Forensic Science MiniLab
Foodborne Outbreak Investigation
(Bad Food at a Good Party)
Student's Guide

Cat# M3006

Version 070518

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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 needed as part of good laboratory practice.
4. Always wash hands thoroughly after handling biological materials or reagents.

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.

Background

This lab is based on a real-world *Shigella* outbreak in the year 2000. Students use scientific reasoning to mimic a foodborne outbreak investigation and design an experiment using gel electrophoresis to determine the source of the outbreak.

The intent of this lab exercise is to engage and encourage you to ask the right questions and develop the skill sets need to approach a problem.

Part I - Observing Phenomena

Objectives

- Translate information expressed as text into visual information to systematically analyze who ate what and who became ill.
- Develop an understanding of foodborne illnesses and foodborne outbreaks.

Case Information

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 are 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 the case Information.

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

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 except for the garden salad, coleslaw, and five layer bean dip. Admits he overate.	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, think 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.

You may choose to go in numerical order, or group "sick" and "healthy" party-goers together.

	Party-Goer											
Food Item												
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 samples 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.gov

Part I - End

Part II - Experimenting

Objectives

- Apply the principles of experimental design to develop a logical experiment and test a hypothesis.
- Analyze the results and collaborate with classmates to determine whether your experimentation adequately supports your hypothesis.

Case Information

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 was 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 from this samples on an agar plate, but did not get a positive result.

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 decided to take another, much more sensitive approach— polymerase chain reaction (PCR).

Molecular-based assays like PCR allow for relatively rapid confirmation of the presence of a particular microorganism, because different species have unique genetic markers. These markers are specific for the DNA sequence of the organism so they an provide accurate identification. This can produce what is called a DNA 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.

Case Information (continued)

You are targeting the ipaH gene and mxiC gene from *Shigella sonnei* by using the primers specific for these genes in PCR. Even when only 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. This will be your *Shigella r*eference standard.

Certain molecules found in food can inhibit the PCR reaction causing it to fail. If the reaction fails no PCR product is produced. When this happens scientists cannot be sure if the lack of PCR product is due to the inhibition of the PCR reaction or because there are not *Shigella* bacteria in the sample. To make sure the PCR is working, scientists use a positive control. This positive control is a lab strain of *Shigella* that is added to all samples. To distinguish between a signal from the positive control and a positive signal from the pathogenic bacteria the scientists use the following information:

Target gene	**Lab Control (+LC)**	***Shigella* Reference Standard (+SRS)**
ipaH gene	175 bp	175 bp
mxiC gene	1800 bp	1000 bp

The presence of a 1800 bp band from the Lab Control mxiC gene would suggest successful PCR amplification from the food sample, with no inhibition. Then, the presence or absence of the 175 bp and 1000 bp bands accurately reports the presence or absence of wild type *Shigella* DNA in the sample.

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.

Materials available to each group of students

1 agarose GreenGel™ Cup (1%)
TBE running buffer (135 mL)
11 DNA samples *(see table below)*
1 MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 micropipette (2-20 µL) and 12 pipette tips

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
+LC	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 with 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 (+LC) as DNA templates	1 tube of 10 µL
C	PCR products using the Cheese Layer and the positive control (+LC) as DNA templates	1 tube of 10 µL
SC	PCR products using the Sour Cream Layer and the positive control (+LC) as DNA templates	1 tube of 10 µL
B	PCR products using the Bean Layer and the positive control (+LC) as DNA templates	1 tube of 10 µL
S	PCR products using the Salsa Layer and the positive control (+LC) as DNA templates	1 tube of 10 µL
G	PCR products using the Guacamole Layer and the positive control (+LC) as DNA templates	1 tube of 10 µL

