

A microscopic view of numerous green, spiky organisms, possibly nematodes or similar small invertebrates, crawling on a textured, brownish surface. The organisms are scattered across the frame, with some showing distinct head and tail regions and many fine, hair-like appendages.

FUTURELAB+

AG/ENVIRONMENTAL

Community Science

Technology Lesson 4: NextGen Sequencing

Developed in partnership with:

Discovery Education and Ignited

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This document is separated into two sections, For Teachers [T], and Student Resources [S], which can be printed independently.

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Cover Image

Bacteria in a water sample is a potential source of environmental DNA (eDNA).

AG/ENVIRONMENTAL/COMMUNITY SCIENCE

Technical (Tech) Lesson 4: NextGen Sequencing

DRIVING QUESTION

How does NextGen sequencing work as a DNA identification technique?

OVERVIEW

The advancement of DNA identification and sequencing technology opens up many possibilities for using it to answer community-focused questions. NextGen Sequencing (NGS) is a large-scale DNA analysis tool that can sequence many DNA fragments rapidly. NGS can be used to identify unknown species in cases of foodborne illnesses or bacterial wastewater contamination. It can also be used to identify species that are invasive through environmental DNA.

In this lesson, students will assume the role of a member of an outbreak investigation team for a local health department. This allows students to integrate their content learning with scientific thinking. Initially they will need to identify potential areas of contamination by reading interviews of multiple community members. From the interviews, they need to decide the locations from which to sample DNA. Students will get the results of sequencing (similar to that of NextGen sequencing) and use the Basic Local Alignment Search Tool (BLAST) from National Center for Biotechnology Information (NCBI) to determine the species associated with the outbreak. Next, they will research the species to determine which ones could cause the issues explained through the community member interviews. They will continue to analyze data to determine which location was the cause of the contamination, and use a Claim-Evidence-Reasoning conclusion format to justify the species and the location. Students will complete a formative assessment where they read about NextGen sequencing technology and compare it to the case study. Students will also add what they learned about the process of NextGen Sequencing to their Decision Tree Assessment.

ACTIVITY DURATION

Four class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

How can NextGen sequencing be used to answer questions related to DNA identification?

How does NextGen sequencing compare to other DNA identification techniques?

How does NextGen sequencing connect to other DNA tools we have learned about (BLAST, PCR)?

OBJECTIVES

Students will be able to:

Explain the purpose of NextGen sequencing.

Identify types of information that can be gathered using NextGen sequencing.

Describe the pros and cons of NextGen sequencing as a DNA identification technique.



Colonies of different bacteria and mold fungi grown on Petri dish with nutrient agar.

Materials

The Next Generation of Food Safety Case Study Capture Sheet

Citizen Interviews

County Health Department—DNA Sequencing Sampling Request Form Capture Sheet

Data Analysis 1 Capture Sheet

Sampled DNA Sequences by Location

Data Analysis 2 Capture Sheet

NextGen Sequencing Reading

NextGen Sequencing Check for Understanding Capture Sheet

Technology Overview Capture Sheet, Part 1: DNA Identification

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students must use self-discipline and self-motivation to stay on task and complete the case study. Students will use evidence to support their decisions. Those decisions will guide their learning.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson connects to real-world issues identified by students in their own communities. Throughout the lesson, students are encouraged to reflect on different perspectives within their community and how to include all stakeholders in decision making. All students are also held to the high expectation of understanding the complex topic of NextGen sequencing.

ADVANCING INCLUSIVE RESEARCH

Throughout this lesson, students have the opportunity to discuss how the lack of data from underrepresented groups can put those groups at a disadvantage when it comes to the utilization of NGS and BLAST technologies. Students can consider the potential harmful effects a community can endure as a result of this lack of access and outreach, as well as the limitations of affordable healthcare those groups may experience. In order to help as many people as possible, it is important that the scientific community develops outreach programs that connect patients with the healthcare and therapies they need.

COMPUTATIONAL THINKING PRACTICES

Students collect and analyze data using a public DNA data analysis tool (BLAST). Students use that data to draw an evidence-based conclusion. In addition, students must use critical thinking to compare the process in the case study to the process of NextGen sequencing.

CONNECTION TO THE PRODUCT LIFE CYCLE

In this lesson, students take on the roles of epidemiologists and environmental health specialists as they use DNA sequencing to identify the microbes behind a food poisoning outbreak. This connects to the **discover** phase of the product life cycle as students determine how existing biotechnology tools are used in public health fields.

Have you ever wondered...

Who helps when there is a foodborne illness outbreak?

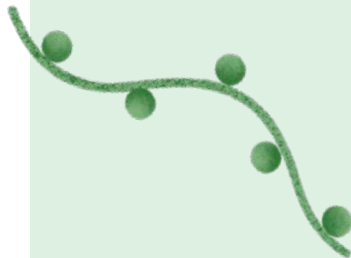
As DNA sequencing becomes less expensive and the technology advances, it can be used to solve problems like never before. Public health officials are harnessing these new technologies to help with their role in keeping food safe for the public.



MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

This lesson is the fourth lesson in a series on major technologies used in DNA identification. Students will be selecting among these technologies as they prepare their final artifact proposals.



How does this connect to careers?

Epidemiologists are disease detectives that search for the cause of disease, identify people who are at risk, determine how to control the spread, and stop it from happening again. They use methods like lab analysis, patient and community interviews, and field studies in their work to control outbreaks.

Environmental health specialists are local officials that enforce health and safety regulations and educate their communities to decrease risks of outbreak. They use their expertise on human health to track air, food, and water quality. They are especially interested in vectors found in air, food, and water that can cause disease.

How does this relate to the product development life cycle?

Food safety is an issue for every community and all individuals. Students will see how NextGen sequencing can be used to identify the source of contamination to restore food safety.

Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

Explain the role of an epidemiologist and an environmental health specialist.

