Identification of Pathogenic Islands using Comparative Genomics Based Tools

Author's Note

Every year I take my bioscience students on a field trip to the University of Florida's campus in Gainesville. The students tour research labs, talk with graduate students, and get to perform a laboratory experiment on campus. It is the highlight of their year! During our field trip in November of 2012, we happened to have some down time between activities. I had noticed a flyer for an open lecture titled "From tRNA to protein modification: Linking gene function by comparative genomics" by Dr. Valérie de Crécy-Lagard. I didn't know how much my students or myself would get out of it, but we went for it. It was there that I first met Valérie, an insanely energetic woman with enough enthusiasm about her research topic to fill an ocean. As a teacher, I recognized that comparative genomics and bioinformatics would be important to my students who chose to continue their education in the field of bioscience. My educational experience with CPET had provided me with a superficial background knowledge of comparative genomics and its importance as a research tool. During the lecture, Valérie mentioned a workshop that she was giving over the summer and afterwards I approached her for more information. She was more than happy to chat about it and told me I could email her. So I did. The response I got stated that the course was intensive and they were not sure I had the 'necessary training in biochemistry and bioinformatics to participate'. I persisted. I believe their decision to allow me to attend the workshop was based purely on my own enthusiasm. I will say that they were absolutely right. I sat at my computer that summer trying desperately to follow the instructions, but to little avail. I'm sure the only thing I got out of it was that I was even more determined that this was something I needed to learn more about.

A full year later I received an email from Valérie asking if I would be interested in developing some bioinformatics modules for high school students. I said yes, of course. To make a long story short, I ended up in Valérie's lab in the summer of 2015 working with one of her amazing and very patient post docs, Dr. Jo Marie Bacusmo. We spent three weeks writing a week long module for high school students using PATRIC (Pathosystems Resource Integration Center). We weren't sure that my students had the 'necessary training in biochemistry and bioinformatics' to get anything out of it, but we presented our module to them in January of 2016. We were thrilled that, not only did they 'get it', but they also enjoyed it. The highlights of the module for the students was the Pathogen Survivor game and the PATRIC tutorial. Plus, they adored Jo Marie.

As a teacher, the moral of this story is to never underestimate the capabilities of motivated high school students and that any complex subject can be presented in a way that is understandable to its audience.

Introduction

In 2014, The World Health Organization (WHO) published its first global report on the global threat of antibiotic resistance. Bacteria are developing antibiotic resistant traits at alarming rates, primarily due to the overuse of antibiotics worldwide. Furthermore, this is happening at alarming rates with resistant strains gaining a foothold anywhere from two to thirty years after an antibiotic is first introduced. Given that no new classes of antibiotics have been discovered since 1987, 27 years ago, this trend is particularly alarming. This 27 year dry spell is referred to as the 'discovery void'.

In the United States alone, more than 2,000,000 people develop antibiotic resistant infections resulting in 23,000 deaths annually. It has been estimated that failure to address this problem will result in more than 10 million deaths annually worldwide by the year 2050 surpassing Ischemic heart disease as the number one leading cause of death. The associated costs will exceed 100 trillion dollars

Given this dire prediction, understanding how bacteria acquire antibiotic resistance could help scientists develop new classes of antibiotics, breaking the 27 year dry spell and minimizing the overwhelming number of predicted deaths.

This curriculum unit was created with the purpose of introducing high school students to comparative genomics and the computer based tools that scientists use to identify genomic islands.

Specifically, this unit is meant to guide students to discover virulent genes and proteins found in pathogenicity islands within the genomes of disease causing bacteria

Explore concepts such as benefits and disadvantages of diversifying the genome. Relating genome diversity to bacterial survival and fitness. Modes of gene transfer. How do bacteria gain or lose traits? What is the driving force behind genome diversity?

Familiarity with common pathogenic factors and the significance of these genes to pathogenesis. What makes a good pathogen? What are the basic characteristics of a good pathogen?

Gain a better understanding of the global impact of disease outbreaks as well as a realistic comprehension of the caveats in pharmaceutical advancements and the significance of the comparative genomics in accelerating identification of targets and drug development.

Opens discussion about natural products, cancer research, and pharmaceutical synthesis, and ethics.

LESSON SEQUENCING GUIDE AND SUMMARIES:

All lessons are based on a 50 minute class period and 24 students per class.

Day 1	**Lesson #1:** Bad Bacteria	Students will take a pretest over the content presented in these six lessons. Students will then watch short videos of a pathogenic bacterium invading a host cell to identify the behaviors and biological mechanisms (virulence factors) exhibited by the bacterium that make it successful.
Day 2	**Lesson #2:** Pathogen Prototype	Student groups build a bacterial prototype expressing virulence factors and then compare their prototype to others. Students will assess the potential success of each prototype by voting for the most successful and least successful prototype and justifying their choices.
Day 3	**Lesson #3:** *Activity #1*: Video 'Gene Transfer'	Students watch a video on horizontal gene transfer and answer three questions. The teacher can choose to discuss the answers to these questions after the worksheet has been collected.
	Lesson #3: *Activity #2*: Activity 'Bacteria Survivor'	Students team up and play a teacher directed game demonstrating genome diversification via gene transfer, highlighting its impact on bacterial fitness and survival
Day 4	**Lesson #4:** *Activity #1*: Video 'The Power of Comparative Genomics (7:07) https://www.youtube.com/watch?v=mU9ROpm6d70	This video introduces comparative genomics as a tool to help scientists focus their research
	Lesson #4: Activity #2 Video 'Comparison of Genomes of Eight Enteroaggregative E.coli O104:H4 Isolates' (2:07) https://youtu.be/6VTxmnZQXgU	This video shows how comparative genomics facilitates identification genomic islands contributing to pathogenesis of disease outbreak strains
	Lesson # 4: *Activity #3*: Video Tutorials on Pathosystems Resource Integration Center (PATRIC)	Tutorial of PATRIC, a comparative genomics web based tool
Day 5	**Lesson # 5a:** Student group research on specific pathogenic bacteria species	Students work in groups using PATRIC to research virulent genes and disease outbreaks for an assigned bacteria species
Day 6	**Lesson #5b:** Student presentations	Students present their research to the class and are graded according to a rubric (provided)

Key Question(s): What is comparative genomics and how can it help scientists narrow the search for virulent genes in disease causing bacteria? What are some of the tools scientists use to compare bacterial genomes and how do they work? How do bacteria diversify their genomes? What are the various modes of gene transfer? What are the common virulence factors and their significance to disease causing bacteria?

Science Subject: Bioscience, Biotechnology, Biology

Grade and Ability Level: 10-12 grade Honors/Advanced Placement or Undergraduate College Level Introductory

Science Concepts: Pathogenesis, Genomics, Proteomics, Genetic Evolution

Overall Time Estimate: This unit will take six class periods of 50 minutes each.

Learning Styles: Visual, auditory, kinetic and cooperative

Vocabulary:

Analog: Analogs are similar genes due to convergent evolution rather than a common ancestor

Antibiotic Resistance: Antibiotic resistance refers specifically to the resistance to antibiotics that occurs in common bacteria that cause infections.

Antimicrobial Resistance: Antimicrobial resistance is a broader term, encompassing resistance to drugs to treat infections caused by other microbes as well, such as parasites (e.g. malaria), viruses (e.g. HIV) and fungi (e.g. *Candida*).

Arabidopsis: a small invasive self-pollinating weed with small white flowers; much studied by plant geneticists; the first higher plant whose complete genome sequence was described.

Basal bodies: a cylindrical organelle, within the cytoplasm of flagellated and ciliated cells, that contains microtubules and forms the base of a flagellum orcilium: identical in internal structure to a centriole.

Cilia: minute hair-like organelles, identical in structure to flagella, that line the surfaces of certain cells and beat in rhythmic waves, providing locomotion to ciliate protozoans and moving liquids along internal epithelial tissue in animals.

Chlamydomona: a common single-celled green algae that lives in water and moist soil and typically has two flagella for swimming.

Comparative Genomics: the field of biological research in which the genomic features of different organisms are compared. The genomic features may include the DNA sequence, genes, gene order, regulatory sequences, and other genomic structural landmarks. Genomic regions that are not present within related strains suggest that the region was horizontally transferred.

Conserved (genes): similar or identical sequences that occur within nucleic acid sequences (such as RNA and DNA sequences), protein sequences, protein structures or polymeric carbohydrates across species (orthologous sequences) or within different molecules produced by the same organism (paralogous sequences)

Epidemiology: the branch of medicine that deals with the incidence, distribution, and possible control of diseases and other factors relating to health.

Flagellum: a slender threadlike structure, especially a microscopic whip-like appendage that enables many protozoa, bacteria, spermatozoa, etc., to swim.

Genomic Island (GI): large genomic regions (typically >8kb), that are thought to have horizontal origins. These regions can often contain genes that are related to antibiotic resistance and/or virulence.

Homolog: A gene related to a second gene by descent from a common ancestral DNA sequence.

Horizontal Gene Transfer: the transfer of genes between organisms in a manner other than traditional reproduction

Morphology: The branch of biology that deals with the form and structure of organisms without consideration of function.

Ortholog: Orthologs are genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

Paralog: Paralogs are genes related by duplication within a genome. Orthologs retain the same function in the course of evolution, whereas paralogs evolve new functions, even if these are related to the original one.

Pathogenicity: refers to the ability of an organism to cause disease (i.e., harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

Pathogenicity Island (PAI): a distinct class of genomic islands acquired by microorganisms through horizontal gene transfer. Pathogenicity islands are found in both animal and plant pathogens. Additionally, PAIs are found in gram positive and gram negative bacteria.

Pathology: the typical behavior of a disease.

Vertical Gene Transfer: the transmission of genes from the parental generation to offspring via sexual or asexual reproduction.

Virulence: the relative ability of a microorganism to cause disease; degree of pathogenicity, the capability of a microorganism to cause disease.

Virulence Gene: a gene whose presence or activity in an organism's genome is responsible for the pathogenicity of an infective agent.