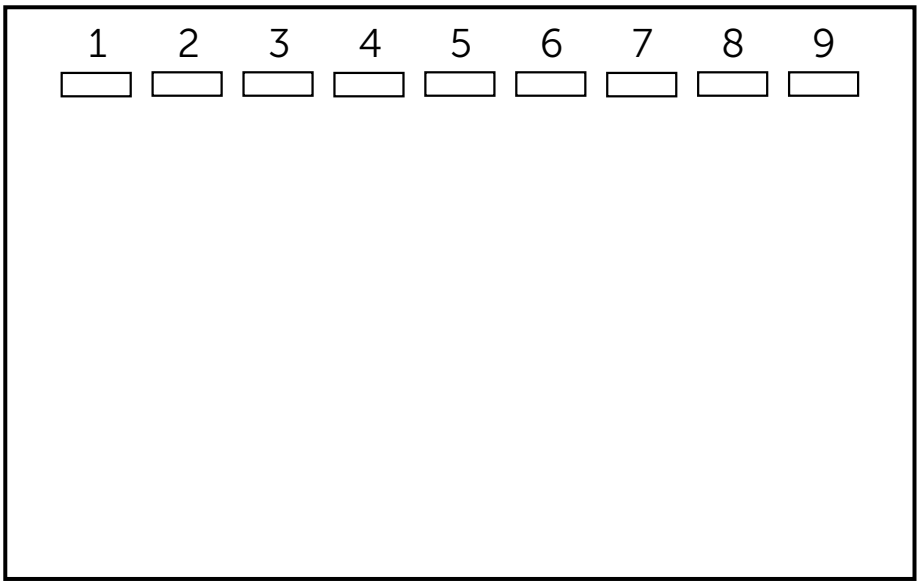
Activity

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.



LOCATION OF SAMPLES

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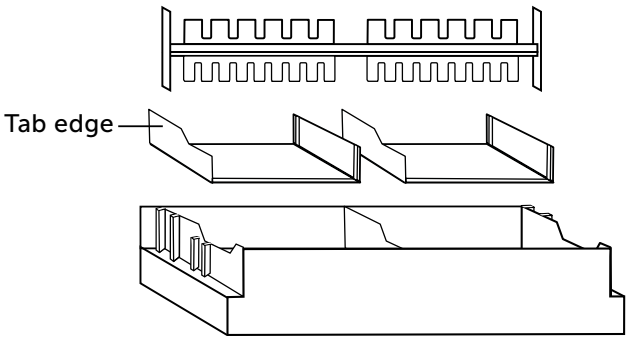
Did You Know?

Like the scientific process, the steps to solving foodborne outbreak cases do 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.

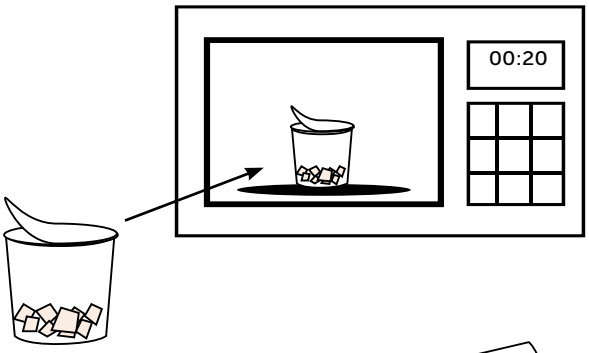
How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 9-well side facing down.

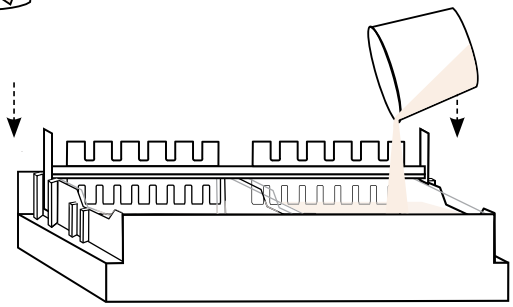


2. Partially peel the film of a GreenGel™ Cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**

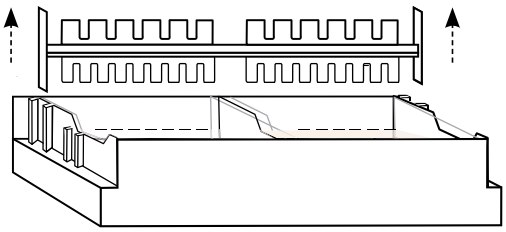
   ⚠**Safety requirement:** Adult supervision required if students are handling gel cups!

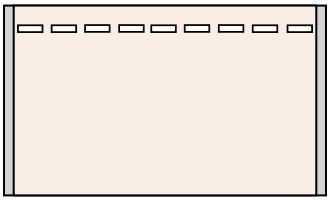


3. **One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 mins or until opaque. **DO NOT disturb the gel until time is up.**



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





How to Load a Gel

1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**

2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.