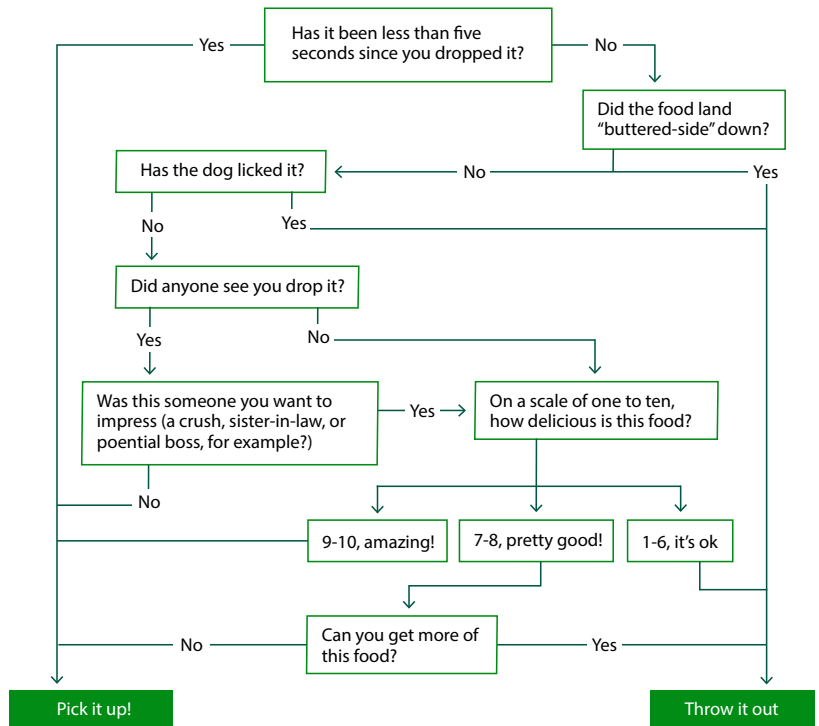
Analyze interview evidence to determine how to proceed in a case study.

Teacher Note > *If you did not use the lab in the Behind the Scenes of Scientific Breakthroughs unit where students examine a SNP in the human genome to determine if there is an association with longevity, it might be good to reference those resources for some background information on BLAST.*

Whole Group (15 minutes)

- 1 Project the “You Dropped Food on the Floor” flowchart as students enter the room. Ask the class:
 - Have you ever eaten food you’ve dropped on the floor?
 - Have you ever had food poisoning?
Do you know or think you know where you got it?
 - What do you think food poisoning has to do with DNA identification?
 - Have you ever worked in a restaurant?
What safety concerns do employees need to consider?

You dropped food on the floor.
Do you pick it up or throw it out?



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Day 1

Continued

Procedure

-
- 2 Pass out the *Next Generation of Food Safety Case Study Capture Sheet*.
-
- 3 Introduce the career of an epidemiologist and an environmental health specialist. The resources below will provide recent information on these careers for students. Students will then answer the first two questions on the capture sheet.
- a. *Who are epidemiologists?* from the CDC
 - b. *Epidemiologists* from the US Bureau of Labor Statistics
 - c. *About the Environmental Health Specialists Network* from the CDC
 - d. *Job Profile: Environmental Health Specialists* from the CDC
-
- 4 Ask students to share their answers to the third question on the *Next Generation of Food Safety Case Study Capture Sheet*: *Have you ever experienced contact tracing?*
-
- 5 Next, students will do a Think-Pair-Share to answer the fourth question on the *Next Generation of Food Safety Case Study Capture Sheet*: *If you were an epidemiologist or environmental health specialist, what questions would you want to ask community members?*

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Day 1

Continued

Procedure

Small Group (30 minutes)

- 1 Tell students that they will role play these careers as they work through the case study in the next couple days. Explain that this will allow them to integrate their content learning with the process of doing science while completing the case study.

- 2 Pass out the *Citizen Interviews*. Place students into small groups of three or four. Students will take turns role playing the environmental health specialist and the person being interviewed. While two group members are role playing, other group members should read the interviews and take notes to complete the Interview Analysis table in the *Next Generation of Food Safety Case Study Capture Sheet*. Tell students to rotate through the positions so they all have a chance to do each task.

Teacher Note > Before the lesson, assign names to the citizens being interviewed in the *Citizen Interviews* to help increase relevance for students.

- 3 Groups will identify which locations they should “collect” DNA samples from to investigate the food poisoning outbreak.

- 4 Groups should provide justification for their sampling locations and submit the *County Health Department, DNA Sequencing Sampling Request Form Capture Sheet* to receive sequencing data. Students must rank their requests according to level of importance. Students will be identifying evidence to support their claim of which locations to sample.

Day 2

Procedure

LEARNING OUTCOMES

Students will be able to:

Analyze DNA sequencing data using BLAST.

Justify the location of bacterial contamination using sequencing evidence.

Identify the type of bacteria causing the foodborne illness in the case study.

Small Group (40 minutes)

- 1 Give groups digital copies of the *Data Analysis 1 Capture Sheet* and the *Sampled DNA Sequences by Location* of specimens found at several locations. To facilitate the case study, you can change the locations to match students' requests.
- 2 Groups will visit the NCBI (National Center for Biotechnology Information) site to use *Nucleotide BLAST* and determine the species present in the DNA and then conduct further research to identify if each species is virulent.

Teacher Note > *BLAST was used in the Behind the Scenes of Scientific Breakthroughs Unit. Please refer to those lessons for further instructions. There are many explanatory videos available online if more explanation is needed.*

Individual Work (10 minutes)

- 1 Students will complete a CER conclusion for the *Data Analysis 1 Capture Sheet*, using the CER framework provided.



Day 3

Procedure

LEARNING OUTCOMES

Students will be able to:

Analyze DNA sequencing data using BLAST.

Justify the location of bacterial contamination using sequencing evidence.

Identify the type of bacteria causing the foodborne illness in the case study.

