MassBioEd / miniPCR™ Food Safety Lab

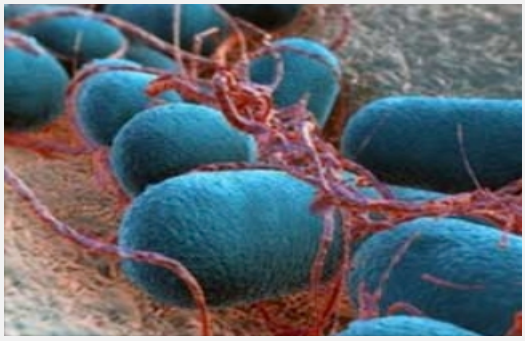
An *E. coli* outbreak in ground beef has tainted the food chain. DNA samples from two meat processing plants are analyzed to evaluate whether they might be responsible for the outbreak.

miniPCR news

Free

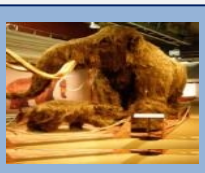
Tainted patties – burger lovers beware

An *E. coli* outbreak befuddles USDA investigators. With samples from two suspect meat processing plants, local students volunteer to try to find the facility behind the food poisoning of at least 26 unwitting burger lovers.



Boston, October 30th, 2025. A maker of frozen patties has recalled more than 180,000 pounds of its products after some were linked to several cases of enterohemorrhagic *E. coli* infection. Massachusetts-based Happy Cow Catering has recalled products distributed in Arizona, California, Nevada, Texas, Utah, Oregon, and New Mexico, the U.S. Dept. of Agriculture says.

So far, 26 people in three states have been diagnosed with enterohemorrhagic *E. coli* infection, the USDA says. Fifteen of them claim having eaten beef patties traced to Happy Cow Catering, which supplies mid-sized grocery store chains such as Shopper's Heaven with "gourmet" products. The Food Safety and Inspection Services has collected samples from two beef processing plants that supply meat to Happy Cow Catering. A godsend for science students, who volunteer with high-tech DNA analysis equipment to identify the plant responsible for this hard-to-digest outbreak.



Experiment overview and instructions

A. PCR set up

1. **Check that you have six 1.5 mL tubes** on your bench:
    - PCR Master Mix
    - DNA Primer Mix
    - Meat Processing Plant A DNA
    - Meat Processing Plant B DNA
    - Control P DNA
    - Control NP DNA

2. **Label 4 small plastic PCR tubes** (200 µL tubes), on the side wall of the tubes:
    - Tube "A": PCR for meat processing plant A DNA
    - Tube "B": PCR for meat processing plant B DNA
    - Tube "P": Control PCR for pathogenic *E. coli* DNA
    - Tube "NP": Control PCR for non-pathogenic *E. coli* DNA

3. **Add the following to each PCR tube:**

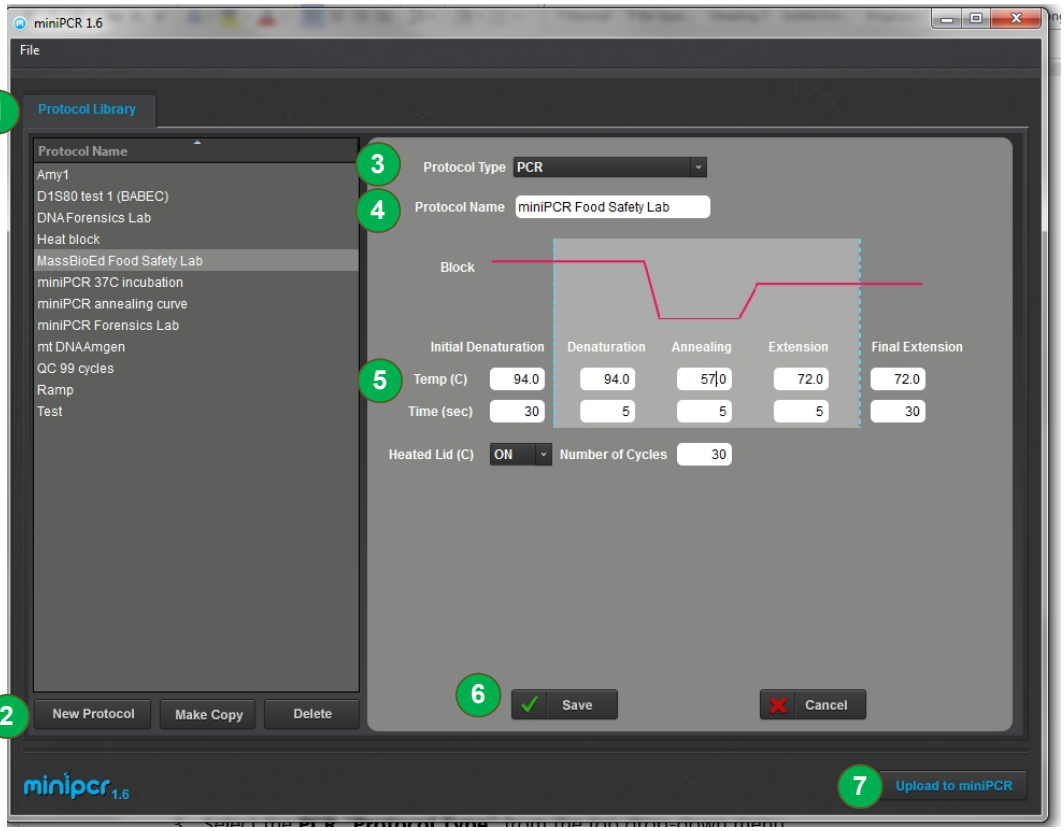
- Pipette each reagent up and down 3-4 times to mix with other liquids in the tube
- Change pipette tips at each pipetting step