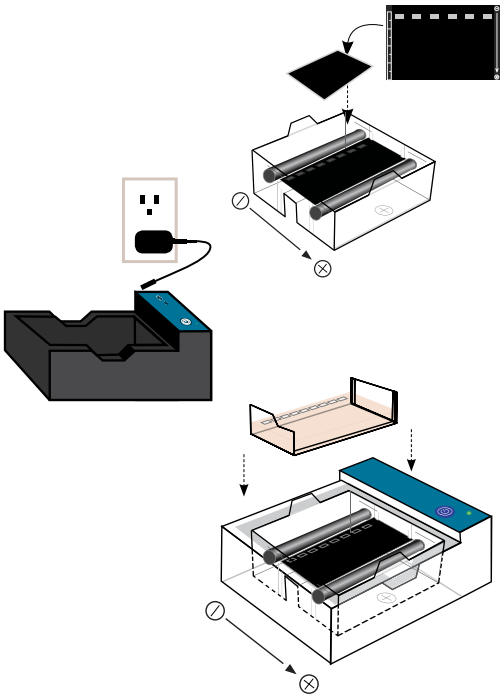
5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TBE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side** of the gel tank.

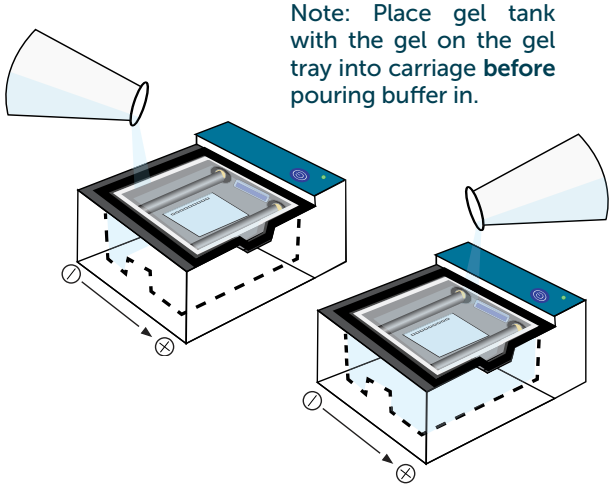
7. Place photo hood on the carriage.

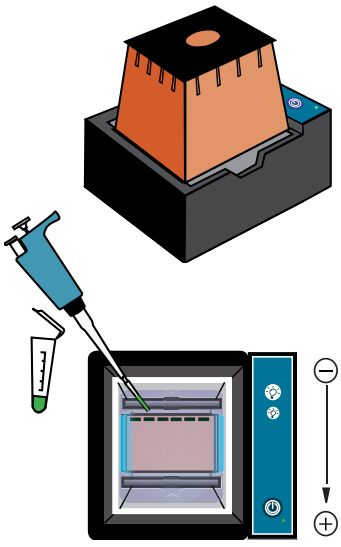
8. Press the power button which should now be a solid green light. If green light is **solid,** turn off the unit and proceed to loading gels. If the green light is **blinking,** see the Troubleshooting Guide.

9. 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 on page 13.**

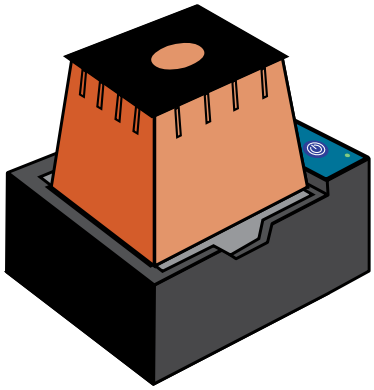


Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.





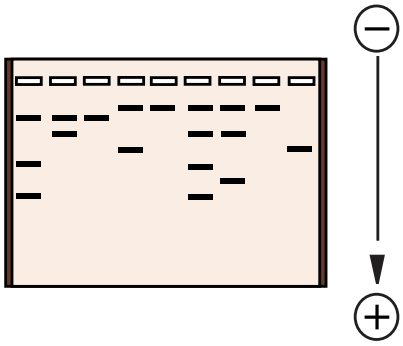
Run, Visualize, and Capture Image



1. 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:

