FORENSIC SCIENCE
Foodborne Outbreak Investigation

Bad Food at a Good Party

Student's Guide

Laboratory Safety

1. Exercise caution when heating or melting reagents.

2. Exercise caution when working with electrical equipment.

3. Gloves and eye protection should be used whenever possible, as a part of good laboratory practice.

4. Always wash hands thoroughly after handling laboratory materials or reagents.

Experiment Objectives

- Translate information expressed as text into other visual information forms, such as tables or charts, in order to systematically analyze data.
- Apply the principles of experimental design to develop a logical experiment and test a hypothesis.
- Develop an understanding of foodborne illnesses and foodborne outbreaks.

LAB Part I – OBSERVING PHENOMENA

Daily Objectives

1. Translate information expressed as text into visual information to systematically analyze who ate what and who became ill.

2. Develop an understanding of foodborne illnesses and foodborne outbreaks.

Case Information I

Several years ago, 406 cases of the same foodborne illness were reported across ten states, mostly on the West Coast.

Results of a cohort study, (observation of a group of people over time) comprised of twelve guests who attended the same party, indicated that a specific food was the common vehicle for transmission of the foodborne pathogen.

Did You Know?
The actual steps taken to solving a Foodborne Outbreak case is very much like the scientific process! The first thing to do is to **DETECT/OBSERVE** an outbreak. The second step is to **FIND** other outbreak cases. Both these pieces are provided for you in the Case Information.

The next step is to **GENERATE A HYPOTHESIS** through interview.

Activity

You are part of the team performing the cohort study. Using the data below, retrieved from interviewing the party-goers, organize a chart on the next page and generate a hypothesis about which food item may be the source of contamination.

Data retrieved from interviewing the party-goers

Party-Goer	What did he/she eat?	Sick?
1	Tortilla chips, guacamole, a burger, five layer bean dip, deviled eggs, coleslaw, and chicken tacos.	Yes
2	Potato chips with French onion dip, tortilla chips with salsa, potato salad, a burger, and deviled eggs.	No
3	Potato chips with buttermilk ranch, tortilla chips with salsa, a burger, five layer bean dip, garden salad with buttermilk ranch dressing, deviled eggs, and coleslaw.	Yes
4	Potato chips with French onion dip, tortilla chips with guacamole, a burger, and deviled eggs.	No
5	Hot dogs and chicken tacos with salsa and guacamole.	No
6	Tortilla chips with guacamole, potato salad, a burger, and a garden salad with buttermilk ranch dressing.	No
7	Ate everything he could get his hands on except for the garden salad, coleslaw, and five layer bean dip. He ate so much that he could barely move.	Yes
8	Tortilla chips, a hot dog, deviled eggs, and chicken tacos.	No
9	Potato chips with French onion dip, tortilla chips with guacamole and five layer bean dip, a burger, and a garden salad with buttermilk ranch dressing.	Yes
10	Tortilla chips with salsa and five layer bean dip, a burger, deviled eggs, coleslaw, and chicken tacos.	Yes
11	Potato chips with French onion dip, tortilla chips with five layer bean dip, a burger, garden salad with buttermilk ranch dressing, and deviled eggs.	Yes
12	Tortilla chips with guacamole, a burger, a hot dog, and chicken tacos.	No

While reading through the interview, students should be thinking about how to construct a chart in order to turn this raw data into usable information.

You may put the party-goers in any order you choose.

Some students may choose to go in numerical order, while others may choose to group "sick" and "healthy" party-goers together.

Potato Chips												
Sick?												

Part I Conclusion: Stop and Think

1. Develop your hypothesis: Which food item do you think is contaminated and why? Remember that the six guests who did not eat this particular food did not become sick.

2. According to the case study, of the six people who were sick only five ate the affected food. How is this possible?

3. What additional information about the party-goers and the food items would help you to develop your hypothesis?

Results of stool sample taken from those who got sick showed that the pathogen responsible for the illness was the bacterium *Shigella sonnei*, which causes diarrhea, fever, and stomach cramps. *Shigella sonnei* is the third leading cause of foodborne outbreaks in the United States.

See Appendix A for more information on *Shigella*

Or you can visit:
<http://www.cdc.com>

- LAB Part I End -

LAB Part II – EXPERIMENTING

Daily Objectives

1. Apply the principles of experimental design to develop a logical experiment and test a hypothesis.

2. Analyze the results and collaborate with classmates to determine whether your experimentation adequately supports your hypothesis.