NEXT GENERATION SUNSHINE STATE STANDARDS - SCIENCE					
BENCHMARK	**LESSON**				
	1	**2**	**3**	**4**	**5**
SC.912.L.14.1 Describe the scientific theory of cells (cell theory) and relate the history of its discovery to the process of science	X				
SC.912.L.14.2 Relate structure to function for the components of plant and animal cells. Explain the role of cell membranes as a highly selective barrier (passive and active transport)	X	X			X
SC.912.L.14.6 Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.	X	X			X
SC.912.L.14.52 Explain the basic functions of the immune system, including specific and nonspecific immune response, vaccines, and antibiotics.	X	X			X
SC.912.L.15.1 Explain how the scientific theory of evolution is supported by the fossil record, comparative anatomy, comparative embryology, biogeography, molecular biology, and observed evolutionary change.			X	X	X
SC.912.L.15.13 Describe the conditions required for natural selection, including: overproduction of offspring, inherited variation, and the struggle to survive, which result in differential reproductive success.			X		
SC.912.L.15.15 Describe how mutation and genetic recombination increase genetic variation.			X		X
SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.				X	
SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.			X		
SC.912.L.17.4 Assess the need for adequate waste management strategies.	X				
SC.912.L.17.6 Compare and contrast the relationships among organisms, including predation, parasitism, competition, commensalism, and mutualism.	X	X	X		X
SC.912.L.18.4 Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.	X				X
SC.912.L.18.11 Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.		X			X
SC.912.N.1.1 Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following: 1. Pose questions about the natural world 2. Conduct systematic observations. 3. Examine books and other sources of information to see what is already known. 4. Review what is known in light of empirical evidence. 5. Plan investigations. 6. Use tools to gather, analyze, and interpret data. 7. Pose answers, explanations, or descriptions of events. 8. Generate explanations that explicate or describe natural phenomena. 9. Use appropriate evidence and reasoning to justify these explanations to others. 10. Communicate results of scientific investigations, and 11. Evaluate the merits of the explanations produced by others.		X	X		X

NEXT GENERATION SUNSHINE STATE STANDARDS - SCIENCE					
BENCHMARK	**LESSON**				
	1	**2**	**3**	**4**	**5**
SC.912.N.2 Describe and explain what characterizes science and its methods.	X			X	
SC.912.N.1.3 Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.				X	X
SC.912.N.1.4 Identify sources of information and assess their reliability according to the strict standards of scientific investigation.	X			X	X
SC.912.N.1.5 Describe and provide examples of how similar investigations conducted in many parts of the world result in the same outcome.				X	
SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.				X	X
SC.912.N.1.7 Recognize the role of creativity in constructing scientific questions, methods and explanations.		X		X	X
SC.912.N.2.4 Explain that scientific knowledge is both durable and robust and open to change. Scientific knowledge can change because it is often examined and re-examined by new investigations and scientific argumentation. Because of these frequent examinations, scientific knowledge becomes stronger, leading to its durability.				X	X
SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.		X		X	X
SC.912.N.4.1 Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.		X		X	

Literature:

Medical Terminology Prefixes and Suffixes
https://quizlet.com/6133174/medical-terminology-prefixes-suffixes-flash-cards/

Bacterial Virulence and Secretion Systems (VetSci Science & Learning)
http://vetsci.co.uk/2011/01/12/bacterial-virulence-factors-secretion-systems/

Micro Exam I – Bacterial Virulence Factors
https://quizlet.com/9931162/micro-exam-1-bacterial-virulence-factors-flash-cards/

Chapter 7: Bacterial Pathogenesis – Virulence Factors
http://www.ncbi.nlm.nih.gov/books/NBK8526/?report=printable

Genetic Transfer – Andrew Boyd (3:13)
https://www.youtube.com/watch?v=Fq0YSTyJlpk

Definitions of homolog, analog, ortholog & paralog
http://homepage.usask.ca/~ctl271/857/def_homolog.shtml

Antibiotic Discovery Timeline
http://www.econlife.com/a-slower-pace-of-technological-innvovation/

Learn.Genetics: How antibiotics work
http://learn.genetics.utah.edu/content/microbiome/antibiotics/

Article: Massive Antibiotic Resistance in China's Rivers "Fueled by Abuse"
http://www.rfa.org/english/news/china/pollution-07072015112452.html

RefSeq Frequently Asked Questions (FAQ)
http://www.ncbi.nlm.nih.gov/books/NBK50679/

Antibodies: Part 1 CRISPR (Radio Lab Podcast Series)
http://www.radiolab.org/story/antibodies-part-1-crispr/

Antibiotic Resistance Learning Site
http://amrls.cvm.msu.edu/overview

Antimicrobial Resistance: WHO Fact Sheet
http://www.who.int/mediacentre/factsheets/fs194/en/

Antibiotic Resistance (includes a table of antibiotic resistant bacteria)
http://www.drugs.com/article/antibiotic-resistance.html

How do viruses and bacteria cause disease?
http://www.ausmed.com.au/blog/entry/how-do-viruses-and-bacteria-cause-disease

Video: Bacteria attacking white blood cells
https://www.youtube.com/watch?v=6AP8yL1qBpw

LESSON ONE: BAD BACTERIA

KEY QUESTION(S): What are virulence factors? How do bacteria use virulence factors to infect an organism and cause disease? How do bacteria gain or lose virulence factors?

OVERALL TIME ESTIMATE:

- Advance Preparation: 10 minutes
- Student Procedure: 50 minutes

LEARNING STYLES: Visual and auditory

VOCABULARY:

Actin: Actin is a cellular protein found especially in microfilaments (as those comprising myofibrils) and active in muscular contraction, cellular movement, and maintenance of cell shape.

Adhesin: Adhesins are cell-surface components or appendages of bacteria that facilitate adhesion or adherence to other cells or to surfaces. Adhesion is required for the colonization of a new host.

Antibiotic Resistance: Antibiotic resistance refers specifically to the resistance to antibiotics that occurs in common bacteria that cause infections.

Antimicrobial Resistance: Antimicrobial resistance is a broader term, encompassing resistance to drugs to treat infections caused by other microbes as well, such as parasites (e.g. malaria), viruses (e.g. HIV) and fungi (e.g. *Candida*).

Apoptosis: the death of cells that occurs as a normal and controlled part of an organism's growth or development and can be induced either by a stimulus, such as irradiation or toxic drugs, or by removal of a repressor agent. The cells disintegrate into membrane-bound particles that are then eliminated by phagocytosis.

Capsule: The capsule is a polysaccharide layer that lies outside the cell envelope of bacteria, and is thus deemed part of the outer envelope of a bacterial cell. It is a well-organized layer, not easily washed off, and it can be the cause of various diseases. A capsule can enhance the ability of a bacterium to cause disease by preventing phagocytosis.

Colonization Factor: A colonization factor is a virulence factor which facilitates bacterial invasion of a host by making the environment more hospitable. For example, *Helicobacter* species counter the low pH of the stomach by producing urease.

Flagella: The flagella are a slender threadlike structure, especially a microscopic whip like appendage that enables many protozoa, bacteria, spermatozoa, etc., to swim.

Motility: In biology, motility is the ability to move spontaneously and actively, consuming energy in the process.

Invasion Factor: An invasin is virulence factor that facilitates bacterial invasion of a host. This is done by disrupting host cell membranes; the result is the facilitation of transport across epithelial layers of tissue and skin. For example, the internalin surface proteins found on *Listeria monocytogenes* allow them to invade mammalian cells via transmembrane proteins.

Pathogenicity: refers to the ability of an organism to cause disease (i.e. harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

Phagocyte: A phagocyte is a type of cell within the body capable of engulfing and absorbing bacteria and other small cells and particles (i.e. white blood cell).

Phagocytosis: Phagocytosis is the engulfing and ingestion of foreign bodies such as bacteria or other cells by a phagocyte.

Secretion System: Secretion in bacterial species means the transport or translocation of effector molecules for example: proteins, enzymes or toxins (such as cholera toxin in pathogenic bacteria for example *Vibrio cholerae*) from across the interior (cytoplasm or cytosol) of a bacterial cell to its exterior. Secretion is a very important mechanism in bacterial functioning and operation in their natural surrounding environment for adaptation and survival.

Toxin: Microbial toxins are toxins produced by micro-organisms, including bacteria and fungi. Microbial toxins promote infection and disease by directly damaging host tissues and by disabling the immune system. Some bacterial toxins, such as Botulinum neurotoxins, are the most potent natural toxins known.

Virulence: Virulence is the ability of a microorganism to produce disease. Virulence depends on the number of infecting bacteria, their route of entry into the body, the response of the host immune system and any characteristics specific to that bacterium – its virulence factors.

Virulence Factor: Virulence factors refer to the properties (i.e., gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease. Bacterial virulence factors are typically proteins or molecules synthesized by protein enzymes. Virulence factors include bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates and proteins that protect a bacterium and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium.

Virulence Gene: a gene whose presence or activity in an organism's genome is responsible for the production of a virulence factor or part of a virulence pathway.

LESSON SUMMARY:

Students will take a pretest over the content presented in these six lessons. Students watch short videos of a pathogenic bacterium invading a host cell to identify the behaviors and biological mechanisms (virulence factors) exhibited by the bacterium that make it a successful pathogen. A teacher facilitated discussion will help students summarize and make sense of seven different virulence factors. Finally, students will use the ‘Virulence Factors of Pathogenic Bacteria Database’ to identify the seven virulent factors as associated with specific bacterial species and diseases.

STUDENT LEARNING OBJECTIVES:
The student will be able to...

1. Define virulence factor
2. Identify six different virulence factors used by some bacteria
3. Provide an example of six different virulence factors
4. Explain why some virulence factors are unique to pathogenic bacteria while others are not

GRADE AND ABILITY LEVEL: This is designed for an honors or other upper level biology or other bioscience course, though it could be easily modified for lower levels.

SCIENCE CONCEPTS: Pathogens and virulence

PRIOR KNOWLEDGE: General knowledge about the human body specifically related to the adverse conditions and specific threats a bacterium might encounter prior to and during infection are required (i.e. stomach acid, the immune system, body temperature, etc.)

STANDARDS:

SC.912.L.14.1
SC.912.L.14.2
SC.912.L.14.6
SC.912.L.14.52
SC.912.L.17.6
SC.912.L.18.4

MATERIALS:

- 1 copy of *Pre-test* per student
- 1 copy of *Student Worksheet: Virulence Factors* per student

BACKGROUND INFORMATION:

Virulence factors are proteins and/or molecules produced by a pathogen that contribute to the ability of the microbe to cause disease. These are factors that are necessary for the organism to successfully (1) encounter a host, (2) invade a host, (3) colonize a host, (4) evade detection by the immune system of the host, (5) acquire nutrients from the host, (6) produce toxins in the host, and (7) exit the host. The genomes of some microbes also code for resistance to specific conditions or threats such as the acidic environment of the human gut, heavy metal toxins, or antibiotics. The more of these factors a microbe can accumulate in its lifetime, through vertical and horizontal transfer, the more diverse and aggressive it can be, especially with respect to its ability to cause disease. These virulence factors can be categorized as offensive, defensive, or neutral with respect to their role in the ability of the organism to proliferate in a specific environment. For example, genes coding for toxins that cause red blood cells to lyse would be offensive, genes coding for the ability to avoid detection by phagocytes would be defensive and genes coding for the acquisition of nutrients from the host could be considered neutral providing it doesn't harm the host in the process.

