TS Biology Lab Report | Fall 2021

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Diagram of Main Steps of the Lab Series

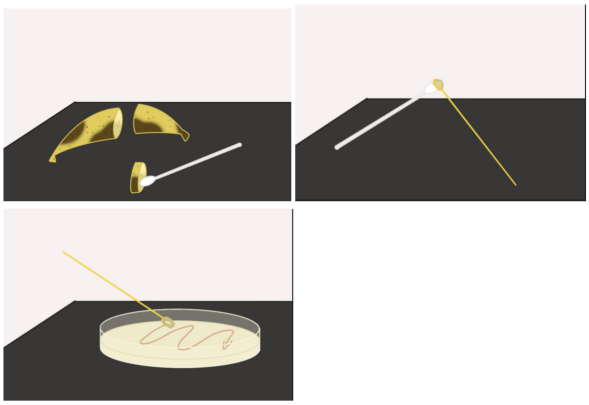
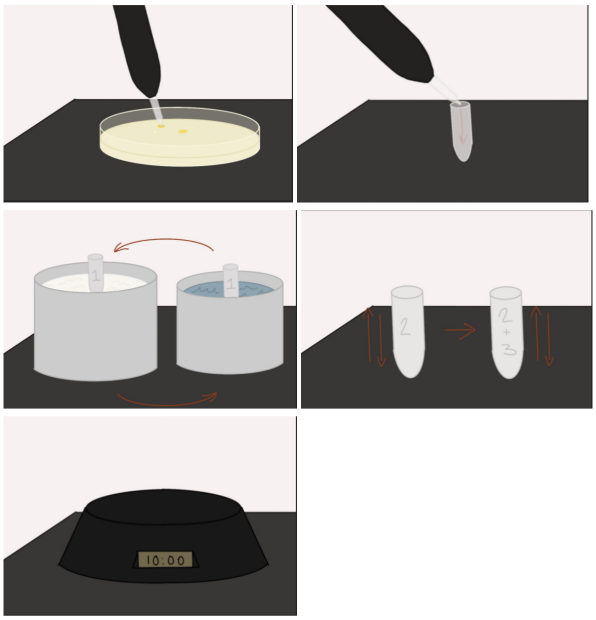
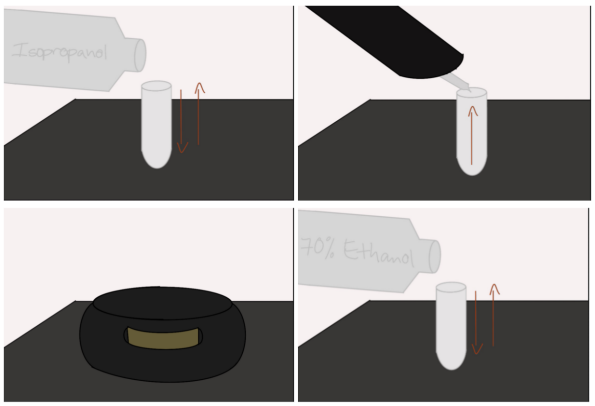
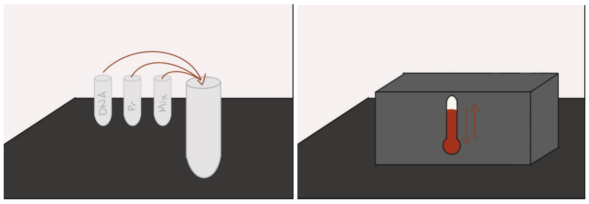
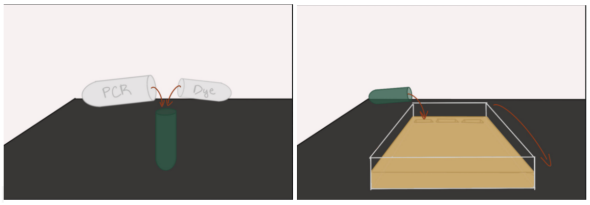
Figure A. Illustration of step 1 (Collecting Samples).	
Figure B. Illustration of step 2 (Extraction of DNA).	