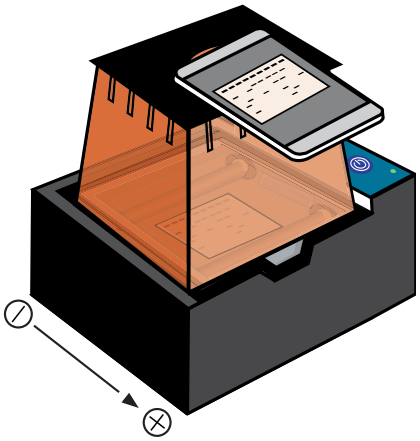
   - **The tank is not properly placed inside the carriage**
   - **There is no buffer in the tank**
   - **The buffer is too diluted**
   - **The photo hood is not on the carriage**
   - **There is too little running buffer**
   - **The power supply is not plugged in. Check by turning on the blue LEDs**



2. Allow the gel to run approximately 20 minutes 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 run. Light will weaken the fluorescent DNA signal.

3. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **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 as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device).



Clean Up

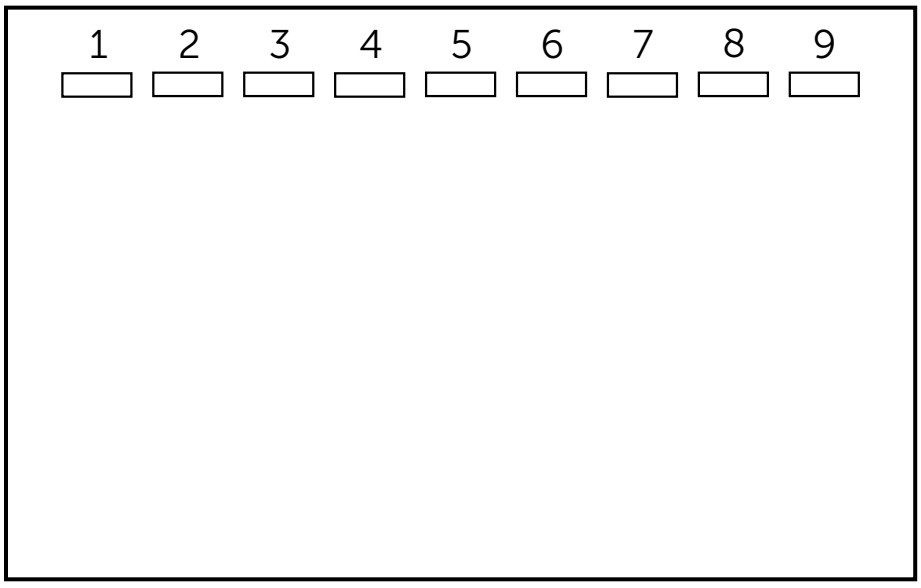
1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.

2. Pour the used running buffer down the drain or into a waste beaker.Throw the gel away. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.

3. Use a paper towel or kimwipe to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for cleanup and storage.

Results, Analysis, and Conclusions

How did your gel turn out? Paste your results here



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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? If you did not run all the controls explain your reasoning.

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? What was the purpose of checking all the layers individually?

6. Did you confirm the contaminated five layer bean dip indeed contained the *Shigella sonnei?* Which layer was contaminated?

LAB Part II - End

Appendix A - *Shigella sonnei*

Foodborne Illness

Foodborne illness (sometimes referred to as "food poisoning") is an infection or irritation of the gastrointestinal tract caused by consuming a biologically or chemically contaminated food or beverage. This contamination can be caused by many microscopic agents, including viruses, bacteria, parasites, or toxins released by such pathogens, or chemicals. In some cases, foodborne illness is due to the proliferation of a microbe itself and is specifically referred to as a foodborne infection, whereas foodborne illness due to toxins produced by the pathogen is referred to as foodborne intoxication. Foodborne intoxication can also be caused by many other harmful chemical contaminants that are not associated with microbes. Contamination can occur at any point in the process of manufacturing food, from the raw ingredients, in the processing plant, during distribution, and even from someone who handles your food.

Foodborne illness affects an estimated 48 million people (1 in 6) in the US every year (CDC, 2014). Depending on the pathogen responsible, foodborne illness can include several different symptoms, most commonly vomiting and diarrhea. For many people, these symptoms are mild and go away on their own. However, for some people symptoms can be severe, requiring treatment and/or hospitalization. Complications can be life threatening or even fatal. In the US, approximately 128,000 people are hospitalized and 3,000 people die each year from foodborne diseases (CDC, 2014).