ADVANCE PREPARATION:

1. Make copies of *Pre-test*
2. Make copies of *Student Worksheet: Virulence Factors*

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Note: It is very important that the teacher use the proper terminology during class discussion in this section (see vocabulary)

1. (10 minutes) Explain to the students that the pre-test does not reflect their grade and that they should choose the answer they think is most likely to be correct. Pass out the pre-test and allow students quiet time to complete.

2. (2-3 minutes) Show the following video to the class with no prior explanation. Salmonella entering the intestinal tract https://www.youtube.com/watch?v=gpLUQza4uWw (2:27) (Note: there is no sound for this video.)

3. (2-3 minutes) Ask the class if anyone thinks they can explain what was going on in the video. Some of them will probably conclude that it was about bacteria and maybe even a bacterial invasion of a cell.

4. (1 minute) Now explain that this was a salmonella bacterium invading an epithelial cell of the small intestine. Ask them to think about offensive and defensive mechanisms that allowed the bacteria to successfully invade the epithelial cell. Tell the students that these mechanisms can be thought of as super powers that allow the tiny bacteria to be successful in overpowering a much larger organism like a human being and wreaking havoc in various systems of the human body.

5. (5 minutes) Now show the following two videos: https://www.youtube.com/watch?v=3UAJS9jzyV4 (3:40) and https://www.youtube.com/watch?v=q5-sxUbEu5M (1:19). Ask the students to jot down in their notes any behaviors or mechanisms the bacterium exhibits that help it be successful in causing disease.

6. (5-10 minutes) After the videos are over, make two columns on the board: Offensive and Defensive. Ask the students what offensive behaviors they came up with. While they are listing these behaviors, write them on the board under the correct column. Some behaviors may be worded differently, but your columns should look similar to the table below. Students may need some guidance in defining or explaining these behaviors and may need to have the correct terminology modeled for them.

Offensive	Defensive
• Flagella or ability to 'swim' • Attachment • Injection • Toxins • Secretion system	• Acid resistance • Antibiotic resistance • Capsule or protective coating

7. (5 minutes) Now replay the first video, pausing at the listed times to ask students to explain what is going on at these specific points. The teacher may wish to allow questions or discussion at each pause. (4 minutes)
    - (0:8) flagella; motility (the bacteria has to be able to get to the specific kinds of cells that it can interact with
    - (0:32) adhesion or attachment (the bacteria attaches itself to the cell wall)
    - (0:46) secretion system (allows the bacteria to inject proteins or enzymes through the cell membrane into the cytoplasm)
    - (1:49) invasion (the bacteria successfully invades the host cell
    - (2:2) toxins (the bacteria emits toxins through a secretion system; toxins may cause apoptosis of the cell or help weaken an organisms immune system allowing the bacteria to evade phagocytosis

8. (15-20 minutes) After discussion, pass out the vocabulary sheet and have students work with a partner and use their notes, Google and the **Virulence Factors of Pathogenic Bacteria** website to define the terms and come up with an example for each term. (Note: if students seem interested in the Virulence Factor website, the teacher may choose to allow more time for investigation and discovery.) This assignment may be collected at the end of 20 minutes or students may be allowed to keep it in their notebook for future reference.

ASSESSMENT SUGGESTIONS:

- Student Worksheet: Virulence Factors can be checked for completion and/or correctness

RESOURCES:

- Medical Terminology Prefixes and Suffixes
  https://quizlet.com/6133174/medical-terminology-prefixes-suffixes-flash-cards/
- Bacterial Virulence and Secretion Systems (VetSci Science & Learning)
  http://vetsci.co.uk/2011/01/12/bacterial-virulence-factors-secretion-systems/
- Micro Exam I – Bacterial Virulence Factors
  https://quizlet.com/9931162/micro-exam-1-bacterial-virulence-factors-flash-cards/
- The Virulence Factor Database
  http://www.mgc.ac.cn/VFs/
- Immune Evasion by Bacteria
  http://primer.crohn.ie/immune-evasion-by-bacteria

STUDENT WORKSHEET: VIRULENCE FACTORS

Name _____ **Date** _____ **Period** _____

Use your notes, Google and the **Virulence Factors of Pathogenic Bacteria** website http://www.mgc.ac.cn/VFs/ to define the following terms and provide a specific example, identifying the bacteria species by name, for each term. Identify the disease caused by each bacterial species.

Adhesin:

Capsule:

Colonization Factor:

Flagella:

Invasion Factor:

Pathogen:

Secretion System:

Toxin:

Virulence Factor:

PRETEST

Name _____ **Date** _____ **Period** _____

1. The ability of a microorganism to cause disease is known as ____.

    a. apoptosis
    b. virulence
    c. phagocytosis
    d. motility

2. Which of the following is defined as the transfer of genes between organisms in a manner other than transmission of genetic material from parent to offspring?

    a. horizontal gene transfer
    b. mRNA transfer
    c. vertical gene transfer
    d. diagonal gene transfer

3. Which one of the following is not a benefit of comparative genomics?

    a. It can help to identify genes that are essential to life.
    b. It provides a powerful tool for studying evolutionary relationships
    c. It can save time by reducing the number of experiments that need to be performed
    d. It requires good quality sequenced and assembled genomes.

4. Genes that are identical or similar between species are said to be ____.

    a. transferred
    b. conserved
    c. resistant
    d. pathogenic

5. A genomic island is

    a. a large region of genes that have been horizontally transferred between organisms and
    code for several functions beneficial to the organism
    b. a large region of genes that have been vertically transferred between organisms and
    code for toxin production
    c. a large region of genes that code for conserved regions that have not been transferred
    between organisms
    d. a large region of genes that are believed to have been vertically transferred between bacterial species

6. PATRIC is

   a. a computer program that allows the user to code for antibiotic resistant genes
   b. an international organization that specializes in regulating patented information between research facilities
   c. a research facility that studies the incidence, distribution, and control of diseases that are resistant to antibiotics
   d. a database that allows the user to compare the genomes of 2 or more bacterial species

7. If you are trying to find a gene that codes for limbs (arms and/or legs) you might compare the genomes of which of the following species

   a. horse, plant, human
   b. human, dog, lizard
   c. fish, plant, worm
   d. worm, plant, amoeba

8. In the lab, scientists can use various methods of identifying gene function in a species. One of the more common methods is

   a. gene transfer
   b. gene knock out
   c. gene annotation
   d. gene programming

9. Housekeeping genes are

   a. transcribed only when needed
   b. transcribed continually at fairly constant levels
   c. typically virulent in nature
   d. cannot be transferred between bacterial species

10. PATRIC uses _____ to help identify genomic islands.

   a. color
   b. letters
   c. percentages
   d. scales

PRETEST TEACHER KEY

1. The ability of a microorganism to cause disease is known as ____.

    a. apoptosis
    b. virulence
    c. phagocytosis
    d. motility

2. Which of the following is defined as the transfer of genes between organisms in a manner other than transmission of genetic material from parent to offspring?

    a. horizontal gene transfer
    b. mRNA transfer
    c. vertical gene transfer
    d. diagonal gene transfer

3. Which one of the following is not a benefit of comparative genomics?

    a. It can help to identify genes that are essential to life.
    b. It provides a powerful tool for studying evolutionary relationships
    c. It can save time by reducing the number of experiments that need to be performed
    d. It requires good quality sequenced and assembled genomes.

4. Genes that are identical or similar between species are said to be ____.

    a. transferred
    b. conserved
    c. resistant
    d. pathogenic

5. A genomic island is

    a. a large region of genes that have been horizontally transferred between organisms and code for several functions beneficial to the organism
    b. a large region of genes that have been vertically transferred between organisms and code for toxin production
    c. a large region of genes that code for conserved regions that have not been transferred between organisms
    d. a large region of genes that are believed to have been vertically transferred between bacterial species

6. PATRIC is

   a. a computer program that allows the user to code for antibiotic resistant genes
   b. an international organization that specializes in regulating patented information between research facilities
   c. a research facility that studies the incidence, distribution, and control of diseases that are resistant to antibiotics
   d. a database that allows the user to compare the genomes of 2 or more bacterial species

7. If you are trying to find a gene that codes for limbs (arms and/or legs) you might compare the genomes of which of the following species

   a. horse, plant, human
   b. human, dog, lizard
   c. fish, plant, worm
   d. worm, plant, amoeba

8. In the lab, scientists can use various methods of identifying gene function in a species. One of the more common methods is

   a. gene transfer
   b. gene knock out
   c. gene annotation
   d. gene programming

9. Housekeeping genes are

   a. transcribed only when needed
   b. transcribed continually at fairly constant levels
   c. typically virulent in nature
   d. cannot be transferred between bacterial species

10. PATRIC uses _____ to help identify genomic islands.

   a. color
   b. letters
   c. percentages
   d. scales

LESSON TWO: PATHOGENIC PROTOTYPE

KEY QUESTION(S): How do virulence factors contribute to the success of pathogenic bacteria?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 15 minutes
- Student Procedure: 50 minutes

LEARNING STYLES: Visual, auditory, kinesthetic and cooperative

LESSON SUMMARY: Student groups build a bacterial prototype expressing virulence factors and then compare their prototype to the others. Students will assess the potential success of each group's bacteria by voting for the most successful and least successful bacteria prototype.

STUDENT LEARNING OBJECTIVES:
The student will be able to...

1. Model the virulence factors learned in the previous lesson
2. Explain the role of each virulence factor as it applies to their model
3. Assess the potential success of the various models as it relates to virulence factors
4. Use content specific prefixes and suffixes to define proteins, enzymes,

STANDARDS:

SC.912.L.14.6
SC.912.N.1.1
SC.912.N.1.7
SC.912.N.3.5
SC.912.N.4.1

MATERIALS:

- 1 desk size whiteboard per student group
- 1 set of seven different colored dry erase markers per student group
- 1 whiteboard eraser per student group
- 1 copy of *Student Worksheet: Pathogen Prototype* per each group
- 1 pair of *Student Cutout: Voting Cards* per each group

ADVANCE PREPARATION:

1. Divide the student list into groups of 3-5 depending on class size.
2. Gather the whiteboards, markers, and erasers and place them in the locations where each group will be working.
3. Decide where students will display their whiteboards when they are finished
4. Make copies of Pathogen Prototype (one per group) and the Voting cards (one per group)

PROCEDURE WITH TIME ESTIMATES:

1. (1 minute) Divide the students into their assigned groups.
2. (1 minute) Pass out a copy of the directions to each student.
3. (30-40 minutes) Allow students 30-40 minutes to work with their group and design a bacterium.
4. (5 minutes) Have the students display their whiteboards by leaning them against and, as a group, evaluate the various models by filling out the voting cards and taping them to the whiteboards they voted for.
5. (10 minutes) Call each group up, one at a time, to explain which prototype they voted for and why.

