2013
Food Science – DNA Fingerprinting: A 2.5 Day Workshop for High School Students

Driving questions:
How can a devastating outbreak of foodborne illness be traced back to the source?
How can practical, relevant food science-based scenarios aid science teaching and learning in the classroom?

Audience: This workshop targets New York State high school 4-H club members. Participants have ranged in grade level from incoming freshman to graduated seniors. Our group typically hosts 15-20 students.

Overview: Students use epidemiological and molecular methods to investigate an outbreak of foodborne illness. This workshop seeks to portray science as a way of learning and knowing rather than a large collection of unrelated facts and seeks to provide a conceptual framework of the field. Student participants should take ownership of their learning and demonstrate independence while coordinators should fade into the background and simply become another member of the problem-solving team.

Calendar: This is a 3 day event. During the first day, students spend two and one half hours in our group, six hours on the second day (excluding time for lunch) and three hours on the third and final day.

Prerequisite knowledge: This workshop is designed to be accessible to a wide range of students from 9th to 12th grade.

Overarching concepts and principles: National Science Education Standards. National Research council, copytight 1996, National Academy Press.

1) The standards call for more than "science as process" (i.e., observing, inferring, experimenting).
2) Inquiry is central to science learning. When engaging in inquiry, students describe objects and events, ask questions, construct explanations, test these explanations against current scientific knowledge, and communicate their assumptions, use critical and logical thinking, and consider alternative explanations.
3) Develop evidence based models, arguments, and explanations.

Day 1: Tuesday, June 28th 2:00-4:30 PM

I. 2:00-2:30 Introductions and Icebreakers (Travis)

Introduce group leaders and students
Provide overview of what we will be doing during the next several days (techniques to investigate/solve an outbreak – sample, fingerprint, analyze)
Icebreaker Activity – led by focus assistant
Ice cream social
Complete Pre-assessments

II. 2:30-2:45 Food microbiology and Food Safety lecturette (Matt S.)

Materials: power point

Learning objectives: Food science and food safety are viable career options for students interested in science and our food supply.

Lesson plan: Present info on the discipline of food science, food microbiology, food safety, foodborne illness and foodborne pathogens.

III. 2:45-4:00 Case study: Foodborne outbreak investigation (Travis)

Materials: power point, Was it something I ate?, Attack Rate table, FDA Foodborne Illness-Causing Organisms in the US,

Learning objectives: Discuss diversity of foodborne pathogens, pathogenesis of foodborne pathogens, applying scientific methods to outbreak investigations, and basic epidemiology.

Lesson plan:
Lecturette on foodborne diseases (organisms, modes of pathogenesis) and basic epidemiology (what are the steps of an outbreak investigation?).
What are some ways that food can become contaminated?
Present case scenario: "Was it something I ate?" Remember the steps of an outbreak investigation? Are you sure an outbreak is occurring? What to do next? Establish case definition?
Are any of these "high-risk" foods? What are some high-risk foods and what makes them high-risk?
Group brainstorming: students decide what information they need. If they ask a logical question, they are given another clue to solving the outbreak…leading to the dissemination of the Attack Rate table and FDA Foodborne Illness Causing Organisms in the US.
Discuss possible causative organisms
Interview cases and matched pairs case controls
Discuss Attack Rate table and likely food source of disease, i.e., formulate hypotheses – and implement preliminary control measures

Has this organism/food combination ever been linked to an outbreak? Can the organism survive/grow in this food? How might it have become contaminated with this organism?

IV. 4:00-4:20 Sample Collection and Processing (Tom D.)

Preparation and Materials: Make sure incubators are set to appropriate temperatures. Students need a lab notebook or sample collection form for record keeping. Hand out Protocol for culturing bacteria from food and environmental samples.

Learning objectives: Bacteria are everywhere, but are microscopically small and hidden in their environment. Generally, bacteria are found in low numbers and much larger numbers are needed to be able to see them and run tests on them. Understand that nutrients and time are required for bacterial growth, also understand principles behind pre-enrichments and selective/differential media.