Foodborne Outbreak

Imagine that a foodborne outbreak is a crime committed by a pathogen-contaminated food. When a crime is committed, detectives and their teams must investigate the scene, gather evidence, identify a suspect, and link them to the crime before they can close the case. However, when foodborne outbreaks occur, the "crime scene" can span multiple states or countries and the "suspect" pool is typically vast. Evidence is usually scarce, and time is always in short supply. These factors can make it much more difficult and complex to conduct an outbreak investigation than to solve most crimes.

Investigation of a foodborne outbreak, like investigation of a crime scene, follows the scientific method (Figure 1). It begins with the observation of cases and detection of the outbreak leading to the question of what caused the outbreak.

Next, background information is gathered and a hypothesis is generated about the source of the contamination. The predictions of the hypothesis are tested against available data and the hypothesis may be revised if it is not supported by the data. If the data indicate that the hypothesized source was responsible for the outbreak, product recalls and other appropriate actions can be used to reduce the impact of the spread of the pathogen and reduce the potential number of people infected. Sometimes, the source is never identified, but if new cases cease, Public Health officials can declare the outbreak to be over (CDC, 2014).

Appendix A - *Shigella sonnei* (continued)

Shigella sonnei

Shigella species are considered genetically identical to E. coli but because of historical references, the name has been retained. There are four serotypes (or variations) of *Shigella: Shigella dysenteriae* (Serotype A), *Shigella flexner*i (Serotype B), *Shigella boydii* (Serotype C) and *Shigella sonnei* (Serotype D). These bacteria are members of the family *Enterobacteriaceae,* and as such, are described as being Gram negative, small rods not producing spores, and surviving in atmosphere with oxygen and reduced amounts of oxygen. *Shigella,* unlike *E. coli,* occupy a very narrow niche of hosts-primarily humans.

The illness that *Shigella* cause (shigellosis) range from mild diarrhea to severe dysentery, usually accompanied with severe dehydration, seizures, and tenesmus. If the illnesses progress, reactive arthritis can occur. Some *Shigella* strains contain the Shiga toxins, potent cytotoxins that can severely affect people, i.e. cause hemolytic uremic syndrome, and in some individuals, death. Typically, shigellosis is self limiting, that is, no medication is prescribed, but the patient should remain hydrated. In some cases, antibiotic treatment may be necessary and the antibiotic of choice is trimethoprim sulfamethoxazole, but the rise of multiple drug resistant strains has complicated patient treatment. Currently, no vaccine is available although a number of laboratories are in the process of developing one.

Shigella species are spread through the fecal-oral route and broadly categorized through the 5 F's: food, fingers, feces, flies and fomites. Person to person spread is common, particularly in crowded and unsanitary conditions. Institutions, such as prisons, nursing homes and day-care centers are common sites for rapid spread of the pathogen.

It is the genetic material of the bacteria that determines if it can infect an individual. Bacteria that can cause an infection are called pathogenic. The pathogenic genes of *Shigella* are found in the plasmid (circular DNA that is separate and can replicate independent of the bacterial chromosome). The bacterial genome is approximately 4.5 million base pairs whereas the virulence plasmid ranges from 180,000 to 220,000 base pairs. Since most of the virulence genes reside on this large plasmid, any *Shigella* that loses that plasmid or has a virulence gene mutated, will be non-virulent (not able to cause disease). Therefore, any *Shigella* strain that causes illness in a human host must be virulent (contains the plasmid).

There is one *E. coli* strain that is very similar to *Shigella* species and also retains some characteristics of *E. coli. Enteroinvasive E. coli* (EIEC) carries the large virulence plasmid that is nearly identical to that virulence plasmid found in *Shigella.*

Today, one of the major public health concerns is focused on the acquisition of antibiotic and Shiga toxin genes by *Shigella* species that enable these pathogens to be multiple antibiotic resistant strains as well as becoming more virulent. This is a worldwide phenomenon and extends to many other pathogenic bacteria. Imagine that a patient is ill due to a multiple drug resistant bacterium – how will this person be treated if no antibiotic is effective?