RESOURCES:

- Medical Terminology Prefixes and Suffixes
  https://quizlet.com/6133174/medical-terminology-prefixes-suffixes-flash-cards/
- Bacterial Virulence and Secretion Systems (VetSci Science & Learning)
  http://vetsci.co.uk/2011/01/12/bacterial-virulence-factors-secretion-systems/
- Micro Exam I – Bacterial Virulence Factors
  https://quizlet.com/9931162/micro-exam-1-bacterial-virulence-factors-flash-cards/
- The Virulence Factor Database
  http://www.mgc.ac.cn/VFs/
- Immune Evasion by Bacteria
  http://primer.crohn.ie/immune-evasion-by-bacteria
- How microbes cause disease
  http://www.geocities.ws/micro2052000/disease.htm

ASSESSMENT SUGGESTIONS:

- Students can be assessed on participation within their group and/or on completion of a good bacterial model that illustrates the virulence factors creatively and realistically.

TEACHERS NOTES:

The goal of the "Pathogen Prototype" activity is to have the students recognize that bacteria need specific biological mechanisms to accomplish tasks that contribute to virulence. Ideally, their whiteboard image should turn out something like the image below, but with creative specific names for each virulence factor. The teacher should walk around the classroom while student groups are working on their prototype and ask them questions that help them to get satisfactory and thoughtful results. The goal of the activity itself is to get students to think rather than for them to get the 'right' answers.

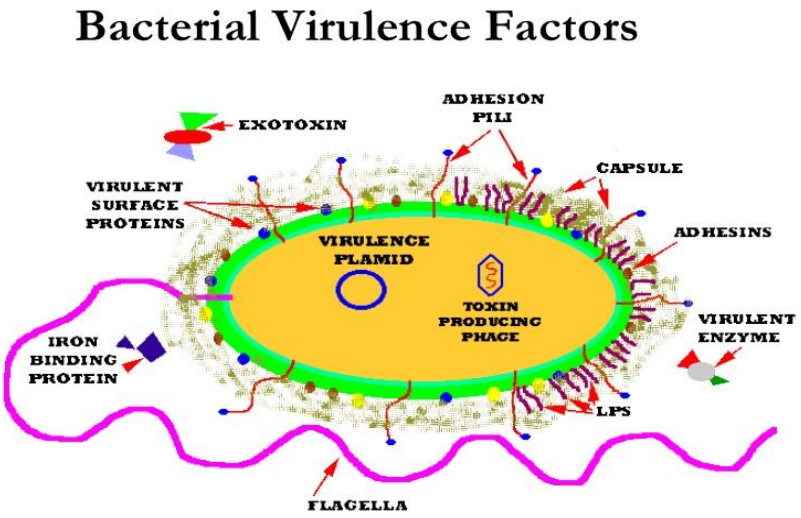


Image Source: http://www.geocities.ws/micro2052000/disease.htm

STUDENT WORKSHEET: PATHOGEN PROTOTYPE

Pathogen Prototype

You are the minions of a savage and evil scientist. You have been tasked with developing a bacterial species that would be capable of infecting the animal species of your choice. The prototype needs to include proteins that would be capable of carrying out the action required to allow the bacterial species to survive, reproduce, and cause an imaginary disease in the animal you have chosen.

You many name your bacteria, disease, and proteins anything you like, but should use the common prefixes, suffixes and identifying terms in the list below for as many of them as possible. Be creative! Once you have completed the design of your pathogenic prototype on your whiteboard, hang it up in the classroom where the teacher has provided space for you to do so. Walk around with your group and evaluate the other prototypes. Try to identify which prototype you think would be most successful and which prototype might be least successful. Be able to justify your choices. You will be asked to explain to the teacher and the class the prototypes you have chosen and explain your reasons for doing so.

Prefixes

anti-	a prefix meaning “against” as in antibody or “opposite of” as in ‘antiparticle
intra-	a prefix meaning inside; i.e. intracellular
inter-	a prefix meaning between; i.e.

Suffixes

-ase	a suffix that means enzyme; i.e. protease
-cide	a suffix that means ‘to kill’
-itis	inflammation; as in appendicitis
-gen	a suffix meaning a substance that produces; i.e. antigen

Identifiers

Inhibitor	a protein molecule that causes a decrease in the speed at which a biochemical reaction can occur
Receptor	identifies a protein molecule usually found embedded within the plasma membrane of a cell surface and receives chemical signals which trigger a cellular response
Factor	identifies a biochemical protein that is necessary for a cellular process

STUDENT CUTOUTS: VOTING CARDS

Voting Card	
Name of Group Members	
Most Successful Bacteria Name	
Justification	

Voting Card	
Name of Group Members	
Least Successful Bacteria Name	
Justification	

Voting Card	
Name of Group Members	
Most Successful Bacteria Name	
Justification	

Voting Card	
Name of Group Members	
Least Successful Bacteria Name	
Justification	

LESSON THREE: PATHOGEN SURVIVOR

KEY QUESTION(S): How and why do bacteria evolve to gain or lose traits so quickly? What drives bacteria to lose or acquire traits? How do different virulence factors affect the fitness of bacteria in different environments?

OVERALL TIME ESTIMATE: 1- 50 minute class period

LEARNING STYLES: Visual, auditory, kinesthetic and group work

VOCABULARY:

Antibiotic Resistance: Antibiotic resistance refers specifically to the resistance to antibiotics that occurs in common bacteria that cause infections.

Antimicrobial Resistance: Antimicrobial resistance is a broader term, encompassing resistance to drugs to treat infections caused by other microbes as well, such as parasites (e.g. malaria), viruses (e.g. HIV) and fungi (e.g. *Candida*).

Conserved (genes): similar or identical sequences that occur within nucleic acid sequences (such as RNA and DNA sequences), protein sequences, protein structures or polymeric carbohydrates across species (orthologous sequences) or within different molecules produced by the same organism (paralogous sequences)

Flagellum: a slender threadlike structure, especially a microscopic whip-like appendage that enables many protozoa, bacteria, spermatozoa, etc., to swim.

Genomic Island (GI): large genomic regions (typically >8kb), that are thought to have horizontal origins. These regions can often contain genes that are related to antibiotic resistance and/or virulence.

Horizontal Gene Transfer: the transfer of genes between organisms in a manner other than traditional reproduction

Ortholog: Orthologs are genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

Pathogenicity: refers to the ability of an organism to cause disease (i.e., harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

Pathogenicity Island (PAI): a distinct class of genomic islands acquired by microorganisms through horizontal gene transfer. Pathogenicity islands are found in both animal and plant pathogens. Additionally, PAIs are found in gram positive and gram negative bacteria.

Pathology: the typical behavior of a disease.

Phage: A bacteriophage: a virus capable of infecting a bacterial cell, and may cause lysis to its host cell.

Vertical Gene Transfer: the transmission of genes from the parental generation to offspring via sexual or asexual reproduction.

Virulence: the relative ability of a microorganism to cause disease; degree of pathogenicity, the capability of a microorganism to cause disease.

Virulence Gene: a gene whose presence or activity in an organism's genome is responsible for the pathogenicity of an infective agent.

LESSON SUMMARY: Students will form teams and play a Bacteria Survivor game that illustrates the concepts and relationships between virulence factors, horizontal gene transfer and fitness.

STUDENT LEARNING OBJECTIVES:
The student will be able to…

1. List common virulence factors
2. Classify genes as essential (housekeeping) or virulence factors
3. Explain the difference between transduction and transformation for gene transfer
4. List some selective pressures that act on a population
5. Describe a genomic island

STANDARDS:
SC.912.L.14.2
SC.912.L.14.6
SC.912.L.14.52
SC.912.L.15.1
SC.912.L.15.13
SC.912.L.15.15
SC.912.L.16.7
SC.912.L.18.11
SC.912.N.1.1

MATERIALS:

- Game cards
- 4 location markers
- Worksheet: Pathogen Survivor (1 copy per group)
- Bacteria Survivor Reference Sheets (1 copy per group)
- Blue and orange markers or highlighters (1 set per group)

BACKGROUND INFORMATION:

In humans, genetic information is transferred vertically from parent to offspring. Considering the average life span of an average human being is approximately 65 years, the evolutionary process of diversifying the genome from one generation to another occurs in a long period of time. On the other hand, bacteria life spans are significantly shorter compared to humans thus allowing bacteria to replicate rapidly. While humans are limited to vertical gene transfer, bacteria are capable of both vertical and horizontal gene transfer. In comparison to humans, bacteria undergo what may be considered as an "accelerated evolution". This is evident in the case of antibiotic resistance, bacteria have evolved faster than pharmaceutical development can keep up. This lesson is designed to demonstrate the various ways bacteria increase genetic diversity and allow students to discover and understand the underlying factors that drive bacteria to diversify their genome.

ADVANCE PREPARATION: (Total prep time 26-36 minutes)

- (20 minutes) The teacher is encouraged to spend some time running through the steps of the game to get a good understanding before playing with the class.

- (2 minutes) Print off the 'Pathogen Survivor' worksheet (1 per student group)
- (2-10 minutes) Print off Game Cards. Print off cards in regular printing paper. Cut along lines and insert into Card Sleeves or Deck Protector Sleeves (66mm x 91 mm). Make sure the printed cards will measure smaller than the Card/Deck Sleeves.
- (1 minute) Print the Bacteria Survivor Reference Sheets and distribute one copy per group.
- (1 minute) Set up groups of desks or lab stations as each of the four environments (water, soil, insect, human).
  (1 minute) Set up a teacher table in the middle of the four environments

Procedure with Time Estimates:

1. (1 minute) Divide students into 6-8 groups.
2. (2 minutes) Deal the cards to each group and distribute worksheets and Bacteria Survivor Reference Sheet.
3. (5 minutes) Go over the rules of the game with the students using the Bacteria Survivor Reference Sheet.
4. (30 minutes) Pathogen survivor activity.
5. (10 minutes) Discussion.

Discussion Questions

1. How many (and which) genes did you retain throughout all of your mutations?

2. Which type of gene transfer resulted in the most random mutations?

3. Rank the virulence factors that were in each surviving bacteria in order of most common to least common. What inferences can you make from this list?

4. What mutations seemed to be fatal?

5. What was your final ratio of virulent genes to non-virulent genes?

6. Did we learn more from the survivors or the deceased bacteria?

7. How many (and which) genes are conserved (the same in all species)? Why do you think these genes are conserved?

PATHOGEN SURVIVOR

Overview of Game:

The game is divided into 3 phases. Each represents an important step in bacterial life cycle.

The **first phase**, is the Replication phase. In this phase, selected genes will be replicated based on fitness.