Individual Work (10 minutes)

- 1 Students should complete the CER conclusion for *Data Analysis 1 Capture Sheet* for Day 2 if they haven't already.

Small Group (20 minutes)

- 1 Based on the lab order submitted on Day 1, give groups additional information about species differences in selected locations.
- 2 Students should compare the *Sampled DNA Sequences by Location* to their list of potentially virulent species to identify the starting location for the outbreak.
- 3 Students should use the information from the interviews (the Interview Analysis table from *The Next Generation of Food Safety Case Study Capture Sheet*) as evidence to support their claims.

Individual Work (10 minutes)

- 1 Students will complete a CER conclusion for the *Data Analysis 2 Capture Sheet* using the CER framework provided.

Teacher Note > *There are multiple opportunities in Day 2 and Day 3 where students could provide peer feedback. If time allows, students could incorporate the feedback to strengthen their arguments (CER).*

Day 4

Procedure

LEARNING OUTCOMES

Students will be able to:

Describe what NextGen sequencing is and how it works.

Explain how the case study was similar to the steps of NextGen sequencing.

Individual Work (20 minutes)

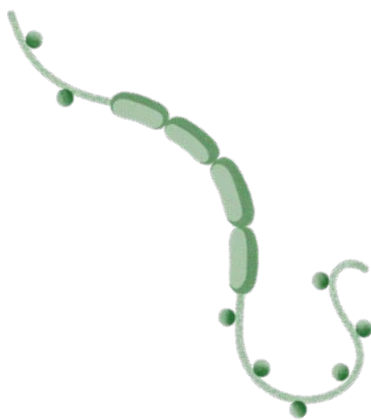
- 1 Students will read the *NextGen Sequencing Reading* and use the reading strategy “*Notice and Note Signposts*” from *Kylene Beers and Bob Probst*.
- 2 Instruct students to take quick notes on the signposts they identify in the reading.
- 3 When done, students should compare their notes with a partner to help understand what NextGen sequencing is and how it works.

Individual Work (10 minutes)

- 1 Students will complete the *NextGen Sequencing Check for Understanding Capture Sheet* as a formative assessment on how the process of NextGen sequencing compares to the process described in the case study.
- 2 Review the capture sheets and assess for overall understanding.

Individual Work (15 minutes)

- 1 Tell students to complete the *Technology Overview Capture Sheet, Part 1: DNA Identification* for NextGen sequencing (Tech Lesson 4).
- 2 Inform students that this will be used to help identify what DNA identification technology they will use for their project.



National Standards

Next Generation Science Standards

LS-1-1 From Molecules to Organisms: Structures and Processes

Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

ETS1-2 Engineering Design

Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.

Science and Engineering Practices

Engage in argument from evidence

Compare and evaluate competing arguments or design solutions in light of currently accepted explanations, new evidence, limitations (e.g., trade-offs), constraints, and ethical issues.

Career and Technical Education (CTE)

A1.6

Explore and outline the various science and non-science fields and careers associated with biotechnology.

A3.3

Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.

A4.0

Recognize basic concepts in cell biology and become familiar with the laboratory tools used for their analysis.

A5.0

Integrate computer skills into program components.

A8.7

Determine which equipment is appropriate to use for a given task and the units of measurement used.

4.3

Use information and communication technologies to synthesize, summarize, compare, and contrast information from multiple sources.

The Next Generation of Food Safety Case Study Capture Sheet

ANSWER KEY

Do not share with students

Directions

Over the next few days, you will play the role of an epidemiologist and an environmental health specialist as you work to investigate a food poisoning outbreak. Follow the steps below.

Interactive Background

You are a current employee of the county health department named below. Due to budget cuts, you have the role of epidemiologist and environmental health specialist for the outbreak investigation team.

Name of your county health department:

Answers will vary.

1. What is an epidemiologist?

Epidemiologists search for the cause of disease, identify people who are at risk, determine how to control or stop the spread of disease, and try to prevent it from happening again.

2. What are some responsibilities of an environmental health specialist?
 - Track food safety and water quality
 - Environmental hazard evaluation
 - Disease control

Over the past two days, the health department has received multiple phone calls and emails from citizens with concerns of food poisoning. Other members of your team followed up with several of the citizens and conducted interviews to contact trace.

3. Have you ever experienced contact tracing?
Write what you know about contract tracing below.

Answers will vary.

Contact tracing: identifying people who may have come into contact with an infection or infected person to collect further information

4. If you were conducting the interview, what questions might you ask and why?

Answers will vary.

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The Next Generation of Food Safety Case Study Capture Sheet

ANSWER KEY

Do not share with students

Continued

Analyze the *Citizen Interviews* provided. Your goal on the investigation team is to identify patterns and start to determine what is causing the problem. After you have identified the problem, an infection control team will take over. Because this is a threat to public health, it is critical to act quickly. These are the only interviews your team is able to collect in the short time frame.

- Use the organization tool below to help with taking notes on the interviews.

Interview Analysis

Interview Number	Relevant Evidence <i>Statements or facts of interest from the interview</i>	Possible Next Step <i>Based on evidence</i>
1	Gloves are worn, surfaces sanitized. Do not always use thermometer to ensure temperature of food Gets food from Smith's sometimes	Check Smith's
2	Ate chicken, vegetables, beef Symptoms: Diarrhea, cramps, no fever Did wash hands	Check with co-workers
3	Ate chicken, spinach artichoke dip Symptoms: Diarrhea, cramps, no fever Smith's meat market Slow cooker stopped working (temperature issue).	Check Smith's Check residence
4	Follows CDC protocols No issues in record books Gets meat from meat market and then package	Check Parkway
5	No issues with food safety Do process poultry on site but is aware of hazards with meat processing (intestine content contamination) Smith's is a direct sale.	Check chicken from facility Check Smith's
6	Shared plate of chicken wings (all ate some chicken) Symptoms: Diarrhea, cramps, no fever, (for everyone) Daughter had a headache from dehydration.	Check Champs

**County Health Department, DNA Sequencing Sampling
Request Form Capture Sheet**
ANSWER KEY
Do not share with students
Directions

Based on the information that you collected from the interviews, fill in the table below to request DNA samples from locations of interest. Make sure you are specific in the location for your request and provide reasoning to support the request. Because the health department is under budget cuts, the employees have limited time and resources, so you will need to rank your requests (1 being most important, and 4 least).