Lesson plan:
Form hypotheses before collecting samples so sample collection is not random. Collect samples from foods and food contact surfaces as well as from a patient.
Streak selective and differential plates and incubate overnight.
What are we sampling for? What are good places to collect samples (if we were in a food processing plant)? What information do you need to have before you begin to think about collecting samples? How should you handle the sample until you bring it in for processing? How quickly do you need to bring it in for processing?

V. 4:20-4:30 Review (Teresa)

Main points from Day 1:
Diversity of foodborne pathogens (different epidemiology, incubation times, symptoms)
Why do we need to collect samples and enrich them?

Investigate source of outbreak, confirm source, samples need to be enriched because organisms may be present in low numbers (they may have been damaged by sanitizing solutions or may not have had an opportunity to grow to high numbers in the foods)
Steps of an outbreak investigation:

1. Ensure that the problem has been properly diagnosed and rule out the possibility of lab error.
2. Confirm an outbreak is occurring; does the number of observed cases exceed the number of cases that normally occur?
3. Implement control measures to prevent the outbreak from spreading and to prevent future outbreaks.

Day 2: Wednesday, June 29th 9:00-4:30PM

I. 9:00-9:15 Review (Teresa)

Main points from Day 1:
Diversity of foodborne pathogens (different epidemiology, incubation times, symptoms)
Why do we need to collect samples and enrich them?

Investigate source of outbreak, confirm source, samples need to be enriched because organisms may be present in low numbers (they may have been damaged by sanitizing solutions or may not have had an opportunity to grow to high numbers in the foods)
Steps of an outbreak investigation:

4. Ensure that the problem has been properly diagnosed and rule out the possibility of lab error.
5. Confirm an outbreak is occurring; does the number of observed cases exceed the number of cases that normally occur?
6. Implement control measures to prevent the outbreak from spreading and to prevent future outbreaks.

Symptoms of foodborne illness (STEC, Salmonella, Listeria) – complications leading to cardiac arrest has been shown to be possible but not typical.

II. 9:15-9:30 Examine Sample Enrichments and Gather Data (Matt S.)

Learning objectives: Think like a scientist – demand evidence, skepticism, blend of logic and imagination, tentative but durable

Lesson plan:
Note: (finish streaking plates)
Make a table of results and describe colony morphologies.
Does the data make sense in light of the outbreak scenario? What do the selective/differential media tell us? Was our hypothesis about the organism rejected or not? What about our hypothesis of the food source? Does it look like the contamination occurred at the ice cream social or a food processing plant? Are there any signs of additional cross-contamination at the ice cream social?

III. 9:30-10:00 Strawberry DNA Extraction (Tom D.)

adapted from Scientific American science buddies
www.sciencebuddies.org/science-fair-project_ideas/BioChem_p015.shtml
Preparation and Materials:

- scissors for each table
- strawberries (~3-5/student)
- ziploc bags (1/student)

- stir sticks (1/student)
- rubbing alcohol or ethanol; keep ON ICE (~10 ml/student)
- dish washer detergent or shampoo with EDTA (~5ml/student)
- salt
- coffee filters
- small jars or beaker
- protocol to extract DNA from a strawberry

Learning objectives: All cells contain DNA, which is the code that instructs the cell on how to grow, survive, and replicate. Each type of cell contains a unique DNA sequence that has evolved specifically to allow the cell to survive in its environment.

Lesson plan: We are going to use the DNA from bacterial cells suspected of causing a foodborne disease to identify the food source that was responsible for causing the outbreak. But first, the DNA must be isolated from the other cellular components. To simulate the type of DNA isolation that we would use for DNA fingerprinting, we will extract the DNA from a strawberry. The techniques used to extract DNA from strawberry cells are the same techniques used to extract DNA from bacterial cells such as those that may have caused foodborne disease.