Case Information II

Multiple cases very similar to that of the party-goers were reported along the West Coast - all pointing to the five layer bean dip as the culprit. The FDA was notified and a public warning about the product was immediately issued. When implicated, the manufacturing company started a voluntary recall. No new cases of *Shigella* were reported after the recall and publicly the outbreak came to an end. However for food safety analysts, the investigation is not over.

Since the bean dip is compiled from five different parts that are manufactured and stored separately (See Bean Dip: General Production Information, page 3), analysts must first figure out whether the point of contamination was in all five layers after assembly or whether it stemmed from an individual layer.

Analysts first sliced down through all five layers of the dip, then thoroughly mixed the sample until it was homogenous. They tried to isolate the bacteria and grow it on an agar plate, but was unsuccessful in getting a positive results.

Remember that the results of stool sample tests showed that *Shigella sonnei* was the pathogen responsible for the illness. This particular strain of bacteria is notorious for having an extremely low infectious dose; only 10–100 cells are sufficient to cause illness. Due to their initial failure in isolating the bacteria, analysts thought to take another, much more sensitive approach - polymerase chain reaction (PCR).

Case Information II - continued

Molecular-based assays like PCR allow for relatively rapid confirmation of the presence of a particular microorganism, because different species have unique genetic markers that can enable accurate identification, much like a fingerprint. Precise primers can be designed according to the DNA sequence of these specific genes and then used to amplify the particular region(s) of these genes using PCR.

The sizes of these gene fragments in base pairs (bp), can be measured and visualized using gel electrophoresis.

You are targeting the **ipaH gene** and **mxiC gene** from *Shigella sonnei* by using the primers specific for these genes in PCR. When a tiny trace of *Shigella sonnei* DNA is present in the sample, PCR will yield the PCR products of **175 bp** for the ipaH gene and **1,000 bp** for the mxiC gene.

Certain molecules found in food can inhibit PCR causing the PCR reaction to fail. A positive control is included within each bean dip layer and the five layer mixed sample to differentiate between a negative result due to lack of *Shigella* in the sample, from a negative result due to total failure of the PCR reaction. This inhibition is especially apparent when trying to detect a low number of bacteria in the sample. To distinguish between the signal from the positive control and a positive signal from a contaminated sample, the positive control you will use is a strain created in the lab, not found in nature. This strain also produces the **175 bp** fragment for the ipaH gene, but the mxiC gene has been modified to produce a fragment of **1800 bp**.

Bean Dip: General Production Information

Prior to assembly, each layer of the bean dip was individually prepared and stored refrigerated. The guacamole and salsa layers were made with fresh, raw ingredients. The containers they were being prepared in were not cleaned or sterilized between batches during the same production day.

The cheese was prepared once or twice a week in big batches. Big chunks of cheese were cut with a knife and broken up by hand prior to being fed into a colloid mill that turned the chunks into a paste. The mill had parts that were difficult to clean properly, and was kept in a room without air conditioning.

The beans were the only layer that contained pre-cooked ingredients. After cooking, the beans were cooled at room temperature and then refrigerated until the dip was assembled.

The sour cream was made from pasteurized dairy ingredients and live pure Lactobacillus cultures. The sour cream was always made and stored in the same container, which was cleaned twice a month. The sour cream was stored refrigerated until assembly.

Supplies Available to you

1% GreenGel-in-a-Cup - one per group
TBE Running Buffer - 135 mL per group

DNA Samples:

DNA SAMPLE	DESCRIPTION	QUANTITY
M1M	MiniOne DNA Marker: Contains 5 DNA fragments of 100, 300, 500, 1000 and 2000 bp sizes	1 tube of 10 μL
1 Kb Ladder	1 Kb DNA Ladder: Contains 15 DNA fragments ranging from 1000 bp to 15000 bp sizes	1 tube of 10 μL
SRS	*Shigella* Reference Standard: PCR product of *Shigella sonnei* (as found in nature) which contains two targeted gene products at 175 bp and 1000 bp sizes	1 tube of 10 μL
+IC	Positive control: PCR product of the lab-made *Shigella* strain which contains two targeted gene products 175 bp and 1800 bp sizes	1 tube of 10 μL
-C	Negative Control: PCR Product of the reagent mixture only. No DNA template	1 tube of 10 μL
5L	PCR products using a homogenous mixture of the five layer Bean Dip and the positive control (+IC) as DNA templates	1 tube of 10 μL
C	PCR products using the Cheese Layer and the positive control (+IC) as DNA templates	1 tube of 10 μL
SC	PCR products using the Sour Cream Layer and the positive control (+IC) as DNA templates	1 tube of 10 μL
B	PCR products using the Bean Layer and the positive control (+IC) as DNA templates	1 tube of 10 μL
S	PCR products using the Salsa Layer and the positive control (+IC) as DNA templates	1 tube of 10 μL
G	PCR products using the Guacamole Layer and the positive control (+IC) as DNA templates	1 tube of 10 μL

Activity

Even a tiny trace of *Shigella sonnei* DNA should be picked up by PCR, but initial results from the five layer mixed sample failed to detect the bacteria. Analysts were sure that the bean dip was the source and were baffled by the negative test results. Analysts suspect that the failure was due to the low infectious dose of *Shigella sonnei*.

How will you revise your hypothesis from Part I to reflect the new case information and bean dip production information you are given?

Using your knowledge of PCR and gel electrophoresis, design an experiment to test your hypothesis. Specifically, how will you tell whether the food item you suspect of being contaminated does indeed contain the microbe? Keep in mind, there are only 9 wells in the gel. Therefore you have to choose which 9 samples to run.

Draw a picture of what you expect the gel to look like at the end of the run:

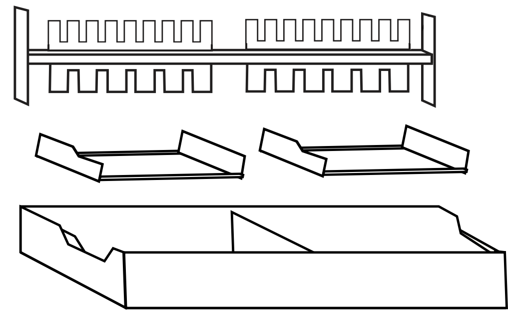
1 2 3 4 5 6 7 8 9

1. ______________
2. ______________
3. ______________
4. ______________
5. ______________
6. ______________
7. ______________
8. ______________
9. ______________

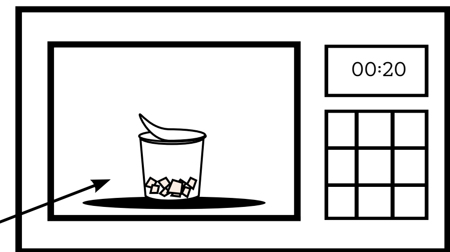
Did You Know?
Like the scientific process, the steps to solving a foodborne outbreak cases does not necessarily follow linear process. In our case here, we have identified a contaminated food source and have CONTROL of the outbreak through recalls. Because the identified food has different components, we now have to narrow down which component(s) to TEST and SOLVE the case by determining the vehicle of contamination.

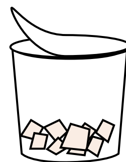
How to Cast a Gel

1. Place the MiniOne Casting Stand on a level surface and place gel trays in the two cavities. The straight edge should be on the Right side. Insert the comb into the slots at the top of the casting stand with the 9 well side facing down.

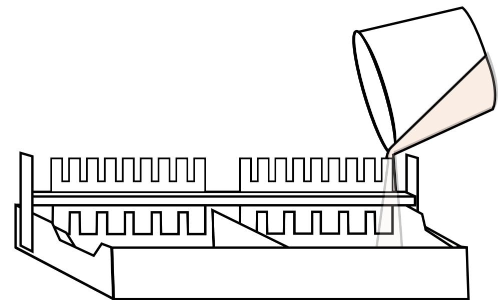


2. Partially peel the film of a GreenGel in-a-Cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 GelCups at a time.**

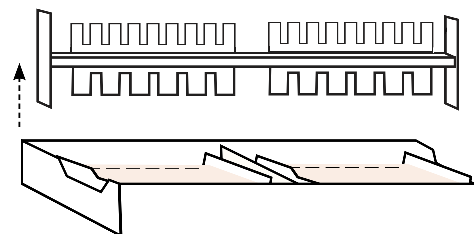


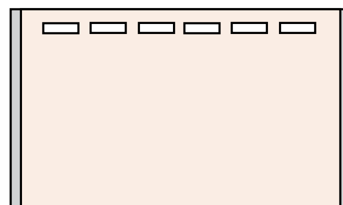


3. Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 mins or until opaque. **DO NOT disturb the gel until time is up.**