Answers in the Rank column may vary.

DNA Sampling Request Form

General Location	Specific Locations <i>Exactly where should the DNA be collected?</i>	Relevant Evidence <i>Why is this location a sampling priority? Support this with information from the interviews.</i>	Rank
Parkway Meat Distribution Center	Poultry processing division	Parkway processes poultry on-site (contamination possible) and sells to Smith's.	1
Champs Restaurant	Sample of the chicken wings	Champs purchases from Smith's and admits to not always using a meat thermometer—the chicken in the chicken wings could have been undercooked, leaving the bacteria alive.	3
Residence of Citizen 1	Sample of the buffalo chicken dip	Citizen 1 purchased from Smith's, and their slow cooker turned off—the chicken in the buffalo chicken dip could have been undercooked, leaving the bacteria alive.	4
Smith's Meat Market & Grocery	Chicken in the meat department	Smith's purchased chicken from Parkway Meat and sold to Champs and to Citizen 1. Smith's does not cook the chicken, so any bacterial contamination from Parkway would remain.	2

Data Analysis 1 Capture Sheet**ANSWER KEY****Do not share with students****Directions**

Now you assume the role of the Lab Scientist to determine the bacterial species at the locations in the case. Follow the directions below to gather information from the *Sampled DNA Sequences by Location* provided by your teacher.

1. Use the Basic Local Alignment Search Tool (BLAST) to find regions of similarity between the sequences on the *Sampled DNA Sequences by Location* and the sequences in the database. This “downstream” analysis of the NGS data will help identify the species found at the different locations. Fill in the data table below.
2. Once you have the scientific name, finish filling in the table with characteristics of the bacteria.

Table 2: Sequences Identified by Species with Characteristics

Sequence Reference Number	Species BLAST Result Scientific Name	Bacterial Characteristics <i>Where is it found? Does it cause illnesses? If so, what are some symptoms of the illness they cause?</i>
1	<i>Staphylococcus epidermidis</i>	Non-virulent
2	<i>Staphylococcus aureus</i>	Can be virulent but does not cause the symptoms described in the interviews
3	<i>Clostridium perfringens</i>	Virulent and has the symptoms described in the interviews
4	<i>Enterobacter roggenkampii</i>	Non-virulent
5	<i>Cutibacterium acne</i>	Non-virulent

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Data Analysis 1 Capture Sheet

ANSWER KEY

Do not share with students

Continued

3. Use the data that you generated in in questions 1 and 2 to conclude which species is causing the issue. Use the Claim-Evidence-Reasoning prompts below.

Claim	Evidence-Reasoning	
<p><i>Make a claim of the scientific name of the species that is causing the issues.</i></p> <p><i>Clostridium perfringens</i> is the species of interest in this foodborne illness outbreak.</p>	<p><i>Explain the illness (symptoms) caused by the species in the claim from CDC's Prevent Illness From C. perfringens.</i></p> <p>This bacteria can cause diarrhea and stomach cramps. It does not cause fever or vomiting.</p>	<p><i>Identify evidence from the case study interviews that are similar to the symptoms caused by the illness.</i></p> <p>Citizens 1, 2, and 3's families all had these symptoms and also did not have a fever. None of them mentioned vomiting either.</p>

Data Analysis 2 Capture Sheet**ANSWER KEY****Do not share with students***Continued*

1. Use the NextGen Sequencing analysis to identify the initial source of the contamination.

Claim	Evidence-Reasoning
<p><i>Claim the source of the contamination.</i></p> <p>Parkway Meat Distribution Center is the initial source of contamination.</p>	<p><i>Explain why this is the location of the contamination using evidence from the table on the previous page. Use your notes from the Interview Analysis table (The Next Generation of Food Safety Case Study Capture Sheet) for reference as well.</i></p> <p><i>Clostridium perfringens</i> was found in all locations except Citizen 1's residence. That means that it must have started at the distribution center, which provided chicken to Smith's, and Smith's provided it to Champs and to Citizen 2. Citizen 1 did not purchase any of the chicken but went to Citizen 2's residence for a potluck.</p> <p>None of the chicken was confirmed to be cooked at the appropriate heat to kill the bacteria.</p> <p>It is known that bacteria can get onto the meat from the intestine of the chicken (or other animal).</p>

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Data Analysis 2 Capture Sheet**ANSWER KEY****Do not share with students***Continued*

2. The infection control team will take over from here. The last thing you need to do is to make a recommendation of the next steps. Based on your research, what is a reasonable first step for the infection control team?

Possible answers:

- Have Smith's remove all chicken from sale.
- Go to Parkway and confirm contamination.
- Go to Smith's and confirm contamination with more sequencing.

NextGen Sequencing Check for Understanding Capture Sheet

ANSWER KEY**Do not share with students****Directions**

Each numbered step below explains a part of the NextGen sequencing DNA identification technology. This case study modeled many of the steps scientists would have done when using NextGen Sequencing to identify the source of a foodborne illness.

To show your understanding, explain the part of the case study that was comparable to NextGen sequencing.

1. The DNA is collected or sampled from the area of interest. What in the case study is similar to this step?

Collecting the DNA from the areas of interest requested in the DNA Sequencing Sampling Request form

2. The DNA is isolated and sequenced quickly in short segments. What in the case study is similar to this step?

The sequences of the segments on the *Data Analysis 1*

3. The sequences are compared to a known DNA library. What in the case study is similar to this step?

Note: A DNA library is a collection of known DNA fragments that can be used for research. The sequence order and species are already identified. It acts like a reference section in a real library but for DNA.