Figure C. Illustration of step 2 (Purification of DNA).	
Figure D. Illustration of step 3 (PCR).	
Figure E. Illustration of step 4 (Gel Electrophoresis).	

Description of the Samples Tested in the Lab Series

Step 1: Sample Collection	Step 2 (refer to Figures 2 and 3): Extraction and Purification of DNA	Step 3: PCR	Step 4 (refer to Figure 1): Gel Electrophoresis
Banana Swab	A: white-yellow, center. A△: white, bottom left. A△: white, bottom left. A●: yellow, top left. A◯: smallest	A A△ A● A◯	A: located in the second well from the left. A△: located in the third well from the left. A●: located in the last well from the left. A◯: located in the

	sample, top center.		first well on the very left.
Face Swab	Did not yield any samples.		

Materials & Methods

For sample collection (step 1):

- 2 agar plates: The samples were placed on agar plates in order for them to grow bacteria.
- Sterile inoculation loops: These were used to transfer the samples to the agar plates.

Using the sterile inoculation loops, samples brought in were swabbed and transferred to two agar plates (Figure A). The agar plate holding the banana peel samples was named plate A; the agar plate holding the face swab samples was named plate D.

For extraction and purification of DNA (step 2):

- Pipettes: These were used for an accurate measurement of liquids.
- Pipette tips: Pipette tips were used to transfer the liquid.
- 1.5 ml tubes: These were used to hold samples of DNA and solutions.
- Solutions:
    - Solution 1 was used to resuspend the pellet into single cell suspension.
    - Solution 2 was used to dissolve the cell membranes.
    - Solution 3 was used to stop the lysis process.
- Dry ice: This was used to freeze the water inside the cells.
- Warm water: This was used to thaw frozen DNA.
- Centrifuge: This machine was used to separate DNA from supernatant.
- Isopropanol 96%: This was used to make DNA precipitate.
- Ethanol: This was used to separate lipids and proteins from DNA.

The material grown on the agar plates was transferred to 1.5mL tubes using pipettes. Then, the cell aggregate was resuspended in Solution 1. It was frozen using dry ice and thawed using warm water. Solutions 2 and 3 were added to the aggregate and mixed. The tubes were put in the centrifuge for 10 minutes; after, the supernatant was poured out (Figure B). Isopropanol was added to the tubes, and mixed through inversion. The DNA was spun in the microfuge, and then the supernatant was removed (this was where the mistake resulting in the loss of Sample A⚠ occurred; a pipette was not used to remove the supernatant). 70% ethanol was put into the tubes, and the tubes were spun in the microfuge again. Finally, we removed the supernatant, let the tubes dry, and then resuspended the DNA (Figure C).

For PCR (step 3):

- PCR mix: The mix with components necessary for PCR.
- Small PCR tubes: These were used to contain DNA and loading dye movement.
- Primer mix: This used to start the process of DNA synthesis.

20 nanoliters of DNA, 20μL of Primer mix, and 60μL of PCR mix were combined in a PCR tube. The tubes were then transferred to a thermocycler, where they were heated and cooled to various temperatures (Figure D).

For gel electrophoresis (step 4):

- Agarose gel: This was used during gel electrophoresis, and this is the gel that the DNA moved through when the base pair lengths were being measured.
- DNA ladder: This was used as a reference to measure the DNA base pair length during gel electrophoresis.
- Loading dye: This was used to make DNA easier to see and add to wells.
- Running buffer: This was used to carry electrical current during gel electrophoresis.
- Stain: This was used to see the DNA with the UV light during gel electrophoresis.

50μL of the fluid in the PCR tubes were transferred to different PCR tubes. Then, 10μL of loading dye were put in the new tubes. After this, the dye and PCR reactions were mixed together. 10μL of this solution was transferred into the gel using pipettes. The current was then run through the gel for ~30 minutes (Figure E).