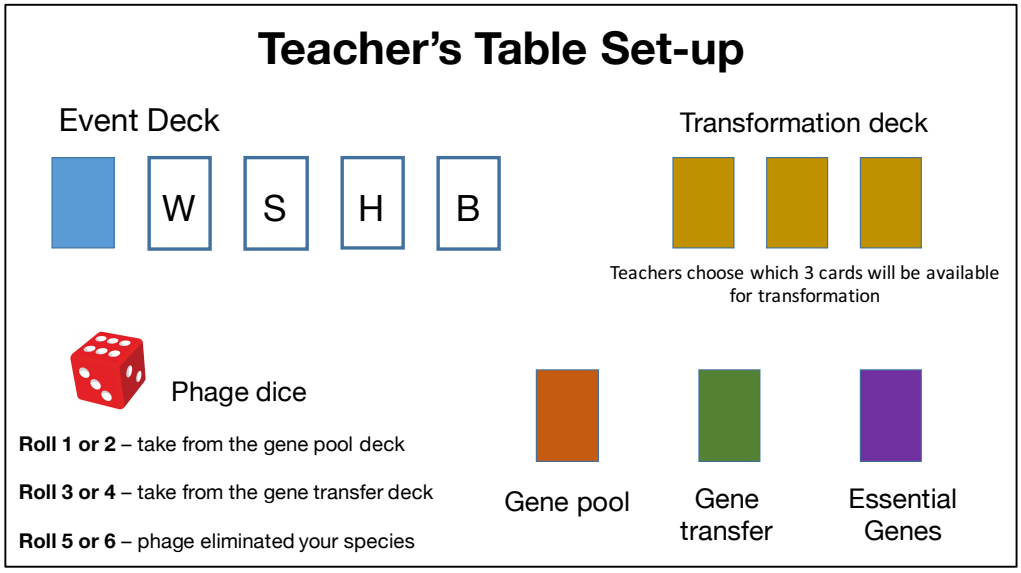
The **second phase** is Gene Transfer. Groups will go around the classroom and exchange genes. In this phase, each group is encouraged to diversify their genome as best possible.

The **third phase** is Gene conservation. The genome size of any organism is always conserved (acquisition of new genes results in loss of other genes in order to maintain genome size). In this phase, each group will decide which 8 genes they will keep.

At the end of the **third phase**, a series of events occur in each location. The events represent stress and environmental factors that drive bacteria to gain and lose traits (evolve).

Activity Set-up:

1. Divide the classroom into 4 locations (Human, Bug, Water, Dirt/Soil) and mark each locations using the printed location markers
2. Divide the class into 8 groups.
3. Distribute one (1) of each of the three **Essential Genes** (consists of Metabolism, Resource Gathering, Gene Replication) to each group.
4. Deal 2 random **Gene Transfer** cards (consists of Conjugation, Transformation, Phage Transduction, Phage Resistance) to each group.
5. Deal 3 random **Gene Pool** cards (consists of Antibiotic Resistance, Toxin Production, Secretion System, Metal resistance, Cell Surface Remodeling, Adhesion, Anti-toxin) to each group.
6. Each group should start with three (3) **Essential Genes**, two (2) **Gene Transfer** cards, and three (3) **Gene Pool** cards.
7. Place one table in the middle of the room. This will be the Game Master's/Teacher's Table. Set up the table as shown in the diagram below.
8. Supplementary PowerPoint presentation is provided to aid in facilitating activity.



9. Distribute worksheets to each of the groups.
10. Each group does the following:
    a. Give their bacterial species a name.
    b. Write down their starting genome (composed of the 8 gene cards they were given at the beginning of the activity composed of 3 essential genes, 2 gene transfer, and 3 from the gene pool).

ROUND START:

Phase I – Replication

Bacteria MUST have Gene Replication card in order to replicate. The number of genes it can replicate is determined by its fitness in its current environment.

When a bacteria satisfies the list of required genes in a given location (see diagram below), it is deemed fit. As an example, let us compare Bacteria A and B located in Water (Required genes: Antibiotic, Toxin, and Metal Resistance).

Bacteria A	**Bacteria B**
Antibiotic resistance	Toxin Resistance
Toxin resistance	Adhesion
Metal resistance	Cell Surface Remodeling
Conjugation	Phage Transduction
Transformation	Conjugation
Metabolism	Metabolism
Resource Gathering	Resource Gathering
Gene Replication	Gene Replication

Bacteria A's genome contains Antibiotic, Toxin, and Metal Resistance, which satisfies the entire list of required genes in Water, while Bacteria B only satisfies one out of the three; Toxin Resistance. Thus, Bacteria A is more fit and is able to replicate three (3) genes while Bacteria B can only replicate one (1) gene.

Make sure to copy the set of bacteria initials onto the newly replicated gene.

Alternative: In lieu of time constraints, the teacher may opt to disregard fitness requirements in this phase and standardize replication to 3 genes.

Water	Dirt/Soil
Required genes: • Antibiotic resistance • Metal resistance • Toxin production	Required genes: • Antibiotic resistance • Metal resistance • Toxin production
Human	**Bug**
Required genes: • Antibiotic resistance • Metal resistance • Toxin production • Cell surface remodelling • Adhesion proteins • Toxin resistance/Anti-toxin • Secretion system	Required genes: • Antibiotic resistance • Metal resistance • Toxin production • Cell surface remodelling • Adhesion proteins • Toxin resistance/Anti-toxin • Secretion system

Phase II: Gene exchange

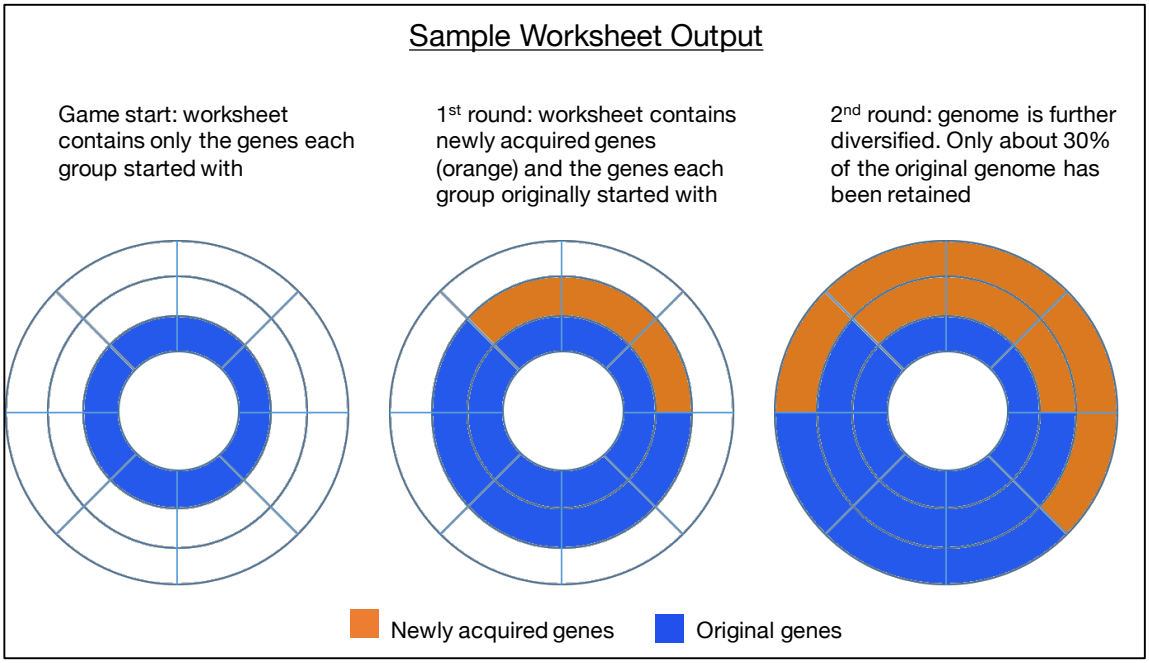
Gene transfer only occurs between bacteria within the same location. Students gain or lose genes (exchange cards) as indicated in their Gene Transfer cards.

a. Conjugation – Any bacteria containing this gene can donate one gene card to any bacterial species of their choosing.
b. Transformation – Any bacteria containing this gene chooses one of the 3 face-up cards in the Transformation Deck
c. Phage transduction – Any bacteria holding this gene can pick a random gene from other bacteria except from those holding phage resistance.
d. Phage resistance – Gives bacteria immunity from phage transduction.

Phase III: Gene conservation /Consolidate genes

Genomes are limited to a specific size. This means that when a new gene is acquired, another must be discarded to accommodate the new gene.

a. Each of the bacteria will now decide which 8 genes they will keep.
b. Discarded genes will be returned to the gene pool (teacher's desk).
c. Write down new genome composition on the worksheet. Write down the newly acquired genes next to each other.
d. On the worksheet, highlight the newly acquired genes Orange and the original genes Blue (see sample worksheet below).



e. Indicate on the worksheet the bacteria's current location (Water, Human, Bug, Dirt/Soil)
f. On each of the gene cards, write your bacteria's initials on the upper left corner of each of your newly acquired genes.

Event Cards. At the end of Phase III, the teacher reveals cards from the event deck, one for each of the 4 locations. These events are then resolved before beginning the following round.

Events:

1. Antibiotics have been administered – Only the bacteria with antibiotic resistance survive.
2. Toxic level of metals have been introduced – Only the bacteria with metal resistance genes survive.
3. Skirmish – Bacteria in the same location battle each other using their toxins and secretion systems.
4. Predator – a paramecium is out to get all the bacteria. Only those with Toxin producing genes and Secretion systems will survive.
5. Immune system fights back – Only those with cell surface remodeling will survive.
6. Starvation conditions – Resource Gathering and Metabolism will be required for replication in the following round
7. Flood - Rushing liquid flows against bacteria. Species containing adhesion gene survives. All other species are removed from this location and eliminated.
8. Nothing happens.

Round ends. A new round is initiated.