Using BLAST on NCBI

4. The species are identified (even if there are mutations). What in the case study is similar to this step?

Reading the information on NCBI to determine species as identified by the DNA sequence

Technology Overview Capture Sheet*Part 1: DNA Identification***ANSWER KEY****Do not share with students****Directions**

After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.

This capture sheet was assigned to students in Tech Lesson 2. Students should keep this sheet and others for use in Lesson 9.

Tech Lesson 2		Sanger Sequencing	
Describe	Summarize how this technique works.	<p>This process determines the sequence of DNA nucleotides through PCR. As the PCR reaction copies the fluorescent chain, terminator nucleotides mark the end of different segment lengths. The segment lengths are put in order and a program reads the end nucleotide fluorescence to determine sequence.</p>	
Discuss	List the pros and cons you identify for the technique.	<p>Pros</p> <ul style="list-style-type: none"> Easily accessible Most established technology Good at cloning individual genes Easily manipulated for initial plasmid research Good for checking that insertion of a gene in a plasmid worked 	<p>Cons</p> <ul style="list-style-type: none"> Slow Expensive per region you want to sequence Can only sequence the target region because you have to design a primer
Support	Provide examples.	<ul style="list-style-type: none"> Forensics—animals or humans Genotyping—Determining presence of different alleles (sequences) Determining viral variants Other examples not mentioned in the lesson do exist. 	

Technology Overview Capture Sheet*Part 1: DNA Identification***ANSWER KEY****Do not share with students****Directions**

After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lessons 9.

This capture sheet was assigned to students in Tech Lesson 3. Students should keep this sheet and others for use in Lesson 9.

Tech Lesson 3		Digital PCR	
Describe	Summarize how this technique works.	<p>Digital PCR is a technique used to detect low levels of DNA or other nucleic acid.</p> <p>It can also be used to quantify the amount of DNA found in a sample.</p> <p>It can be used to detect the presence of species (find a needle in a haystack).</p>	
Discuss	List the pros and cons you identify for the technique.	<p>Pros</p> <p>Only need a small amount of DNA</p> <p>Can be used in large bodies of water where DNA could be diluted</p> <p>Accurate in detecting species</p> <p>It can detect low levels of the target DNA sequence.</p>	<p>Cons</p> <p>Specialized and expensive equipment</p> <p>Can only do a few samples at a time (96)</p> <p>Need to know what kind of DNA you want to detect</p> <p>Can be inhibited by mutations because primers don't work</p>
Support	Provide examples.	<p>Finding invasive species in bodies of water or plant matter</p> <p>Accurately determining amount of virus in a sample (can be used to correlate viral symptoms with amount of virus in the infected individual)</p> <p>Detecting SARS-CoV-2 in sewage</p> <p>Other examples not mentioned in the lesson do exist.</p>	

Technology Overview Capture Sheet*Part 1: DNA Identification***ANSWER KEY****Do not share with students****Directions**

After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.

This answer key is for the current lesson, Tech Lesson 4. Students should keep this sheet and others for use in Lesson 9.

Tech Lesson 4	NextGen Sequencing											
Describe	Summarize how this technique works.	<p>NextGen sequencing is a method to sequence DNA using short sequencing reads.</p> <p>The fast speed of the sequencing allows for all the DNA to be sequenced from the collection sample. Then the detected DNA sequences can be compared to a library to determine the species of origin.</p>										
Discuss	List the pros and cons you identify for the technique.	<table border="1"> <thead> <tr> <th data-bbox="496 940 997 970">Pros</th> <th data-bbox="1003 940 1487 970">Cons</th> </tr> </thead> <tbody> <tr> <td data-bbox="496 999 954 1029">Do not have to know the species</td> <td data-bbox="1003 999 1487 1066">Cannot quantify how much DNA is there, just if it is there</td> </tr> <tr> <td data-bbox="496 1087 954 1180">Can do targeted sequencing to look for specific sets of sequences, such as SARS-CoV-2 variants</td> <td data-bbox="1003 1087 1487 1180">Need to have a high enough quantities to sequence it (no detection of low-level quantities)</td> </tr> <tr> <td data-bbox="496 1201 906 1230">Can do blanket general sequencing</td> <td data-bbox="1003 1201 1273 1230">Expensive instruments</td> </tr> <tr> <td data-bbox="496 1285 813 1314">Not impacted by mutations</td> <td data-bbox="1003 1285 1487 1388">Short reads—if you do not have a full genome to compare it to, then you may not detect what it is.</td> </tr> </tbody> </table>	Pros	Cons	Do not have to know the species	Cannot quantify how much DNA is there, just if it is there	Can do targeted sequencing to look for specific sets of sequences, such as SARS-CoV-2 variants	Need to have a high enough quantities to sequence it (no detection of low-level quantities)	Can do blanket general sequencing	Expensive instruments	Not impacted by mutations	Short reads—if you do not have a full genome to compare it to, then you may not detect what it is.
Pros	Cons											
Do not have to know the species	Cannot quantify how much DNA is there, just if it is there											
Can do targeted sequencing to look for specific sets of sequences, such as SARS-CoV-2 variants	Need to have a high enough quantities to sequence it (no detection of low-level quantities)											
Can do blanket general sequencing	Expensive instruments											
Not impacted by mutations	Short reads—if you do not have a full genome to compare it to, then you may not detect what it is.											
Support	Provide examples.	<p>Detecting foodborne illnesses</p> <p>Detecting microbial water pollution or water contamination</p> <p>Determining viral variants</p> <p>Determining a breed of cat or dog, population ancestry analysis</p> <p>Forensics—animals</p> <p>Genotyping—Determining presence of different alleles (sequences)</p> <p>Other examples not mentioned in this lesson may exist.</p>										

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The Next Generation of Food Safety Case Study Capture Sheet

Directions

Over the next few days, you will play the role of an epidemiologist and an environmental health specialist as you work to investigate a food poisoning outbreak. Follow the steps below.