Appendix A - *Shigella sonnei* (continued)

Genes selected for amplification and identification

The PCR assay for the detection of *Shigella* targets the ipaH gene. Why this gene? First, this gene is present only in *Shigella* (and EIEC) and no other bacteria. Second, this gene has 9-11 copies in both the bacterial chromosome and virulence plasmid which gives the PCR assay a built-in advantage-has more than one target per cell. Although theoretically it is possible to detect one bacterial pathogen, this is not the case in real world applications. Usually it takes several hundred or more bacteria in a sample to yield a positive PCR result. In many cases, many PCR assays target only a single copy of one gene. In the *Shigella* PCR assay, there is an additional factor that increases the likelihood of amplification and detection-nearly a 10 fold increase in target per cell. Another advantage to target the ipaH genes in *Shigella* is that in the event the plasmid is lost from *Shigella* during routine laboratory cultivation, there are 5 other ipaH genes present in the chromosome. Therefore, the loss of the plasmid-borne genes will not affect the final analysis of whether the pathogen is present or absent.

The mxiC gene is one of about 30 or so genes on the virulence plasmid that is essential for *Shigella* to be pathogenic. What else is important is that this gene is also unique to *Shigella.* The mxiC gene was genetically altered in the laboratory so that it now has the genetic information to confer resistance to the antibiotic kanamycin. This strain is used in the food analytical laboratory as a control. This bacterium, and is one of a kind, is used to ensure that the entire analytical process, that is from the extraction of the food to PCR, was properly performed. Therefore, any *Shigella* isolated from people, environment, etc. will not have this specific arrangement of the mxiC gene, which makes this a unique target for the PCR assay and an excellent control strain. By PCR, all unaltered *Shigella* will yield a PCR product of about 1000 bp whereas the altered mxiC will be at about 1800 bp. Therefore, this strain can be differentiated from all other *Shigella* species and as a control, it ensures that we detected only those real, pathogenic *Shigella.*

Check This Out

CDC has an interactive database for the public to track and reports on foodborne illnesses and outbreaks by year and by state:

http://wwwn.cdc.gov/foodborneoutbreaks/

Stop and Think

1. What is foodborne illness and what is the definition of a foodborne outbreak?

2. Name some ways that food might become contaminated with a microbe.

3. Why do methods of mass food production increase the chances of a foodborne outbreak?

Appendix B - Scientific Method

The Scientific Method

The scientific method or scientific process is a set of practices and techniques used to investigate and explain observed phenomena. Although referred to as scientific, these principles can be applied to almost any type of inquiry. The application of scientific reasoning is a cornerstone of all science-based disciplines as a means to discover the hidden mysteries of life and world around us. If you are challenged to think about everyday uses of the scientific method, you might be surprised to see how common its use is in your daily life!

The basic steps of the scientific method are:

Observation and Purpose – Ask a question and then form an objective based on your observation

Background Research – Gain background knowledge about subject

Hypothesis – Make an informed and testable prediction

Experiment – Test your hypothesis by doing experiments and collecting data

Results and Conclusion – Analyze your data and compare to hypothesis—were you right? If not, try to determine why.

Communicate – share your hypothesis, experiments, results and conclusions with others, seek feedback

A thorough understanding of the scientific process will allow you to ask meaningful, testable questions and to think critically and analytically about the causes and explanations for phenomena, including in this case, an outbreak of foodborne illness.

Experimental Design

The best way to test a hypothesis is through controlled, systematic, and reproducible experiments. Proper experimental design is critical for obtaining usable, reliable, and applicable data. There are two important components to be considered: variables and controls.

Variables

Variables are the conditions and components that are changeable and controllable during an experiment. There are both independent variables and dependent variables.

Independent variables are factors that can be intentionally changed and their impact can be measured on the dependent variable.

Dependent variables are the outcomes or what happens as a result of the independent variable(s).

There are typically many independent and dependent variables to consider when performing an experiment, but it is often best to alter only one independent variable at a time to accurately gauge the effect of that variable on the dependent variable. Multiple independent variables can be changed if you are interested determining a combined effect.

Appendix B - Scientific Method (continued)

Controls

Controls are the components and conditions that are known and kept constant during an experiment. Controls are used for a point of reference and they are often safeguards against internal factors that may influence the outcome of an experiment. Different types of experiments may require different types of controls, depending on the testing procedures. The three main types of controls are positive, negative, and experimental controls.

