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

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.

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 (A)
   - 1 tube labeled "B": for Space Burgers DNA 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***

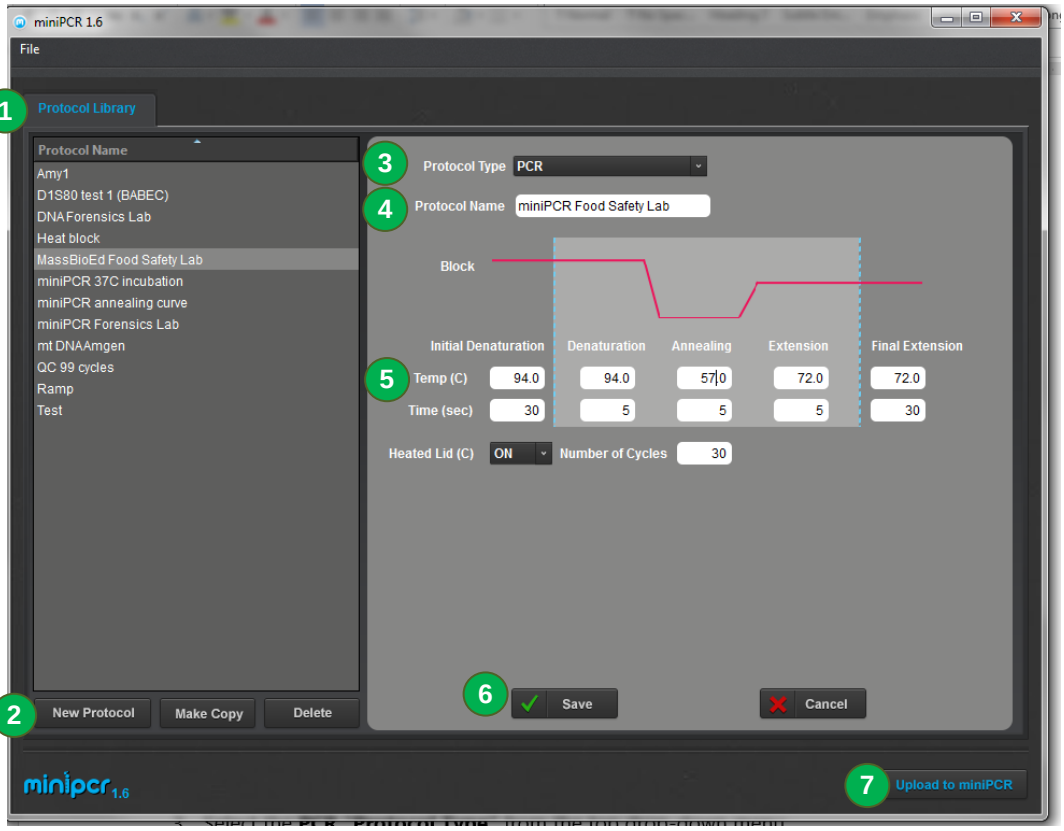
 Use a micropipette to add each of the reagents.
Remember to change tips at each step!

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

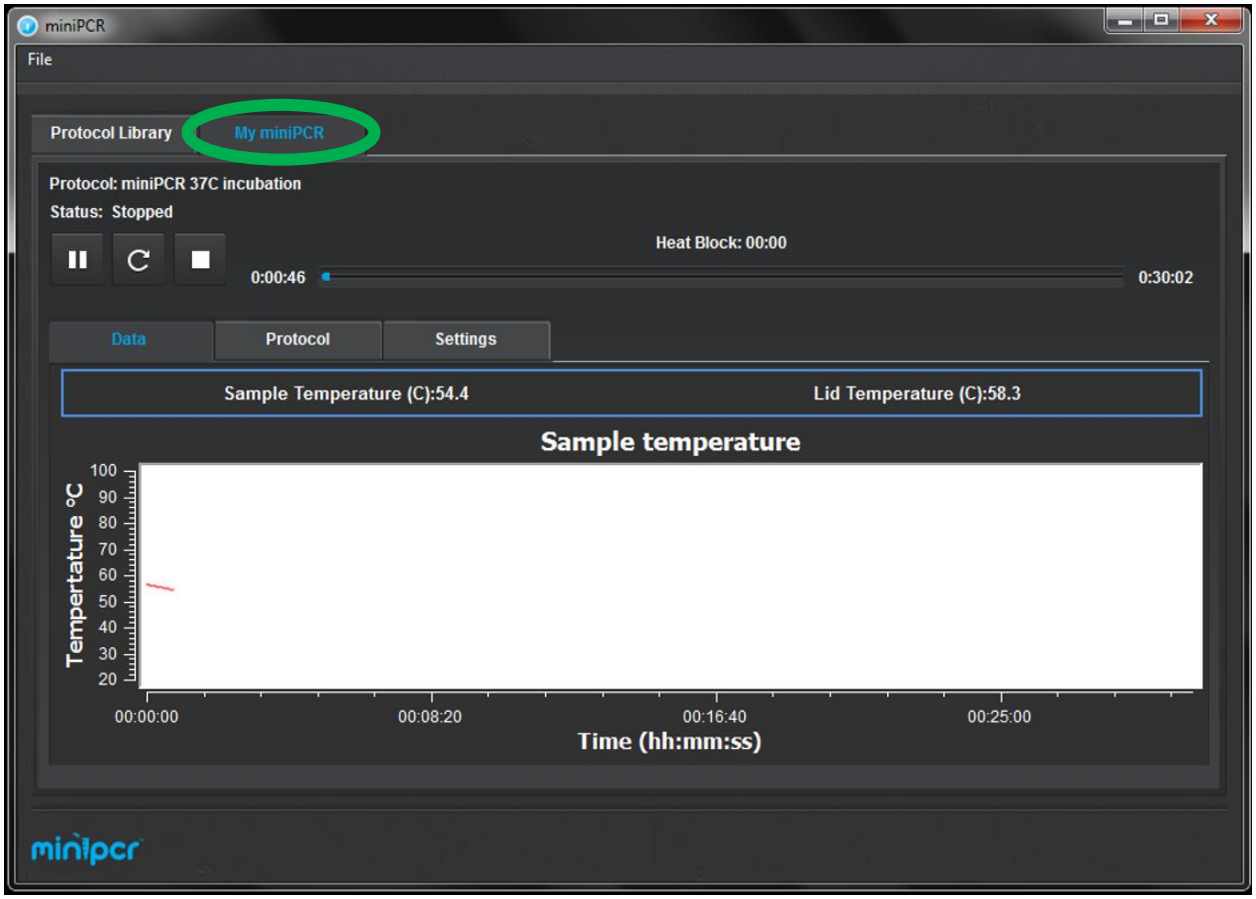
4. Enter the Protocol Name; for example **"Group 1 – Food Safety Lab"**

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 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 – Running the gel*

1. Make sure the gel is completely submerged in 1X 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)

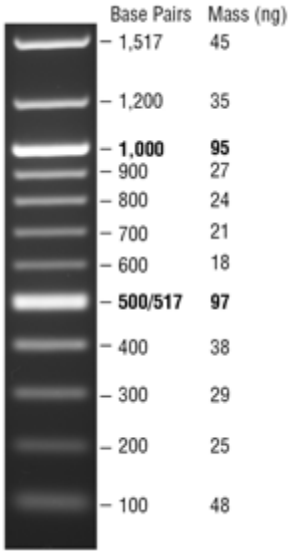
E *Size determination and interpretation*

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 1 through 4)

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