Interactive Background

You are a current employee of the county health department named below. Due to budget cuts, you have the role of epidemiologist and environmental health specialist for the outbreak investigation team.

Name of your county health department:

1. What is an epidemiologist?

2. What are some responsibilities of an environmental health specialist?

Over the past two days, the health department has received multiple phone calls and emails from citizens with concerns of food poisoning. Other members of your team followed up with several of the citizens and conducted interviews to contact trace.

3. Have you ever experienced contact tracing?
Write what you know about contract tracing below.

4. If you were conducting the interview, what questions might you ask and why?

Continues next page >

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The Next Generation of Food Safety Case Study Capture Sheet

Continued

Analyze the *Citizen Interviews* provided. Your goal on the investigation team is to identify patterns and start to determine what is causing the problem. After you have identified the problem, an infection control team will take over. Because this is a threat to public health, it is critical to act quickly. These are the only interviews your team is able to collect in the short time frame.

- Use the organization tool below to help with taking notes on the interviews.

Interview Analysis

Interview Number	Relevant Evidence <i>Statements or facts of interest from the interview</i>	Possible Next Step <i>Based on evidence</i>

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Citizen Interviews

Directions

The following are the important excerpts from interviews conducted by the environmental health team. An environmental health specialist (EHS) is the health department employee conducting the interviews. Insert the name of the interviewee in the space provided.

1 Interview with Champs Restaurant Employee (CE):

EHS	Where do you work and what is your job?
CE	I am a line cook at Champs restaurant in town. So I help with food preparation and sometimes help with the cooking.
EHS	What are the food safety protocols?
CE	We wash our hands and wear gloves but we do not wear masks. Every night we use bleach to clean all the countertops and cooktops.
EHS	Do you use a meat thermometer?
CE	We are supposed to but sometimes we are so busy that we forget or in general just assume that the food is cooked.
EHS	Was it busy in the restaurant last night?
CE	Yes, I think so. It was a Sunday and there were a lot of people who liked to come and watch games and hang out. So we were definitely busy.
EHS	Where do you get your meat and vegetables?
CE	We normally get a food shipment two times a week, but sometimes at the end of the week if we are running low on any items we will stop by Smith's Meat Market and Grocery to pick up any items.

2 Interview with Anonymous Citizen 1 (C1):

EHS	What and where did you eat before you got sick?
C1	Last night I went to a potluck with my coworkers at a friend's house. There was a lot of different food there. I know I ate some carrots off the vegetable tray but I didn't eat that dip. I had some taco dip and buffalo chicken dip with chips. I also had a glass of water and punch with ice cream in it.
EHS	What are your symptoms? How long did you have your symptoms and when did they start?
C1	I woke up at like 2 am with really bad stomach cramps. I had diarrhea and I thought I was going to puke but I didn't. I kept getting hot and then cold and then hot again. I took my temperature, but it was normal.
EHS	Did you wash your hands before you ate?
C1	Yes and no. I went to the restroom and then washed my hands. It was a lot of eating and talking so I didn't wash them before everytime I ate.
EHS	Do you know if anyone else at the party got sick?
C1	My coworker that hosted the party said that he also got sick, but I am not sure other than that. They just posted that they didn't feel well on social media.

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Citizen Interviews

Continued

3 Interview with Anonymous Citizen 2 (C2):

EHS What and where did you eat before you got sick?

C2 It was my night to host the monthly staff potluck so I was home before I got sick but there were several people at my house. I made the buffalo chicken dip so I ate a lot of that. I don't fully remember what else I ate, but I know I had a sugar cookie. Bruce, the administrative assistant, makes the best sugar cookies. I think I also had the spinach artichoke dip.

EHS What are your symptoms? How long did you have your symptoms and when did they start?

C2 I woke up sweating, but I didn't have a temperature. Then I got a terrible stomach ache, but the pain was sharp. Then I started to have loose stools.

EHS You said that you made the buffalo chicken dip—where did you get the chicken from and how do you make it?

C2 I got it at Smith's Meat Market a couple of days ago. It is a really easy recipe. You just put the chicken in the slow cooker on low for eight hours and then add the buffalo sauce and the cheese. My slow cooker stopped working half-way through so that was unfortunate.

EHS Did you eat any fruits or vegetables?

C2 I probably should have, but no I don't think I actually did. Unless you count the spinach artichoke dip.

EHS Any other information that would be helpful?

C2 Overall I think I am feeling better so I was only sick for less than 24 hours. I posted on social media that I was sick. I will go back and see if anyone else had the same problem.

EHS Here is my card. Call me if you find out anyone else also has issues. Thank you.

4 Interview with Smith's Meat Market and Grocery Head Butcher (HB):

EHS Where do you work and what is your job?

HB I am the head butcher at Smith's and I oversee the meat department here. I can't talk that long. We are about to head into the after-work shopping rush.

EHS Thanks for meeting, I will try to be fast. What are your basic safety protocols and do you know if you have had any issues with them?

HB We follow the required food safety protocols set up by the health department and I am pretty sure they get their guidance from the CDC (Center for Disease Control). If we have any issues, we log them in a record book, but there haven't been any entries since one of our freezers stopped working last summer.

EHS Where do you get your meat from?

HB One of the reasons we are able to keep prices competitive is that we buy our meat directly from Parkway Meat Distribution Center. I am sorry I have to go. I could meet tomorrow afternoon, but I can't leave customers waiting.

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Citizen Interviews

Continued

5 Interview with Parkway Meat Distribution Manager Head of Product Safety (PMD):

EHS Thank you for meeting on a short notice. We believe we have a foodborne illness outbreak and we are trying to get it figured out as quickly as possible.

PMD No problem, I can assure you we have not had any breach of safety protocol.

EHS That is reassuring. But we know that in the natural meat production process, the bacteria from the animals' outsides or insides—intestines—can sometimes make its way onto the food. What types of meats do you process at this facility?