1. Lyse the strawberry cells to free the contents of the cells.
    a. Place a few (3-5) strawberries in a small whirl-pack bag.
    b. Grab the strawberries from outside the bag and mash the berries until the bag is filled with strawberry slurry.
    c. Cut a small hole in the corner of the bag and drain out just the liquid portion of the slurry into a beaker. Separate out as much pulp as possible.
2. Dissolve the nucleus of the strawberry cells to free the DNA.
    a. Add one medium squirt of dishwashing liquid to the beaker of strawberry juice.
    b. Stir the juice vigorously for 30 seconds using a sterile wooden stick.
3. Precipitate the DNA apart from the rest of the strawberry cell juice.
    a. Slowly pour cold ice cold rubbing alcohol down the side of the beaker to create a clear layer on top of the berry juice.
    b. As DNA rises from the berry juice, pull it out using a sterile wooden stick.

Discussion: The detergent helps to dissolve the cell membrane. Sodium chloride removes proteins that are bound to DNA and keeps proteins dissolved so they don't precipitate with the DNA in ethanol. DNA is soluble in water, but not ethanol so precipitates out of solution in ethanol.

IV. 10:00-10:30 Pipet Practice Activity (Tom D.)

Preparation and Materials: Have several beakers of water dyed with different food colors. Pipets, pipet tips, 1.5 ml and 0.2 ml eppendorf tubes.

Learning objectives: Students will gain experience using carefully calibrated scientific instruments. Scientists must use precise measurements for durability and repeatability of results. Very small volumes of liquid are measured in microliters by instruments called pipets (1000 microliters = 1 milliliter, 1,000,000 microliters = 1 liter, 1000 milliliters = 1 liter; "micro" means one millionth or 10^6, "milli" means one thousandth or 10^3. Slightly larger volumes can be measured by milliliters. Students should understand when to use a given pipet (p20, p200, p1000) and how to adjust the volume. Students also will understand the difference between sterile and non-sterile surfaces and the importance of changing tips before pipetting a different reagent and the importance of not contaminating the pipet or stock reagents. Practice aseptic technique!!!

Adapted from Rainin "Reduce pipetting errors"
Work in the 35-100% range.
Avoid too rapid aspiration.
Pipette with consistent rhythm and plunger pressure.
Hold tip in sample for 1 second after aspiration and withdraw tip slowly and smoothly.
Pre-rinse tip twice before aspirating sample.
Don't immerse anything but the tip in sample.
Don't twist the volume higher than the maximum for the pipette.
If filled, don't lay the pipette down.
Don't drop pipettes.

Lesson plan: Practice pipetting various volumes of food dye-colored liquids (water) into various sized eppendorf tubes (0.2 ml and 1.5 ml). Ex. 100 ul yellow dye in 20 ul red dye should make orange and the stock yellow reagent should not get contaminated with red dye.

V. 10:30-11:30 invA PCR (Teresa)

Preparation and Materials:
Prepare stock PCR reagents for each group.

Learning objectives: DNA can be replicated, or amplified in test tubes in the lab. PCR does not amplify the entire genome as occurs in the cell.

Primers are used to target specific sequences of DNA to amplify many times over. The first step of PCR is denaturation (high temperatures separate DNA strands), followed by annealing (primers attach to the target DNA sequence) and elongation (DNA polymerase extends the new DNA strand). This cycle is generally repeated 30 times.

Lesson plan: Students will follow the invA PCR protocol to make a master mix and add pre-prepared DNA lysates.

VI. 11:30-1:00 Lunch

VII. 1:00-2:00 DNA Model: Structure, Replication, and PCR (Travis)

Preparation and Materials: Powerpoint presentation, DNA model.

Learning objectives:

- DNA is hereditary genetic material
- DNA is found in humans, plants, bacteria, and other living organisms (excluding some viruses)
- DNA is also typically very stable and can often even be utilized from historic remains.
- DNA is a double strand of **nucleotides**. Nucleic acids is a broad term for DNA and RNA; amino acids are the building blocks of **proteins**.
- A nucleotide is composed of a phosphate, deoxyribose sugar, and a nitrogen base.

