of different bacterial serotypes[1]. We use plasmid DNA instead of bacterial genomic DNA as the PCR template in order to increase the protocol's experimental robustness. We use a single-site cutter rather than a complex restriction banding pattern to simplify interpretation and discussion of the results. Similar designs have been incredibly effective for other very successful educational lab courses, to which we owe inspiration[2].

Starting on a modest scale working with Massachusetts schools, miniPCR™ Learning Labs have been received well, and their use is growing rapidly through academic and outreach collaborations.

Authors: Ezequiel Alvarez Saavedra, Ph.D., and Sebastian Kraves, Ph.D.

[1] See, for example: Wang L. *et al*., Sequence diversity of the *Escherichia coli* H7 fliC genes: implication for a DNA-based typing scheme for *E. coli* O157:H7. J Clin Microbiol. 2000 May;38(5):1786-90. PubMed PMID: 10790100; Machado J, *et al*., Identification of Escherichia coli flagellar types by restriction of the amplified fliC gene. Res Microbiol. 2000 Sep;151(7):535-46. PubMed PMID: 11037131.
[2] See, for example: Bouakaze C, *et al*. "OpenLAB": A 2-hour PCR-based practical for high school students. Biochem Mol Biol Educ. 2010 Sep;38(5):296-302. doi: 10.1002/bmb.20408. PubMed PMID: 21567848.