PMD We have a smaller scale poultry division. Most of the products are used locally. Most of the other products that come through our facility are processed at another facility and this is the distribution center.

EHS Do you remember any issues with the poultry processing last week?

PMD No, like I said earlier there have been no deviations from the protocols.

EHS Who buys your meats in this area?

PMD Here is a list of our direct sales for last week.

Note: Smith's Grocery and Meat Market is on the list.

6 Interview with Anonymous Citizen 3 (C3):

EHS Thanks for contacting me about the issue. Can you tell me where and what you ate?

C3 We went out for my son's 13th birthday to Champs. We got a plate of chicken wings to share and then I ordered the chicken salad, my son had a hamburger, my daughter had a cheese quesadilla, and I think my husband ordered the grilled ham and cheese sandwich.

EHS Who in your family got sick?

C3 We all did. It was a really bad night to be at our house. Overall I think my daughter had it the worst. Everyone had runny poop and stomach pains. My daughter said she had a headache and was really dehydrated. She was finally able to keep fluids down or we were going to have to go to the hospital.

EHS Did anyone have a fever?

C3 I didn't actually check, but I know I didn't have one.

EHS What did your family eat for breakfast that morning?

C3 We got doughnuts and coffee from Rosie's around the corner from our house.

EHS Did anyone eat any eggs?

C3 No, Rosie's only serve doughnuts.

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County Health Department, DNA Sequencing Sampling Request Form Capture Sheet

Directions

Based on the information that you collected from the interviews, fill in the table below to request DNA samples from locations of interest. Make sure you are specific in the location for your request and provide reasoning to support the request. Because the health department is under budget cuts, the employees have limited time and resources, so you will need to rank your requests (1 being most important, and 4 least).

DNA Sampling Request Form

General Location	Specific Locations <i>Exactly where should the DNA be collected?</i>	Relevant Evidence <i>Why is this location a sampling priority? Support this with information from the interviews.</i>	Rank

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Data Analysis 1 Capture Sheet

Directions

Now you assume the role of the Lab Scientist to determine the bacterial species at the locations in the case. Follow the directions below to gather information from the *Sampled DNA Sequences by Location* provided by your teacher.

1. Use the Basic Local Alignment Search Tool (BLAST) to find regions of similarity between the sequences on the *Sampled DNA Sequences by Location* and the sequences in the database. This “downstream” analysis of the NGS data will help identify the species found at the different locations. Fill in the data table below.
2. Once you have the scientific name, finish filling in the table with characteristics of the bacteria.

Sequences Identified by Species with Characteristics

Sequence Reference Number	Species BLAST Result Scientific Name	Bacterial Characteristics <i>Where is it found? Does it cause illnesses? If so, what are some symptoms of the illness?</i>
1		
2		
3		
4		
5		

Continues next page >

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Data Analysis 1 Capture Sheet

Continued

3. Use the data that you generated in questions 1 and 2 to conclude which species is causing the issue. Use the Claim-Evidence-Reasoning prompts below.

Claim	Evidence-Reasoning	
<p><i>Make a claim of the scientific name of the species that is causing the issues.</i></p>	<p><i>Explain the illness (symptoms) caused by the species in the claim from CDC's Prevent Illness From <i>C. perfringens</i>.</i></p>	<p><i>Identify evidence from the case study interviews that are similar to the symptoms caused by the illness.</i></p>

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Sampled DNA Sequences by Location

Directions

Below is a simplified version of a Next Gen sequencing (NGS) file called a FASTQ file. This is the file that scientists analyze to determine the species that are found in the samples. The following five sequences are of the samples collected at your request. Use these sequences to complete the *Data Analysis 1 Capture Sheet*.

Note: All the DNA from the chicken has been removed. These are only microbe sequences.

1
 ATGCGTGTAATTGCGGGTAAGCATAAAAGTAAAGCGCTAGAAAGTTTAGAGGGGCGAAATACTCGTCCAA
 CTATGGATAAAGTTAAAGAAGGTATTTTTAATAGTTTATATGATGTTTCTGGTTTAGGATTAGACTTATT

2
 GTGCAACGTTATTTTCATAGACCAAACGCTGATGTAAGTCAGCGTTTTTTTTATTACAAAAAAGAAGATA
 TTCATCATATAACAAATGTAATGAGATATGATGTTGGTAGTAAAATTATTTTAACTTTTAATGATCAAAC

3
 ATGAATGCAAGAAAAATAATAGTTGAAATATTAGACAATGTCTTATTAATGGAGCATATTCAAATATAG
 AAATAAATAAGCAATTTGCATCTAATGATATAGATCCAAAAGATAAGGGATTAATAACAGAGGTTGTTTA

4
 ATGAAAAAACAAAATCTACGCAGCATGGCGGCCAGGCCGTCGAGCAGGTTATCGAGCAGGGCCAGTCAC
 TGAGCAACGTCCTGCCTCCCCTGCAGCAAAAAGTCTCTGATAAAGATAAAGCCCTGCTTCAGGAGCTCTG

5
 GTGAGTAAGACACAGCCGGGACGAGTTATCCTGGCGGGCACCCCGATTGGCGACCGTCGTAGCGCCTCGC
 CCGCCCTTATCGAGACCTTGCGGGATGCCCGCGTCATTGCCGCTGAGGATACCCGTCGGCTGCGTGACCT

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Data Analysis 2 Capture Sheet

Directions

Use the data from the table below to identify the location of the contamination.