Lesson plan:
Adenine = green
Thymine = red
Guanine = blue
Cytosine = orange

Start at origin of replication (enzyme complex has to stay together (okazaki frag)
Helicase – breaks hydrogen bonds between bases
Single stranded DNA binding proteins
Topoisomerase - breaks and reseals DNA backbone to release tension caused by twisting.
Primase – RNA polymerase (RNA primers)
DNA pol III – copies DNA (can only add to an existing strand)
DNA pol I – removes RNA primers and replaces with DNA nucleotides
DNA ligase – seals up breaks in the DNA backbone by forming covalent bonds between nucleotides
[polymerase goes 3 to 5 on existing leading strand]

Students will learn about DNA structure by building a model of a DNA strand. First, students will construct nucleotide models and then assemble nucleotides into a double-stranded DNA molecule. Students will use this DNA model to recreate the process of DNA replication. First, the DNA molecule is unwound, then primers are added, and the new DNA is synthesized. Students will be assigned to carry out the role of a specific enzyme during the process. Also, the students will use the DNA model to demonstrate how DNA is amplified in a PCR reaction. I will explain the bonds that hold the molecules of DNA together while students are making their models. I will also discuss the roles of the various enzymes.
Also, demonstrate the principle of gel electrophoresis by having different sized groups of students walk down the aisles cluttered with lab stools (without touching any stools).

Discussion: Adenine, thymine, cytosine, and guanine are the four nitrogen bases found in a DNA molecule. Adenine and thymine can interact by forming two hydrogen bonds while cytosine and guanine can form three hydrogen bonds (Chargaff and Davidson, 1955). The phosphate group(s) of any single, unattached nucleotide is always found on the 5-carbon end of its deoxyribose sugar. One end of the double helix coding strand of nucleotides terminates with a 5 carbon that is not attached to another nucleotide, while the opposite end of the same strand terminates with an unattached 3 carbon. Understand DNA replication and the roles of relevant enzymes. Also understand how DNA is amplified in a Polymerase Chain Reaction (PCR).

What makes each person's or organism's DNA unique? Order of bases
Why does DNA need to be so stable? If it broke down easily, would it be a very reliable carrier of the genetic code?
DNA uses it's own template to make exact copy (semi-conservative replication)

Applications of PCR reaction in food safety (routine surveillance or outbreak investigations:

- Detection or confirmation of specific microorganisms in foods
- Molecular subtyping of isolates

Talk about other molecular detection methods and provide subtyping data to investigate the outbreak.

VIII. 2:00-3:00 Restriction Digest (Matt S.)

adapted from Bio-Rad Instruction Manual for Restriction Digestion and Analysis of Lambda DNA kit, Biotechnology explorer, reference number 4006102.
Preparation and Materials:

Set water bath to 37. Prepare stock reagents and DNA samples.

Learning objectives:
The restriction enzyme is a natural, bacterial defense mechanism. A restriction enzyme acts like molecular scissors, making cuts at specific sequence of base pairs that it recognizes. This serves to destroy DNA from invading viruses. A restriction enzyme sits on a DNA molecule and slides along the helix until it recognizes specific sequences of base pairs that signal the enzyme to stop sliding. The enzyme then cuts the DNA at this "restriction site."

Lesson plan: When the DNA from two different bacteria contain the sequence recognize by the restriction enzyme at different places in their DNA code, the restriction enzyme will cut the DNA at those different places. This will generate different sized pieces of DNA for the different bacteria, which can be used to identify those bacteria

Goal In this experiment you will use one restriction enzyme to digest the DNA from bacteria from both a patient sick with salmonellosis and from bacteria found in 5 different foods that the sick patients have eaten.

Procedure

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.
2. Label six tubes: P (Patient), F1 (Food 1), F2, F3, F4, and F5. Also include your group name and date.
3. Using a fresh tip for each sample, pipet 10 ul of each DNA sample from the stock tubes and transfer to the corresponding six tubes (P, F1, F2…)
4. Pipet 10 ul of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well.
5. Tightly cap the tubes and mix components by gently flicking with your finger. Gently tap the tube on the bench top or pulse-spin in microcentrifuge to collect all liquid in the bottom of the tube.
6. Place tubes in a foam microtube holder and incubate for 45 min at 37°C in a water batch.
7. See group leader to pour 1% agarose gels.

IX. 3:00-3:30 DNA Fingerprinting Overview (Teresa)

Materials: DNA model and powerpoint.