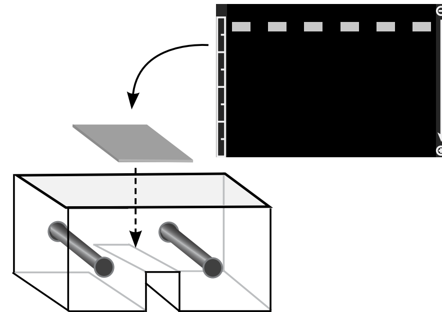


4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any access agarose from the bottom of the tray

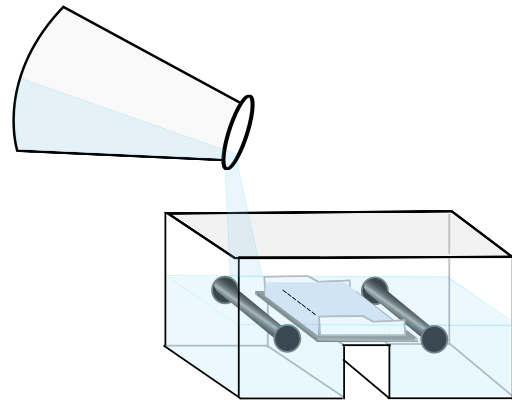




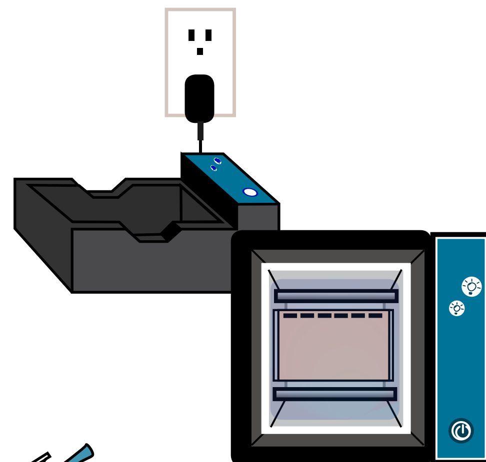
How to Load a Gel



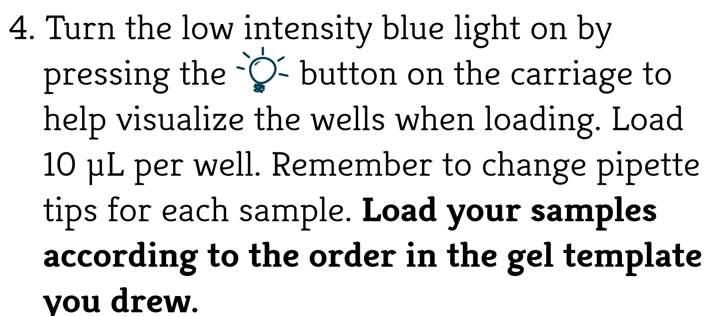
1. Ensure the black viewing platform is in the tank if it is not already installed and put the gel (along with the gel tray) into the tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



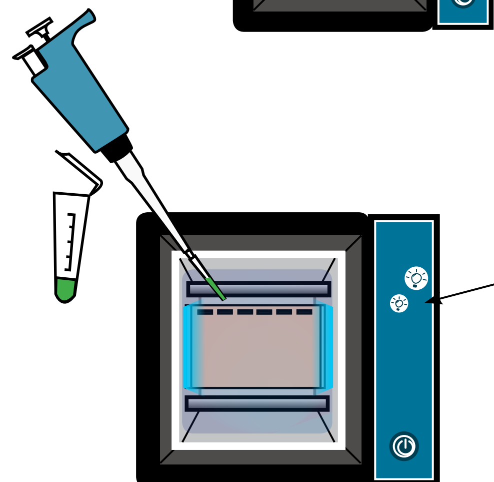
2. Measure 135 mL of TBE running buffer and pour into **one** side of the tank to push out the air, creating a nice even background without air bubbles or air trapped for imaging later.