Species Identified at Locations through NextGen sequencing

Location	Species Identified
Parkway Meat Distribution Center	<i>Staphylococcus epidermidis</i> , <i>Clostridium perfringens</i> , <i>Cutibacterium acnes</i> , <i>Staphylococcus aureus</i>
Champs Restaurant	<i>Clostridium perfringens</i> , <i>Cutibacterium acnes</i>
Residence of Citizen 1	<i>Enterobacter roggenkampii</i> , <i>Cutibacterium acnes</i>
Smith's Meat Market & Grocery	<i>Clostridium perfringens</i> , <i>Cutibacterium acnes</i>
Residence of Citizen 2	<i>Clostridium perfringens</i> , <i>Enterobacter roggenkampii</i>

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Data Analysis 2 Capture Sheet

Continued

1. Use the NextGen Sequencing analysis to identify the initial source of the contamination.

Claim	Evidence-Reasoning
<p><i>Claim the source of the contamination.</i></p>	<p><i>Explain why this is the location of the contamination using evidence from the table on the previous page. Use your notes from the Interview Analysis table (The Next Generation of Food Safety Case Study Capture Sheet) for reference as well.</i></p>

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Data Analysis 2 Capture Sheet

Continued

2. The infection control team will take over from here. The last thing you need to do is to make a recommendation of the next steps. Based on your research, what is a reasonable first step for the infection control team?

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Background Reading: NextGen Sequencing

What is DNA Sequencing?

DNA sequencing is used to determine the order of nucleotide bases in a DNA sample. There are four nucleotide bases: adenine, guanine, cytosine, and thymine. Determining the sequence of nucleotides allows scientists to study genes found in coding and noncoding regions of the DNA. More importantly, scientists can use sequencing to see how the expression of genes changes during development and in disease. Since the completion of the Human Genome Project, technological advancements in DNA sequencing have made this process more accessible and more efficient. It has allowed scientists to sequence over 100,000 billion bases per year and entire genomes for thousands of dollars. Many of these technologies have been developed with support from the Human Genome Research Institute (NHGRI) Genome Technology Program, which works toward developing new sequencing technology in an effort to reduce the cost of sequencing the human genome for less than \$1,000. As advances in DNA sequencing technology become more common, researchers are able to generate vast sequencing libraries to analyze changes in gene expression in a variety of conditions such as cancer. Using this knowledge, researchers can more easily isolate therapeutic targets that can eventually impact human lives.

Paraphrased from National Human Genome Research Institute's [DNA Sequencing Fact Sheet](#).

What is NextGen Sequencing (NGS)?

Next generation sequencing is similar to Sanger sequencing, also known as dideoxy or capillary electrophoresis sequencing, in that they both rely on the incorporation of fluorescent nucleotides by DNA polymerase to determine the DNA template's sequence. However, NGS is much higher efficiency and allows millions of DNA fragments to be sequenced simultaneously per run. NGS has much higher sensitivity to detect low-frequency variants, faster turnaround time for high sample volumes, comprehensive genome coverage, lower limit of detections, higher capacity with sample multiplexing, and the ability to sequence hundreds to thousands of genes simultaneously. The primary advantage of Sanger sequencing is that it is cost-effective for sequencing low numbers of targets, below 20.

Paraphrased from [NGS vs. Sanger Sequencing](#) from Illumina.

How does NGS work for DNA Identification?

Next-generation sequencing platforms sequence million of small fragments of DNA in parallel. Bioinformatics analyses help organize the sequencing data by comparing it to the reference human genome. Next-generation sequencing provides robust genome coverage, sequencing each base in the human genome multiple times. Additionally, NGS can be adapted to sequence entire genomes or specific regions, including all 22,000 coding genes in the exome or a subset of individual genes. Next generation sequencing has the capability to improve patient care in capturing a greater number of mutations compared to Sanger sequencing, defining genomic characteristics of pathogens, and identifying cancer causing mutations in the genome. Next-generation sequencing drastically improves on Sanger sequencing's ability to identify large genomic deletions, translocations, or rearrangements. Sanger sequencing is only capable of identifying small insertions and deletions, thus requiring additional assays to detect any chromosomal changes. Next-generation sequencing also does not require prior knowledge of a gene or locus under investigation, eliminating any bias in the identification of novel genes presented in clinic. In oncology, NGS allows for cancer genomes to be sequenced for a more precise diagnosis and classification of the disease. Ultimately, NGS could provide patients with individualized treatment based on the consensus sequencing libraries. The biggest drawback is the required infrastructure, including computer capacity and personnel needed to analyze and interpret the data.

Paraphrased from [What is next generation sequencing?](#) Arch Dis Child Educ Pract Ed. 2013 Dec; 98(6): 236-238.

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NextGen Sequencing Check for Understanding Capture Sheet

Directions

Each numbered step below explains a part of the NextGen sequencing DNA identification technology. This case study modeled many of the steps scientists would have done when using NextGen Sequencing to identify the source of a foodborne illness.

To show your understanding, explain the part of the case study that was comparable to NextGen sequencing.

- The DNA is collected or sampled from the area of interest. What in the case study is similar to this step?

- The DNA is isolated and sequenced quickly in short segments. What in the case study is similar to this step?

- The sequences are compared to a known DNA library. What in the case study is similar to this step?

Note: A DNA library is a collection of known DNA fragments that can be used for research. The sequence order and species are already identified. It acts like a reference section in a real library but for DNA.

- The species are identified (even if there are mutations). What in the case study is similar to this step?

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Technology Overview Capture Sheet

Part 1: DNA Identification

Directions

After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.

Tech Lesson 4	NextGen Sequencing															
Describe	Summarize how this technique works.														
Discuss	List the pros and cons you identify for the technique.	<table border="1"> <thead> <tr> <th data-bbox="467 966 1006 1029">Pros</th> <th data-bbox="1006 966 1542 1029">Cons</th> </tr> </thead> <tbody> <tr><td>.....</td><td>.....</td></tr> <tr><td>.....</td><td>.....</td></tr> <tr><td>.....</td><td>.....</td></tr> <tr><td>.....</td><td>.....</td></tr> <tr><td>.....</td><td>.....</td></tr> <tr><td>.....</td><td>.....</td></tr> </tbody> </table>	Pros	Cons
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Support	Provide examples.														