- PCR Master Mix — 15 µL per tube
  (Same for all reaction tubes)
- DNA Primer Mix — 10 µL per tube
  (Same for all reaction tubes)
- Template DNA sample — 5 µL per tube
  (Specific for A, B, P, or NP tubes)

4. **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.)

5. **Place the tubes inside the PCR machine**

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

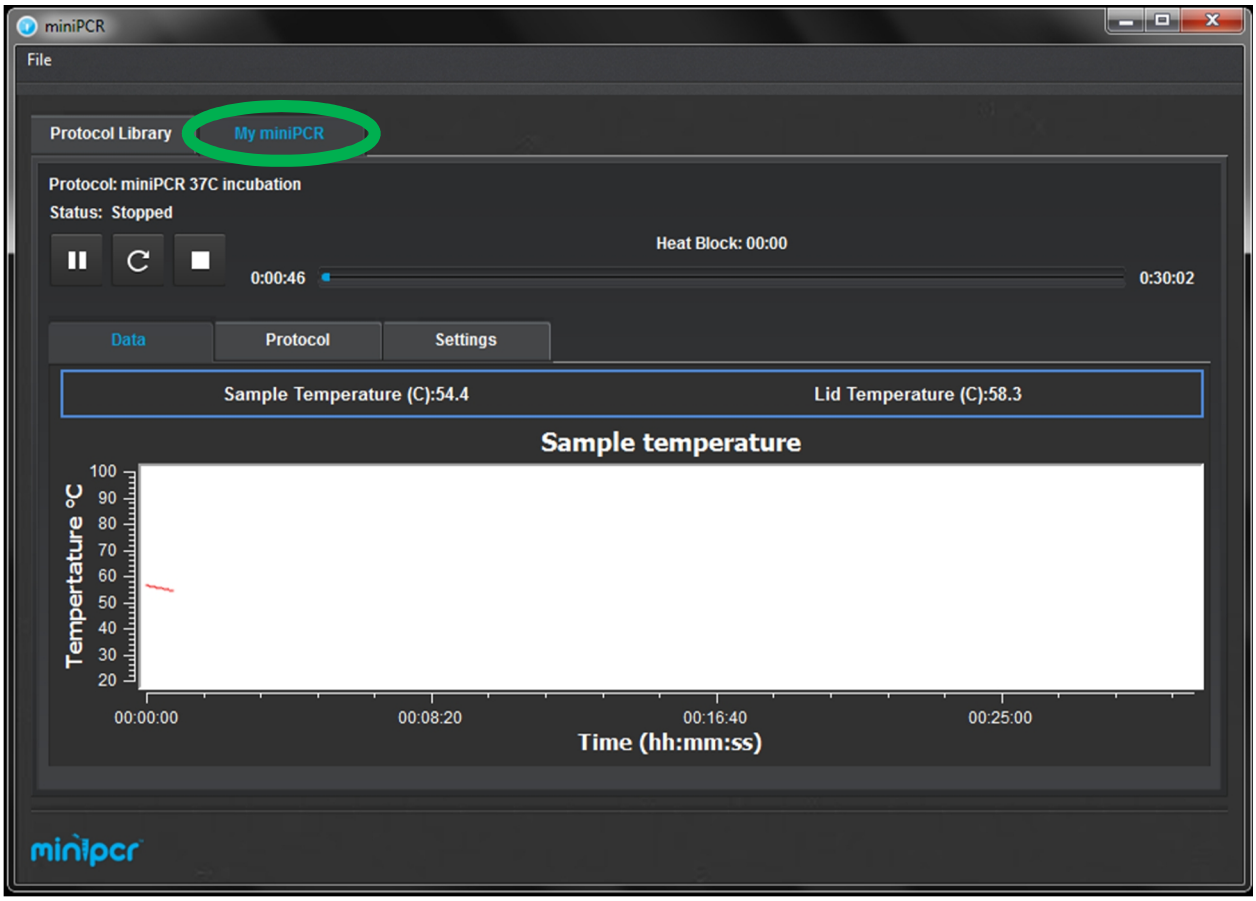
4. Enter the Protocol Name; for example **"Group 1 – Food Safety Lab"**