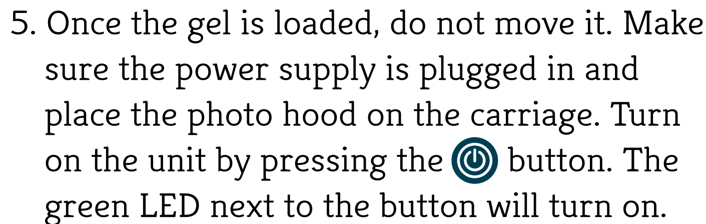
3. Plug the power supply into the wall. Place the tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.



4. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order in the gel template you drew.**



Run, Visualize and Capture Image



5. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the ⏻ button. The green LED next to the button will turn on.

   The green power LED will not turn on if:

   1. The tank is not appropriately inside the carriage

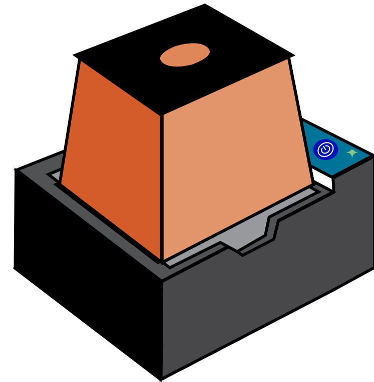
   2. There is no buffer in the tank

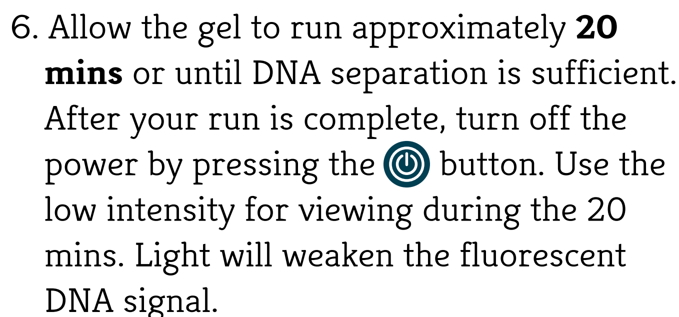
   3. The buffer is too concentrated or too diluted

   4. The photo hood is not on the carriage

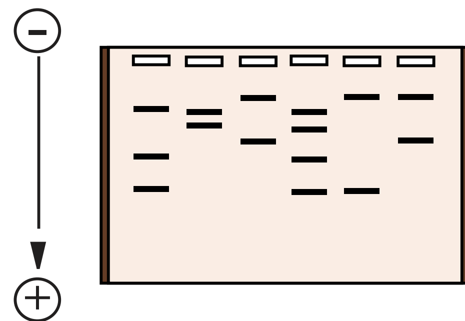
   5. There is too much or too little running buffer

   6. The power supply is not plugged in. Check by turning on the blue LEDs

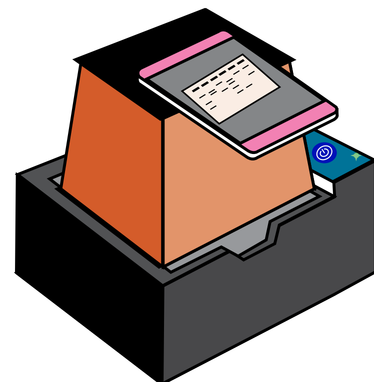




6. Allow the gel to run approximately **20 mins** or until DNA separation is sufficient. After your run is complete, turn off the power by pressing the ⏻ button. Use the low intensity for viewing during the 20 mins. Light will weaken the fluorescent DNA signal.



7. **Document your results.** At the end of 20 mins, wipe off condensation from the inside of the hood with a soft cloth. Turn on the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. DO NOT zoom in. The photo hood is already at the optimal focal length for a smart device.



Results, Analysis and Conclusions

How did your gel turn out? Paste your results here:

1 2 3 4 5 6 7 8 9

1. ______________
2. ______________
3. ______________
4. ______________
5. ______________
6. ______________
7. ______________
8. ______________
9. ______________

Part II Questions: Stop and Think

1. Which sample(s) did you test? Why?

2. Which controls did you use, and why did you choose them? Why did you not run some of the controls?

3. Did all your results support your hypothesis? Why, or why not?

4. Did you encounter any problems? Is there something you would now do differently?

5. What do you believe to be the most likely cause of the negative test results from the mixed sample?

- LAB Part II End -