Bacteria Survivor Reference Sheet

Shorthand Notation for Gene Cards

Essential Genes - Phase I

- **ResG** - Resource Gathering
- **GenR** - Gene Replication
- **Met** - Metabolism

Gene Transfer - Phase II

- **Conj** - Conjugation
- **Tran** - Transformation
- **PTran** - Phage Transduction
- **PRes** - Phage Resistance

Gene Pool - Used in the Event Phase

- **AntR** - Antibiotic Resistance
- **Omn** - Omnitoxin
- **AntO** - Anti-Omnitoxin
- **CSR** - Cell Surface Remodelling
- **SS** - Secretion System
- **MRes** - Metal Resistance
- **Adh** – Adhesion

Order of Play

Phase 1: Replication

- ❖ Assess fitness
- ❖ Replicate genes

Phase 2: Gene Transfer

- ❖ Diversify genome according to gene transfer cards

Phase 3: Consolidate genome

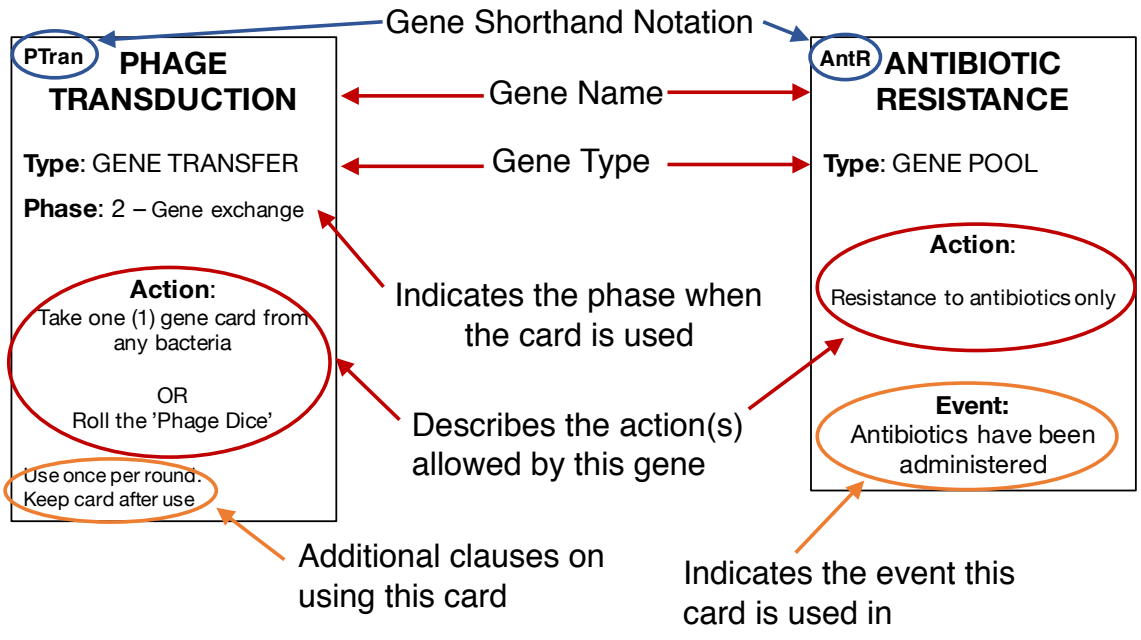
- ❖ Reduce hand back to 8 gene cards

Write new genome on worksheet

- ❖ Color worksheet: [**Blue** – original genes | **Orange** – new genes]

Event Phase

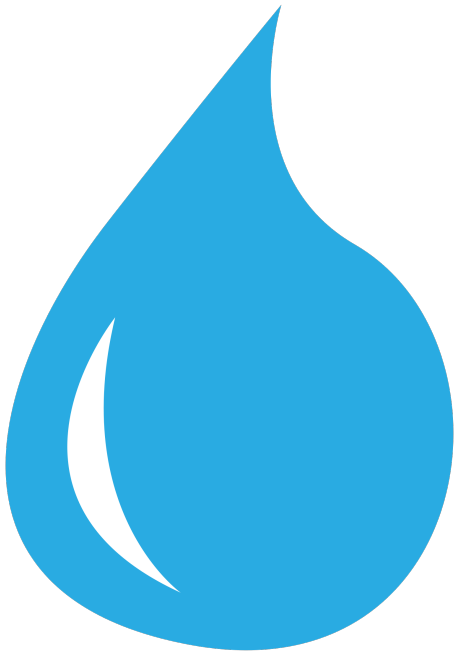
Anatomy Of The Gene Cards



WATER

Required Genes:

- Antibiotic Resistance
- Metal Resistance
- Toxin Production



DIRT/SOIL

Required Genes:

- Antibiotic Resistance
- Metal Resistance
- Toxin Production

HUMAN

Required Genes:

- Antibiotic Resistance
- Metal Resistance
- Toxin Production
- Cell Surface Remodeling
- Adhesion Proteins
- Toxin Resistance/Anti-toxin
- Secretion System

BUG

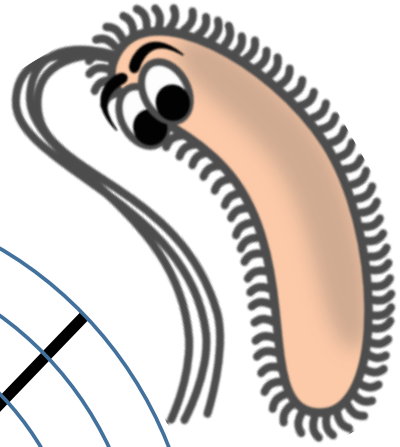
Required Genes:

- Antibiotic Resistance
- Metal Resistance
- Toxin Production
- Cell Surface Remodeling
- Adhesion Proteins
- Toxin Resistance/Anti-toxin
- Secretion System

Pathogen Survivor

Bacterial Species:_____

Group Members:_____



Locations:

Round 0:_____

Round 1:_____

Round 2:_____

Round 3:_____

Round 4:_____

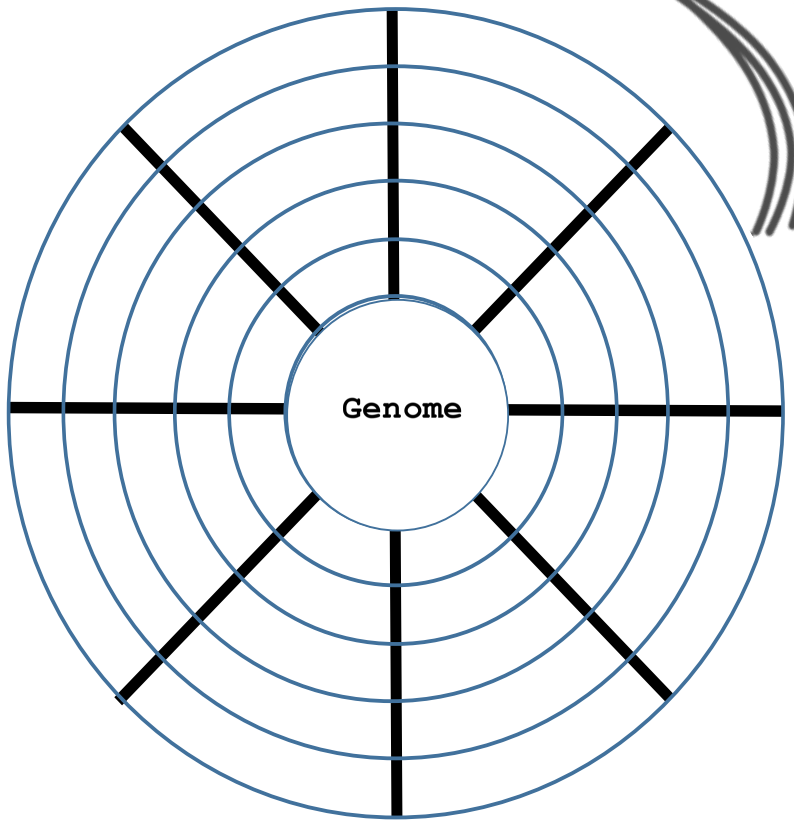


Image sourc : https://openclipart.org/detail/32875/funny-bacillus

LESSON FOUR: COMPARATIVE GENOMICS

KEY QUESTION(S): What can we learn from comparing the genomes of different species, particularly between bacteria? How do scientists compare genomes? How do they know what to look for?

SCIENCE SUBJECT: Bioscience, Biotechnology, Biology

GRADE AND ABILITY LEVEL: This is designed for an honors or other upper level course, though it could be easily modified for lower levels.

SCIENCE CONCEPTS: Comparative Genomics

PRIOR KNOWLEDGE: The concept of genomic islands and pathogenicity islands from the previous lesson is required.

OVERALL TIME ESTIMATE: 1- 50 minute class period

LEARNING STYLES: Visual, auditory, kinesthetic and cooperative

VOCABULARY:

Antibiotic Resistance: Antibiotic resistance refers specifically to the resistance to antibiotics that occurs in common bacteria that cause infections.

Antimicrobial Resistance: Antimicrobial resistance is a broader term, encompassing resistance to drugs to treat infections caused by other microbes as well, such as parasites (e.g. malaria), viruses (e.g. HIV) and fungi (e.g. *Candida*).

Arabodopsis: a small invasive self-pollinating weed with small white flowers; much studied by plant geneticists; the first higher plant whose complete genome sequence was described.

Basal bodies: a cylindrical organelle, within the cytoplasm of flagellated and ciliated cells, that contains microtubules and forms the base of a flagellum orcilium: identical in internal structure to a centriole.

Cilia: minute hair-like organelles, identical in structure to flagella, that line the surfaces of certain cells and beat in rhythmic waves, providing locomotion to ciliate protozoans and moving liquids along internal epithelial tissue in animals.

Chlamydomona: a common single-celled green algae that lives in water and moist soil and typically has two flagella for swimming.

Comparative Genomics: the field of biological research in which the genomic features of different organisms are compared. The genomic features may include the DNA sequence, genes, gene order, regulatory sequences, and other genomic structural landmarks. Genomic regions that are not present within related strains suggest that the region was horizontally transferred.

Conserved (genes): similar or identical sequences that occur within nucleic acid sequences (such as RNA and DNA sequences), protein sequences, protein structures or polymeric carbohydrates across species (orthologous sequences) or within different molecules produced by the same organism (paralogous sequences)

Flagellum: a slender threadlike structure, especially a microscopic whip-like appendage that enables many protozoa, bacteria, spermatozoa, etc., to swim.

Genomic Island (GI): large genomic regions (typically >8kb), that are thought to have horizontal origins. These regions can often contain genes that are related to antibiotic resistance and/or virulence.

Horizontal Gene Transfer: the transfer of genes between organisms in a manner other than traditional reproduction

Pathogenicity: refers to the ability of an organism to cause disease (i.e., harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

Pathogenicity Island (PAI): a distinct class of genomic islands acquired by microorganisms through horizontal gene transfer. Pathogenicity islands are found in both animal and plant pathogens. Additionally, PAIs are found in gram positive and gram negative bacteria.

PATRIC: Pathosystems **R**esource **I**ntegration **C**enter

Vertical Gene Transfer: the transmission of genes from the parental generation to offspring via sexual or asexual reproduction.

Virulence: the relative ability of a microorganism to cause disease; degree of pathogenicity, the capability of a microorganism to cause disease.

Virulence Gene: a gene whose presence or activity in an organism's genome is responsible for the pathogenicity of an infective agent.

LESSON SUMMARY:

This lesson includes two activities. The first activity involves the teacher showing a seven minute video titled “The Power of Comparative Genomics”. This video defines comparative genomics and demonstrates the advantages of applying it to research into gene identification and protein function. The second activity involves several tutorials of the Pathosystems Resource Integration Tool (PATRIC) to compare two staphylococcus aureus bacterial species (MRSA and MSSA) and locate a pathogenicity island and genes that code for antibiotic resistance.