Learning objectives: PCR is used to amplify a single gene and results in presence/absence data, can use to confirm species and even sequence PCR products for further subtyping. DNA fingerprinting (Restriction Digest) is used to distinguish organisms within species. Restriction digest uses the whole genome. Banding patterns are derived from unique DNA

sequences. Restriction enzymes cut specific DNA sequences which results in different sized fragments of DNA depending on where these specific sequences are located. These DNA fragments are separated by gel electrophoresis: smaller bands migrate fastest.

Lesson plan: Briefly go through powerpoint slides, then demonstrate PCR and the action of restriction enzymes with DNA model. Demonstrate the principle of gel electrophoresis by having one single student, a group of ~ 3 students holding hands in a circle (all facing inwards) and a group of ~6 students also holding hands start to run across the length of a football field. Blow a whistle and have them all stop at the same time. The smaller groups should be able to "migrate" faster.

X. 3:30-4:15 Agarose gel electrophoresis (Tom D.)

Learning objectives:
(Adapted from Bio-Rad Instruction Manual, Restriction Digestion and Analysis of Lambda DNA Kit, Biotechnology Explorer) Agarose gel electrophoresis separates DNA fragments by size. The gel rig is filled with a conductive buffer solution and a current is passed between wires at each end. Since DNA fragments are negatively charged, they will be drawn toward the positive end. The gel acts like a sieve, allowing smaller fragments to migrate further faster. Fragments of the same size stay together and migrate in single bands which can be visualized after staining.

Lesson plan: Load gels and run in gel rigs.

XI. 4:15-4:30 Review (Travis)

Main points from Day 1:
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Steps of an outbreak investigation:

7. Ensure that the problem has been properly diagnosed and rule out the possibility of lab error.
8. Confirm an outbreak is occurring; does the number of observed cases exceed the number of cases that normally occur?
9. Implement control measures to prevent the outbreak from spreading and to prevent future outbreaks.

Symptoms of foodborne illness (STEC, Salmonella, Listeria) – complications leading to cardiac arrest has been shown to be possible but not typical.
Nature of Science-tentative (always changing), unable to answer all questions, always based on evidence but also a human endeavor that so that's not to say science can't be wrong or change but all conclusions are based on current evidence), science is a blend of logic and imagination (use imagination to develop new questions/hypotheses, science is also a complex social activity (collaborative effort).

Main points from Day 2:
DNA is hereditary genetic material, found in humans, plants, bacteria, and other living organisms (excluding some viruses). DNA is also typically very stable and can often even be utilized from historic remains. DNA is a double strand of **nucleotides**. Nucleic acids is a broad term for DNA and RNA; amino acids are the building blocks of **proteins**. A nucleotide is composed of a phosphate, deoxyribose sugar, and a nitrogen base.

DNA can be replicated, or amplified in test tubes in the lab. The first step of PCR is denaturation (high temperatures separate DNA strands), followed by annealing (primers attach to the target DNA sequence) and elongation (DNA polymerase extends the new DNA strand).
Molecular detection and subtyping methods are used frequently by the food industry, government, and academia to obtain surveillance data of foodborne pathogens, investigate outbreaks, as well as to study diversity, development of antimicrobial resistance, virulence, etc.

Investigation progress?

Day 3: Thursday, June 30th 9:00-12:00 PM

I. 9:00-9:15 Review (Travis)

Main points from Day 1:
Diversity of foodborne pathogens (different epidemiology, incubation times, symptoms)
Why do we need to collect samples and enrich them?
Investigate source of outbreak, confirm source, samples need to be enriched because organisms may be present in low numbers (they may have been damaged by sanitizing solutions or may not have had an opportunity to grow to high numbers in the foods)
Steps of an outbreak investigation:

10. Ensure that the problem has been properly diagnosed and rule out the possibility of lab error.
11. Confirm an outbreak is occurring; does the number of observed cases exceed the number of cases that normally occur?