A **positive control** is something known to produce a positive result and will often be included (especially for diagnostic tests) to ensure that a negative result is not due to experimental or reaction failure.

A **negative control** is something known to produce a negative result and will often be included to ensure that a positive result is truly positive and not due to contamination or other interference.

Experimental controls (or "control groups") are used in controlled experiments to acquire baseline data. This baseline data can be compared to the experimental data to see the relative effect (if any) of the independent variable(s) on the dependent variable. This type of control is a parallel of the experiment, except no changes are made to any of the independent variables. Sometimes an experimental control is also a negative control, depending on the expected outcome and type of experiment. An experimental control can have an outcome similar to the experimental subject if the independent variable does not greatly impact it, whereas with a negative control, no outcome is expected at all.

Determining what types and how many controls to include in an experiment can affect the reliability and accuracy of your data and ultimately your conclusions.

Data Collection and Analysis

Once the experiment is designed, decide what type of data you need to collect and how you will collect it in order to evaluate your hypothesis. Consistency when making measurements and collecting data is important to ensure accuracy, precision, and ultimately, repeatability of your experiment. When you perform any experiment, be sure to record all your findings, preferably in ink. Good record keeping, observations, and notes will help you make a more thorough and reliable analysis of your data and will give more credibility to your results.

Appendix C - Molecular Method

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a technology and method that enables researchers to produce detectable quantities of a specific DNA sequence by amplifying from a single or a few copies of a DNA template in less than two hours. A typical PCR reaction can literally make milions of copies of the target DNA.

PCR relies on a heat-stable enzyme called Taq DNA-polymerase, which is capable of copying DNA from a template. The specificity of PCR for a particular gene comes from precisely designed primers, which are short pieces of single stranded DNA, with nucleotide sequences that are complementary to the sequence of the gene that is being targeted.

A PCR reaction requires the following components:

1. **Template or target DNA,** often genomic DNA isolated from an organism being studied (in the case of the Foodborne Outbreak Lab, the template is the bacterial DNA extracted from the contaminated food.
2. **Primers** that are specific for the gene that is being amplified
3. **dNTPs,** nucleotides, the building blocks for making DNA
4. ***Taq* DNA polymerase** (heat-stable enzyme)
5. **PCR buffer**, a salt solution that maintains an ideal pH
6. **Mg^{2+},** a co-factor for the DNA polymerase

The components are mixed together in a small tube, which is then placed in a device called a thermal cycler. The thermal cycler is used to repetitively change the temperature of the reaction mixture through the following steps:

1. The first temperature step, usually greater than 94°C, causes the double strands of DNA to separate into two single stands, exposing the bases. This is called denaturation.
2. In the second temperature step is lowered and primers bind, or anneal, to complementary sequences of exposed bases on the target DNA molecules. The annealing temperature has to be carefully selected: it must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that non-specific, or random, binding between primer and template occurs.
3. The third temperature step, called elongation, is higher than the annealing temperature but lower than the denaturation temperature. Now the DNA-polymerase binds to the DNA where the primer has attached and synthesizes a new strand of DNA out of dNTPs by copying the template sequence adjacent to where the primer is bound. This results in a new copy still attached to the template as double-stranded DNA.

Appendix C - Molecular Method (continued)

This sequence of temperatures is repeated over and over again, up to 45 times, with the double-stranded DNA produced in step 3 serving as the template that is copied in the next round. This cycle can be repeated anywhere from 10 to 45 times resulting in hundreds of trillions of copies of the target gene. These products be run on gels and detected.

A number of factors contribute to the success or failure of a PCR reaction. The primers must be carefully designed to be specific to the target gene. If sequences complementary to the primers exist in other parts of the genome you can end up with multiple products, or no product. Similarly, the annealing temperature must be carefully selected to favor specific binding of the primer to the target. pH and Mg^{2+} concentration must be controlled for maximum activity of the DNA polymerase. Finally, even small concentrations of contaminants can interfere with PCR resulting in complete failure of the reaction.

Check This Out

The American Society of Human Genetic (ASHG) has this great forensic activity on Short Tandum Repeats (STR) for Human Identification. This exercise can also be used to explain PCR:

http://www.ashg.org/cgi-bin/gena/glesson.pl?s=LSN&t=10&l=2&c=0

Appendix D - What is Gel Electrophoresis?