STUDENT LEARNING OBJECTIVES:
The student will be able to…

- Understand the basic principles of comparative genomics
- Enumerate advantages to research and discovery presented by comparative genomics
- Use PATRIC to browse and compare genomes
- Use the Proteome Comparison Tool in PATRIC
- Identify candidate genomic islands
- Identify potential virulence factors within said genomic islands

STANDARDS:

SC.912.L.15.1
SC.912.L.16.10
SC.912.N.1.1
SC.912.N.1.6
SC.912.N.1.7
SC.912.N.2.4

MATERIALS:

- Computers
- Copies of Worksheet “The Power of Comparative Genomics” for each student

RESOURCES:

Comparative Genomics
https://www.genome.gov/11509542

The Power of Comparative Genomics (7:07)
https://www.youtube.com/watch?v=mU9ROpm6d70

MATERIALS:

- A class set of laptops or reserved computer lab
- Worksheets “The Power of Comparative Genomics”
- Worksheets “Meet PATRIC”

ADVANCE PREPARATION: Total time estimate is 1 hour and 12 minutes

- (1 hour) Read through the transcripts of the videos and go through the PATRIC tutorials
- (1 minute) Reserve computers or computer* lab time
- (1-10 minutes) Make sure that all computers will allow popups in PATRIC. If not, the teacher may need to get an authorized individual to change the settings to allow popups.
- (1 minute) Print off enough copies of the worksheet titled “Meet PATRIC” for each team of two students.

ACTIVITY #1: VIDEOS

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATESF:

1. (1 minute) Pass out the worksheet "The Power of Comparative Genomics" to each student.
2. (7:07 minutes) Show the video "The Power of Comparative Genomics" to the class and have students complete the worksheet as the video is shown.
3. (5-10 minutes) Collect the worksheet and ask students to explain in their own words what comparative genomics is and what benefits it has for research scientists.
4. (5-10 minutes) Allow some time for questions or discussion from the students

ACTIVITY #2: MEETING PATRIC

Procedure and Discussion Questions with Time Estimates

1. (2 minutes) Team students up with one partner so you have groups of two.
2. Assign each student a computer and have them sit next to their partner.
3. Pass out the worksheet titled "Meet PATRIC" to each team of students.
4. (30 minutes) Allow students the remainder of the period to go through the tutorials on PATRIC making sure to walk around the room and offer assistance to those students who require it.
5. (1 minute) Collect the worksheet as students finish.

TRANSCRIPT

The Power of Comparative Genomics (7:07)
https://www.youtube.com/watch?v=mU9ROpm6d70

Published in 2001, the human genome project worked out the complete sequence of three billion DNA letters necessary to code for an individual human being. This monumental feat stands as one of the greatest ever achievements in science. As well as knowing the total sequence of human DNA, scientists now know the complete genetic code for more than a thousand other species.

Comparing the genomes of different organisms can help researchers to decide upon the most important experiments to conduct. It may also allow them to shift some of their experiments from working with animal models such as mice and fish onto studies using simpler and less controversial species instead. (Professor Andrew Fry University of Leicester) "Comparative genomics allows us to compare genes that are conserved right from very simple organisms through to higher organisms like man and as well as showing us the similarities, it shows us the differences so it shows us where systems have evolved to develop more complexity so we can begin to understand how genes have specialized, uh, through comparisons between different organisms."

Genes are said to be conserved if their sequences are the same or very similar in different organisms. This can often be an indication that they code for an important protein. If, however, the DNA sequences in various species are very different that can also be highly informative. Many scientific projects today start by using computers to compare the genome sequences of

different organisms. One medically relevant study[1] led by Susan Dutcher of Washington University began with the unlikely sounding comparison of human DNA with the genomes of two plant species, chlamydomonas and Arabidopsis, as a way of focusing on genes involved in the production of hair-like structures called cilia. This work is important because a range of rare but devastating diseases are caused by mutations in proteins within cilia and an associated structure called a basal body which serves as an anchor for cilia within the cell. These diseases have diverse symptoms ranging from kidney disease to progressive blindness.

(Andrew Fry) “So because basal bodies play such a key role in organizing cilia and flagella then any defect in a basal body that actually prevents formations of cilia or functioning of the cilia will lead to various different defects in human development.” Dutcher’s team were very clever when they chose to use chlamydomonas and Arabidopsis for comparison with the human genome. The first plant, chlamydomonas is a single-celled organism that lives in water. It possesses two flagella which it beats in order to move around. Flagella are simply elongated cilia grown from basal bodies. The second plant, Arabidopsis, lives on land. In keeping with most land-based plants Arabidopsis has no need for cilia or flagella and, therefore, it can be assumed it will not have the genes needed to make basal bodies. (Professor Hugh Woodland University of Warwick) “So if you say what’s common to organisms like chlamydomonas and humans, but absent in a land plant, then you immediately get at your genes which you have an initial suspicion might be involved in formation of flagella.” (Andrew Fry) “And this is the principle on which, uh, the scientific group led by Susan Dutcher went about looking for genes which are specifically required for basal body function.”

To begin by screening the entire human genome to try and find a gene coding for a basal body protein would be the equivalent of looking for a needle in a very large haystack. By limiting the search only to genes found in both humans and chlamydomonas, both of which have basal bodies, we can make the haystack much smaller. Then, by excluding genes which are also found in Arabidopsis, which we know does not have basal bodies, the search can be even more focused. By using comparative genomics in this way, Dutcher’s team were able to go from an initial list of several thousand genes and reduce it to between 600 and 700 candidate genes.

(Hugh Woodward) “Now the question is if you have these, um, candidates for genes that are absent in Arabidopsis, candidates for being involved in forming cilia and flagella, well what’s the next step? How do you find out that they are important?” (Fry) So the Dutcher group really had to come up with experimental techniques that allowed them to test potential functions in cilia activity. And one could carry out those experiments in humans, but having shown that those genes in chlamydomonas, it’s much simpler to use the model organism to do those experiments. So for example using experimental techniques you can remove flagella from chlamydomonas and those organisms will simply grow the flagella back again and when they do that they will up regulate those genes that are required for flagella synthesis and so we can look at which of those six hundred gens are regulated when you ask the flagella to regrow.”

(Woodward) “The next thing that you would do to start to knock those genes out in one way or another and find what the affect is. That’s perhaps most easily done in chlamydomonas, you would knock them out and then you would ask then what is the effect then on the formation of the flagella and the function of the flagella.”

(Fry) So it’s through this very elegant approach of comparative genomics a new gene was identified that was involved in human disease and had not been identified in any other approach.”

As this simple example demonstrates possession of the complete genome sequence of many organisms and especially of humans is providing scientists with a hugely valuable research tool. By starting their research with analysis of DNA databases, scientists can save time and save money by reducing the number of unnecessary experiments they might otherwise have carried out. Importantly, this approach can lead to reduction in the number of experiments using mammals and other vertebrate species, by helping to identify lower organisms in which meaningful research can be conducted. (end)

(1) - http://www.sciencedirect.com/science/article/pii/S0092867404004507

TRANSCRIPT
"Comparison of Genomes of Eight Enteroaggregative E. coli O104:H4 Isolates (2:07)
https://www.youtube.com/watch?v=6VTxmnZQXgU

Roscoe and colleagues initially sequenced the genome of an e.coli strain that had caused a German outbreak which was characterized by an unusually high incidence of hemolytic-uremic syndrome or HUS.

The investigators then sequenced the genomes of seven different isolates of enteroaggregative *E. coli* known to cause diarrhea, but not HUS.

To visualize regions of difference between these strains the eight genomes were compared to two closely related genomes.

In the first analysis, investigators matched each of these genomes onto the genome of TY2482, an isolate from the German outbreak that was recently sequenced and assembled by the Beijing Genomics Institute and placed in the public domain. Red indicates regions in the TY2482 genome and absent in the corresponding comparator strains. Comparing the patterns across the genomes divulges region specific to C227-11 that may contain genes pertinent to the pathology. For example, it is clear that the phage like element containing the genes encoding HUS causing Shiga toxin 2 is common to C227-11 and TY 242, but is absent in the genomes of the other seven comparator strains. The presence, indicated here in solid blue, of a different phage-like element in the genome with the outbreak strain and also in most of the other genomes suggest that this element is unlikely to be relevant to the severe pathogenesis of the outbreak strain.

In a second analysis the investigators mapped the genome of each of the sequenced E. coli strains to the genome of the strain 55989 which causes diarrhea but is not known to cause HUS. Red indicates regions present in the 55989 genome and absent in the comparator genomes. Comparison across the genome suggest that the relative absence of sequence in the outbreak strain is unlikely to be relevant to the severe pathogenesis of the outbreak strain.

STUDENT WORKSHEET

"The Power of Comparative Genomics" (7:07)
https://www.youtube.com/watch?v=mU9ROpm6d70
"Comparison of Genomes of Eight Enteroaggregative E. coli O104:H4 Isolates (2:07)
https://www.youtube.com/watch?v=6VTxmnZQXgU

Name _____ **Date** _____ **Period** _____

1. What does comparative genomics compare?

2. What results can a scientist obtain by using comparative genomics?

3. What is the relevance of genes that are conserved?

4. Why did Dutcher choose Chlamydomonas and Arabidopsis genomes to compare to the human genome?

5. If scientists wanted to identify genes that code for fish scales, what species could they use to compare? Explain.

6. What genotypic or phenotypic observations would a scientist expect to see if she 'erased' flagella versus 'knocking out' the genes that code for basal bodies?

7. What are three advantages comparative genomics offers?

TEACHER ANSWER KEY

Name _____ **Date** _____ **Period** _____

1. What does comparative genomics compare?

   Genomes of different organisms.

2. What results can a scientist obtain by using comparative genomics?

   Which genes are conserved between species and which genes are different.

3. What is the relevance of genes that are conserved?

   It means that they most likely have similar functions in those species and that they are probably an essential gene required for basic biological functions.

4. Why did Dutcher choose Chlamydomonas and Arabidopsis genomes to compare to the human genome?

   Dutcher wanted to look for genes that code for cilia or basal bodies whose impaired function is the cause of several serious human diseases. Arabidopsis doesn't have cilia, but Chlamydomonas does so comparing all three genes helped narrow the search from the gene pool.

5. If scientists wanted to identify genes that code for fish scales, what species could they use to compare? Explain.

   Answers will vary, but they should choose two species that are very different, but have scales in common and one species that is similar, but doesn't have scales. For example, they could compare sharks (which don't have scales) and fish (which do have scales) and reptiles (which have scales, but don't swim).

6. What genotypic or phenotypic observations would a scientist expect to see if she 'erased' flagella versus 'knocking out' the genes that code for basal bodies?

   By erasing the flagella, the genes that code for flagella production would be expressed as they tried to produce more. By knocking them out they would expect to see an organism that could not produce flagella and there would be no genes to be expressed.