Results

After plating the samples, we waited one week. Petri dish A yielded 5 samples (Figure 2), while Petri Dish D did not yield any samples (Figure 3). Of the five samples yielded in petri dish A, two were white (samples A◬ and A△), one was yellow (sample A●), and one was white-yellow (sample A). Compared to the other samples, sample A○ was the smallest. Most of the samples were located in the center of the petri dish, save for sample A◯.

After performing gel electrophoresis, samples A◯, A, and A△ traveled to the ~1500 bp marker (Figure 1). Parts of A● traveled to the ~1500 bp marker and parts traveled further to the ~200 bp marker. However, because of errors in the gel, the DNA strand that traveled to the ~200 bp marker was not horizontal like the other strands.

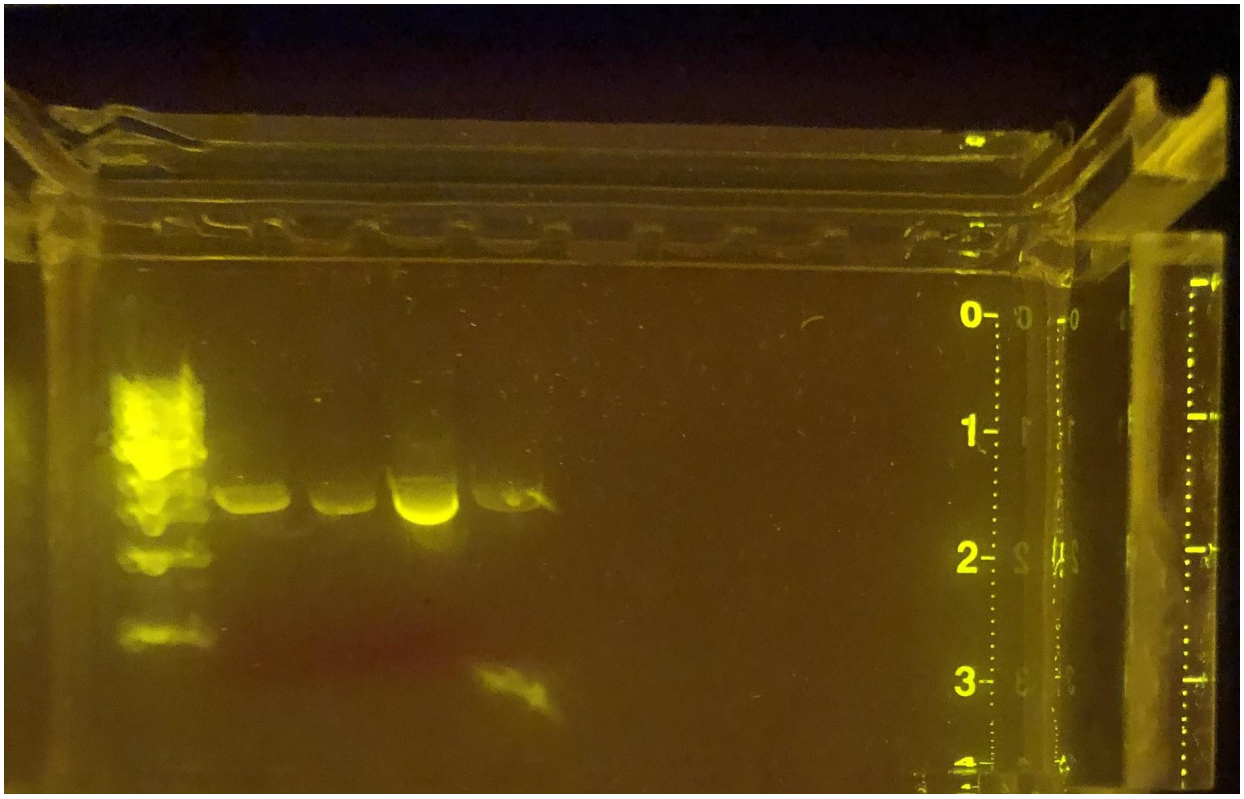


Figure 1. Results for gel electrophoresis. Samples from left to right: A◯, A□, A△, A●.