12. Implement control measures to prevent the outbreak from spreading and to prevent future outbreaks.

Symptoms of foodborne illness (STEC, Salmonella, Listeria) – complications leading to cardiac arrest has been shown to be possible but not typical.
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DNA is hereditary genetic material, found in humans, plants, bacteria, and other living organisms (excluding some viruses). DNA is also typically very stable and can often even be utilized from historic remains. DNA is a double strand of **nucleotides**. Nucleic acids is a broad term for DNA and RNA; amino acids are the building blocks of **proteins**. A nucleotide is composed of a phosphate, deoxyribose sugar, and a nitrogen base.

DNA can be replicated, or amplified in test tubes in the lab. The first step of PCR is denaturation (high temperatures separate DNA strands), followed by annealing (primers attach to the target DNA sequence) and elongation (DNA polymerase extends the new DNA strand).
Molecular detection and subtyping methods are used frequently by the food industry, government, and academia to obtain surveillance data of foodborne pathogens, investigate outbreaks, as well as to study diversity, development of antimicrobial resistance, virulence, etc.

Investigation progress?

II. 9:15-9:30 Analyze Gels and Discuss Results (Tom D.)
Learning objectives: Understand the nature of science – demand evidence, science is a human endeavor and is subject to human mistakes and biases.

Lesson plan: Discuss results, do the results make sense in light of the investigation? Troubleshoot any failed reactions. Also, can look at plates again.

III. 9:30-10:00 Conclusion of Investigation (Teresa)
Learning objectives: steps of an outbreak investigation.

Lesson plan: What food source caused the outbreak? What control measures could be implemented to prevent future outbreaks?

IV. 10:00-10:15 Nature of Science (Travis)

Materials: Pipe model, paper handouts of pipe model diagram, cardboard tubes, string, tape, hole punch, scissors.

Learning objectives:

- Science demands evidence
- Science is tentative
- Science is a blend of logic and imagination
- Science is a collaborative effort
- Science is limited

  Science content standards: Standard A: abilities necessary to do scientific inquiry, understandings about scientific inquiry; Standard G: science as a human endeavor, nature of scientific knowledge.

Lesson plan: Show the model.
What is this?
What do you think is inside?
How do you know?
Pull one string of the pipe model. Ask students to write down their observations. Pull another string. Ask students to draw what they think is going on inside the pipe. Tape the diagrams to the board and discuss.
Which diagrams could explain what is going on in the tube?
How could we test this?
Have students make their own models.
Did anyone change their diagram/hypothesis after receiving more information (i.e., more string pulling)?
Does anyone know what is going on inside now? How do you know?
Your model may be a good explanation for what could be inside but we can't know for sure without being able to look inside (i.e., we are bound by a level of certainty).

Discussion: Food safety is bound by the same principles of the Nature of Science as any other scientific discipline and high profile outbreaks put these limits on full display (don't eat tomatoes, then peppers, then Mexican peppers; same with European O104 outbreak). Science demands evidence but we can only make observations of things we can see or measure and have to formulate hypotheses based on these measurements about what is going on with things we can't see. We can never prove anything definitively. We can only disprove, or reject, hypotheses. Science is tentative so if more or better evidence comes along (maybe an instrument that would allow us to see or take measurements inside the tube) we may change or confirm our hypotheses. Science is also a blend of logic and imagination. How do we

know there is not a little troll
(imagination) in the tube pulling strings? Logic, based on prior knowledge and experiences.

The Nature of Science is tentative (always changing as our knowledge of the world increases; current information may be incomplete and our explanations incorrect), unable to answer all questions (God?), always based on evidence but also a human endeavor so that's not to say science can't be wrong or change but all conclusions are based on current evidence), science is a blend of logic and imagination (use imagination to develop new questions/hypotheses, science is also a complex social activity (collaborative effort).

This activity also demonstrates the process of scientific inquiry by generating questions, designing experiments, making predictions, gathering data (evidence) and communicating findings.

Which diagrams could explain what is going on in the tube?

How could we test this?

Build models

Did anyone change their model after seeing other diagrams or talking to others?

Did anyone change their diagram/hypothesis after receiving more information (i.e., more string pulling)?

V. 10:15-10:45 Overview of Research Programs and Lab Tours (Teresa)

VI. 11:15-12:00 Conclusions and Evaluations
Evaluations, post-assessments, food science careers, food science at Cornell.