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: more robust)
   - **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 begin monitoring the PCR reaction



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

10. At the end of the run, the screen will show "Status: Completed" and all LEDs on your miniPCR machine will light up.
    - You can now open the lid and remove your tubes, ***being careful not to touch the metal lid which may still be hot***

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

1. Obtain from your teacher the **Restriction Enzyme Mix tube**

2. At the end of your PCR run, **label 4 new plastic tubes** (200 µL)
    - 1 tube labeled "A+X": PCR product from meat processing plant A
    - 1 tube labeled "B+X": PCR product from meat processing plant B
    - 1 tube labeled "P+X": PCR product from pathogenic *E. coli* control
    - 1 tube labeled "NP+X": PCR product from non-pathogenic *E. coli*

3. **Add 5 µL of Restriction Enzyme Mix** to the bottom of each tube

4. **Carefully add 15 µL of PCR product** to each tube:

- Pipette each reagent up and down 3-4 times to mix with other liquids in the tube
- Use a new pipette tip for each tube

    - Tube "A+X": Add **15 µL** from PCR Tube "A"
    - Tube "B+X": Add **15 µL** from PCR Tube "B"
    - Tube "P+X": Add **15 µL** from PCR Tube "P"
    - Tube "NP+X": Add **15 µL** from PCR Tube "NP"

5. 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: 37°C
        - Input the desired Time: 15 minutes
        - Save and upload to miniPCR

6. **Insert the 4 tubes** containing Restriction Enzyme Mix and PCR product (A+X, B+X, P+X, NP+X tubes) in the miniPCR or water bath at 37°C

7. **Remove the tubes** after completing the 15 minute incubation

Gel electrophoresis – Pouring agarose gels (Preparatory activity)

1. Prepare a clean and dry agarose gel casting tray
    - Seal off the ends of the tray as indicated for your apparatus
    - Place a well-forming comb at the top of the gel (10 lanes or more)

2. Prepare a 2% agarose gel using 1X TAE buffer
    - Add 1 g of agarose to 50 ml of 1x TAE buffer
    - Mix reagents in glass flask or beaker and swirl

3. Heat the mixture using a microwave or hot plate
    - 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, swirling intermittently

5. Add gel staining dye (DNA intercalating agent)
    - Follow dye manufacturer instructions
    - Typically, 1-10 µL of staining dye per 100 mL of agarose solution
    - *Note: Follow manufacturer's recommendations and state guidelines when 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, 15 minutes

8. Place the gel into the electrophoresis chamber and cover it with 1X TAE buffer

Gel electrophoresis – Running the gel

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

2. Add 6X gel loading buffer into the samples you will electrophorese:
    - 5 µL into each PCR tube (tubes A, B, P, N)
    - 5 µL into each Restriction Digest tube (A+X, B+X, P+X, NP+X)
    - 5 µL into the tube of 100 bp DNA ladder (*provided by your teacher*)

3. Load reactions in the following sequence
    - Lane 1: **10 µL** of 100 bp DNA ladder
    - Lane 2: **15 µL** of PCR product from 'Plant A' DNA
    - Lane 3: **15 µL** of PCR product from 'Plant B' DNA
    - Lane 4: **15 µL** of PCR product from 'Control P' DNA
    - Lane 5: **15 µL** of PCR product from 'Control NP' DNA
    - Lane 6: **20 µL** of Restriction Digest from 'Plant A' PCR product
    - Lane 7: **20 µL** of Restriction Digest from 'Plant B' PCR product
    - Lane 8: **20 µL** of Restriction Digest from 'Control P' PCR product
    - Lane 9: **20 µL** of Restriction Digest from 'Control NP' PCR product
    - Lane 10: **10 µL** of 100 bp DNA ladder

4. Place the cover on the gel electrophoresis box
    - Ensure the electrode terminals fit snugly into place

5. Insert the black and red leads into the power supply

6. Set the voltage at 100-130V and conduct electrophoresis for 15 minutes, or until the loading buffer dye has progressed to about half the length of the gel
    - Check that small bubbles are forming near the terminals in the box

7. Once electrophoresis is completed, turn the power off and remove the gel from the box

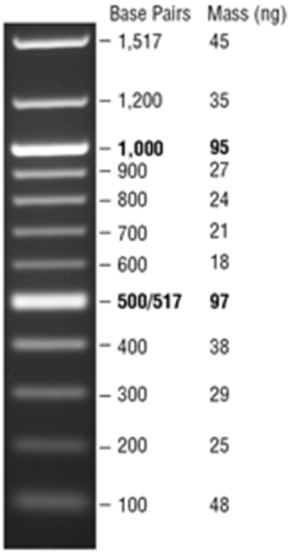
D. Size determination and interpretation

1. Place the gel on the transilluminator
    - *Wear UV-protective goggles if using UV light*

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 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
    - If available, use a Gel Documentation system