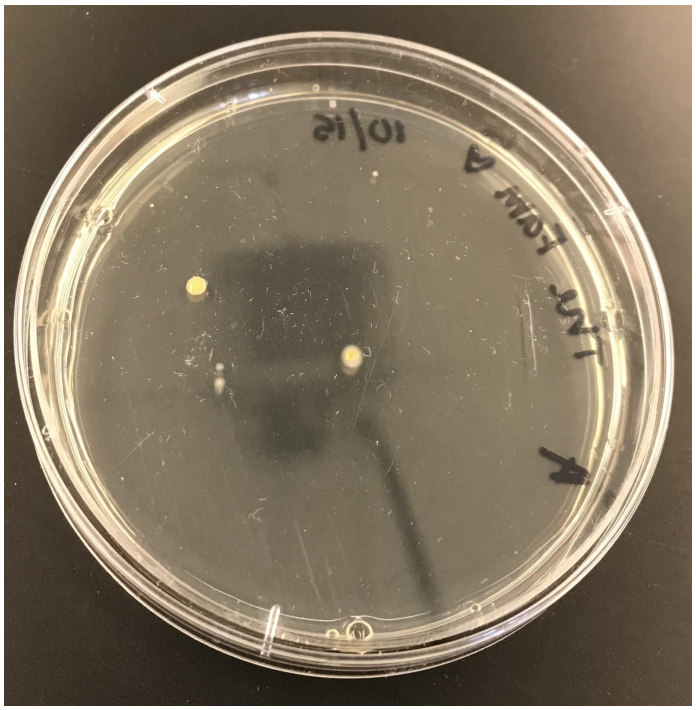


Figure 2. Petri dish A, which we used for the experiment.

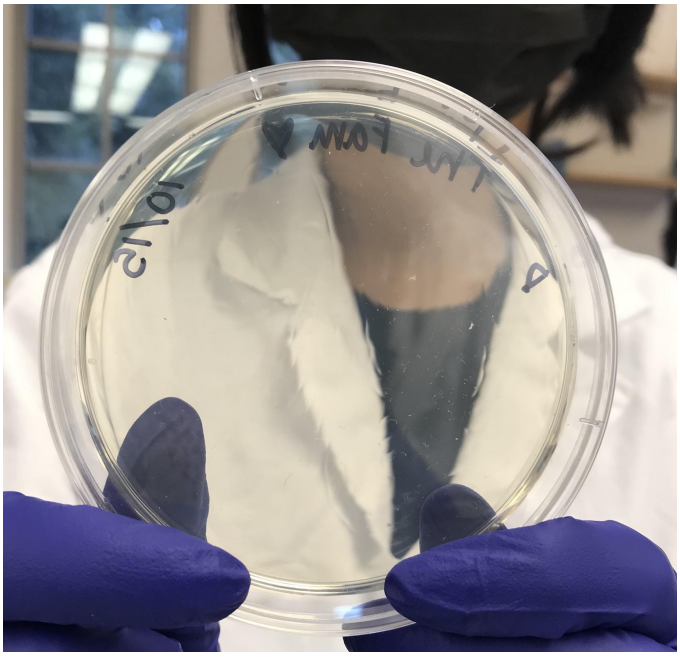


Figure 3. Petri dish D, which did not produce any samples.

Conclusions

The reference gel said that bacterial DNA travels to the ~1500 bp mark, and mold DNA travels to the ~200 bp mark. In our gel, samples A◯, A□, A△, and A● all traveled to the ~1500 bp mark, which indicates that all of our samples are bacteria. Additionally, some strands of sample A● also traveled to the ~200 bp mark, meaning that sample A● also had mold. In this experiment, we collected samples from banana peels and face swabs. The face swabs did not yield any samples, so we tested the samples from the banana peels. After DNA extraction and purification, PCR, and gel electrophoresis, we found that our samples have bacteria and mold.