Electrophoresis

Looking at a sample of purple dye, how can you know if it is really purple? Could it be a mixture of blue and red dyes? Electrophoresis is a technique used in many areas of science to analyze and separate samples by applying a constant electric field across a separation matrix. Biologists or forensic scientists can use this technology to separate mixtures of DNA, RNA, proteins, or dyes into individual components based on size and electrical charge. A tracking dye, or loading dye, is usually included with the sample to make it easier to see whether the sample has been loaded and to judge how the separation is progressing.

The gel used in gel electrophoresis is a sieving matrix through which particles travel. Gels can be made from different substances depending on what is being separated (DNA, RNA, proteins, etc.), but it should be both conductive and have the ability to form a uniform matrix with appropriate pore sizes. The matrix is like a sieve or colander: if the holes are too big or too small it won't work very well. One of the most commonly used and effective reagents for DNA separation is agarose. Agarose gels are usually cast in a tray with molten (melted) agarose. A comb is placed while the agarose is molten and then removed after the gel solidifies to create wells in which to load samples. A DNA stain is used to enable visualization of the DNA.

As an electric field is applied to the agarose gel, the particles in the wells will begin to move. The direction that particles move depends on their charge. DNA has a negative charge, so it will be attracted to a positive electrode. Some dyes and other particles have a positive charge and will thus migrate toward a negative electrode. The relative speed of migration is determined mainly by the size of the particle but also by the strength of the particle's charge. Like an obstacle course, larger particles have more difficulty passing through the matrix due to their bulk and do not travel very far, while shorter and smaller ones can maneuver much more easily and therefore travel faster and farther.

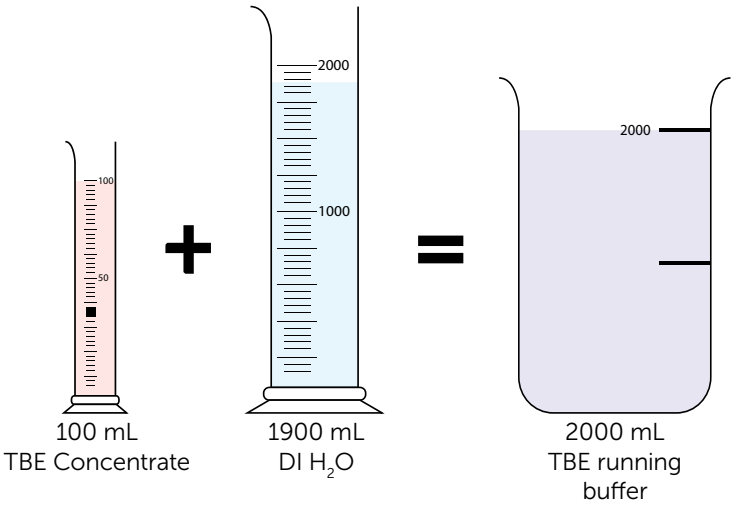
Sometimes a particle with a bigger size migrates faster than a smaller particle. This can happen if the strength of the charge of the larger particle is significantly stronger by comparison to the charge on the smaller particle. An example of this phenomenon is the loading dye Orange G. This dye often runs faster than the smaller DNA fragments and other relatively small particles because it is more negatively charged and has a stronger attraction to the electrode than the smaller particles.

Both particle size and electrical charge can affect the results of gel electrophoresis experiments. In general however, gel electrophoresis separates charged particles and fragments by size.

Appendix E - TBE Concentrate Dilution Instruction

Dilute **1** part TBE Concentrate with **19** parts deionized or distilled water

1. **To make 2000 mL TBE running buffer (1X)** = mix 100 mL TBE Concentrate and 1900 mL deinonized or distilled water



Limited TBE running buffer is stable to store at room temperature.

2. **To make various volumes of TBE concentrate use the formula:**

$$C_1 \times V_1 = C_2 \times V_2$$

Where:

C_1 = Original TBE Concentrate
V_1 = Volume of the Original TBE Concentrate needed
C_2 = Final concentration
V_2 = Total final volume desired

Once you have calculated the volume of TBE Concentrate needed, SUBTRACT that amount from the total volume of TBE running buffer desired to find the volume of water needed.

We recommend diluting buffer in batches for accuracy.

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