7. What are three advantages comparative genomics offers?

   Save time; save money; minimize the amount of research that needs to be done on controversial species

STUDENT WORKSHEET

MEETING PATRIC

Name(s) _____

1. On one computer open up a window to the PATRIC tutorial https://edpuzzle.com/media/55aea4d65f0175a52d3acbb0

2. On the other computer open up a window to PATRIC https://www.patricbrc.org/portal/portal/patric/Home
    a. Login to PATRIC using the following username and password
    b. Username: nsf_cpet
    c. Password: nsfcpet2015

3. On the PATRIC website, go to the "about" tab and click on the "What is PATRIC?" link.

4. In your own words, describe the purpose of the web based tool PATRIC.

5. One of you should go through the tutorial and direct the other student through the steps on the PATRIC website.

6. A genomic island is that part of a genome that exhibits telltale signs of horizontal origins. These islands may or may not contain virulence factors that contribute to an organisms pathogenicity. How does PATRIC allow the user to visualize genomic islands?

7. Switch computers and repeat step #5 so that both of you have gone through the tutorial and directed the other and so each of you have had the opportunity to perform the tutorial of PATRIC. You will be using PATRIC tomorrow to research an assigned known pathogen.

8. Now do the same thing with the 'PATRIC to Excel' tutorial. (On the computer that had the PATRIC tutorial, open the second tutorial ("PATRIC to Excel", while keeping PATRIC open on the other). (Note: this tutorial was done on a Mac and may look slightly different on a PC.) Tutorial: https://edpuzzle.com/media/55b10a414b1a02f843a157c1

9. Switch places and repeat step #8.

10. Now do the same thing again with the "VirulencePred" tutorial as you did in step #8. And switch places like you did in step #9. Tutorial: https://edpuzzle.com/media/55b139b3cf6c3d1028cef675

11. By the end of this worksheet you should each have gone through each of the three tutorials on your own. These will be used in tomorrow's activity.

LESSON FIVE A: BACTERIAL RESEARCH

KEY QUESTION(S): How are genomic islands related to the pathogenicity of specific bacteria? How do web based tools like PATRIC use comparative genomics to find genomic islands in bacteria?

SCIENCE SUBJECT: Bioscience, Biotechnology, Biology

GRADE AND ABILITY LEVEL: This is designed for an honors or other upper level course

SCIENCE CONCEPTS: Genomic Islands, Pathogenicity, and Comparative Genomic Databases

OVERALL TIME ESTIMATE: 1- 50 minute class period (This lesson is research intensive for the students. If the research and poster are not completed by the end of the period, the teacher may choose to assign it as homework or allow an extra class period.

LEARNING STYLES: Visual, auditory, and or kinesthetic.

VOCABULARY:

Antibiotic Resistance: Antibiotic resistance refers specifically to the resistance to antibiotics that occurs in common bacteria that cause infections.

Antimicrobial Resistance: Antimicrobial resistance is a broader term, encompassing resistance to drugs to treat infections caused by other microbes as well, such as parasites (e.g. malaria), viruses (e.g. HIV) and fungi (e.g. *Candida*).

Comparative Genomics: the field of biological research in which the genomic features of different organisms are compared. The genomic features may include the DNA sequence, genes, gene order, regulatory sequences, and other genomic structural landmarks. Genomic regions that are not present within related strains suggest that the region was horizontally transferred.

Conserved (genes): similar or identical sequences that occur within nucleic acid sequences (such as RNA and DNA sequences), protein sequences, protein structures or polymeric carbohydrates across species (orthologous sequences) or within different molecules produced by the same organism (paralogous sequences)

Epidemiology: the branch of medicine that deals with the incidence, distribution, and possible control of diseases and other factors relating to health.

Genomic Island (GI): large genomic regions (typically >8kb), that are thought to have horizontal origins. These regions can often contain genes that are related to antibiotic resistance and/or virulence.

Horizontal Gene Transfer: the transfer of genes between organisms in a manner other than traditional reproduction

Morphology: The branch of biology that deals with the form and structure of organisms without consideration of function.

Pathogenicity: refers to the ability of an organism to cause disease (i.e., harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

Pathogenicity Island (PAI): a distinct class of genomic islands acquired by microorganisms through horizontal gene transfer. Pathogenicity islands are found in both animal and plant pathogens. Additionally, PAIs are found in gram positive and gram negative bacteria.

Pathology: the typical behavior of a disease.

Vertical Gene Transfer: the transmission of genes from the parental generation to offspring via sexual or asexual reproduction.

Virulence: the relative ability of a microorganism to cause disease; degree of pathogenicity, the capability of a microorganism to cause disease.

Virulence Gene: a gene whose presence or activity in an organism's genome is responsible for the pathogenicity of an infective agent.

LESSON SUMMARY:

Groups will use PATRIC, VirulentPred and other databses and web resources to research the pathogenicity of an assigned bacterium and its associated virulent genes.

STUDENT LEARNING OBJECTIVES:

The student will be able to…

- Describe the background and history of the assigned pathogenic species
- Conduct independent research and genome comparisons using PATRIC and other databases/web resources.
- Identify candidate pathogenic islands
- Identify and list virulence factors within the identified pathogenic islands

STANDARDS:

SC.912.L.14.2
SC.912.L.14.6
SC.912.L.14.52
SC.912.L.15.1
SC.912.L.15.15
SC.912.L.17.6
SC.912.L.18.4
SC.912.L.18.11
SC.912.N.1.1
SC.912.N.1.3
SC.912.N.1.4
SC.912.N.1.6
SC.912.N.1.7
SC.912.N.2.4
SC.912.N.3.5

MATERIALS:

- Computers or computer lab
- Poster Board (and materials for creating a poster presentation such as: markers, glue, scissors, printer, colored paper, tape, etc.)
- Copies of directions (enough for each student)
- Copies of bacteria cards (one card per group)

ADVANCE PREPARATION: (5 minutes)

1. (<1 minute) Make enough copies of the directions for each student.
2. (1 minute) Write the name of one of the following bacterial strains on six different index cards. There will be six different index cards each with a different bacterial strain. The six strains are:
    - *Salmonella enterica subsp. enterica serovar Enteritidis str. EC20110223 (1412451.3) vs Salmonella enterica subsp. enterica serovar Typhi strain E99-6478 (90370.563)*
    - *Escherichia coli O104:H4 str. C227-11 (1038927.9) vs Escherichia coli 55989 (585055.8)*
    - *Staphylococcus aureus MRSA 252 vs Staphylococcus aureus MSSA 476*
    - *Vibrio cholera O1 biovar El Tor str N16961 (243277.26) vs Vibrio cholera O1 str. Amazonia*
3. (<1 minute) Reserve computers or computer lab time.
4. (2 minutes) Assign students to a specific bacterium so that each group (bacterial species) has no more than 4 students.

BACTERIAL STRAIN RESEARCH AND POSTER DESIGN

Procedure:

1. (5 minutes) Pass out a copy of the directions to each student. Go over the expectations for student research and group presentations.
2. (2 minutes) Assign students to six groups.
3. (2 minutes) Assign each group (or member of each group) to a computer, depending on computer availability.
4. (1 minute) Pass out a bacteria card to each group. This is their assigned bacteria.
5. (40 minutes) Allow students class time to research their bacteria, create their poster, and prepare their presentation. Walk around the room to assist those students who may be having difficulty.

*If students are not able to complete their research and poster by the end of the class period the teacher may choose to assign it for homework or opt to give students another class period.

PROJECT GUIDELINE

Bacteria Research

Your group will be researching an assigned bacterial species. Use this guideline to ensure you meet the requirements for this project. You must use the five resources listed below as well as one primary resource of your choice. Your resources must be typed up in APA format on a 'Resources Cited' page which will be stapled to the back of your rubric prior to your presentation.

Microbe Wiki https://microbewiki.kenyon.edu/index.php/MicrobeWiki

Bacterial Genome Atlas http://bacmap.wishartlab.com/

PATRIC https://www.patricbrc.org/portal/portal/patric/Home

Virulence Pred http://203.92.44.117/virulent/index.html

Uniprot http://www.uniprot.org/

Once your general research is complete, use PATRIC to identify and locate genomic islands and specific genes that relate to pathogenicity. Complete the table below for your assigned bacteria. Then each student must complete the table for another bacterial strain of their choice from the list provided.

You will present your poster as a group and will be evaluated by your teacher as well as by your classmates. It is just as important that you learn how to effectively communicate information orally as it is to present excellent content.

Virulence Factor Research Presentation Guidelines

Your research, poster and presentation should include, but need not be limited to the information listed in the guidelines below.

1) **Bacteria**
   a) Morphology
      i) Cell Wall Description (gram negative or gram positive)
      ii) Size and shape of bacteria
      iii) Photo Image or Diagram

2) **Disease**
   a) Pathology
      i) What diseases does it cause?
      ii) What are some symptoms?
      iii) What is the prognosis?
   b) Epidemiology of disease
      i) How is it transmitted?
      ii) Is it endemic in any specific population? Where is it usually found and or transmitted?

3) **Story**
   a) Historical Timeline
      i) When was the disease and/or bacteria first discovered?
      ii) Is it resistant to any antibiotics? Which ones?
      iii) When did it become resistant to antibiotics?
   b) (Historical or Current Outbreaks)
      i) When?
      ii) Where?
      iii) Interesting Facts

4) **Genomics**
   a) Total number of Genes
   b) Total number of coding genes
   c) Total number of different plasmid types

5) **Pathogenicity Islands (PAI)**
   a) Genomic Islands
      i) Be able to identify some of the major genomic islands from a PATRIC genome overlap image.
   b) Virulence Factors
      i) What are they?
      ii) How do you think this species of bacteria acquired these virulence factors?
      iii) What is the evidence? (Hint: is it flanked by ‘mobile elements’ or transpons? Is there evidence of a phage

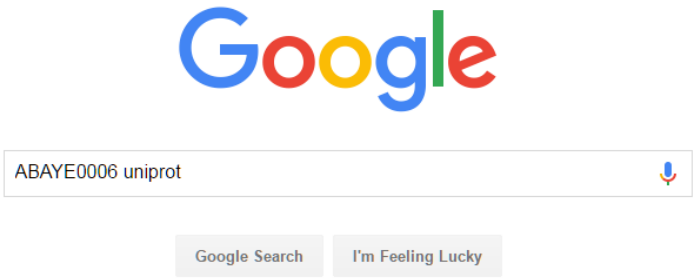
6) **Resources Cited (APA) Style**
   a) Five required sources
   b) One primary research article

Directions for Research / Student Handout

Use the directions below to complete the table *'Virulence Factors within Pathogenicity Islands'*. Once complete, this table should be stapled to the back of your rubric and turned in to your teacher before you present.

VirulentPred (virulence determination) Procedure:

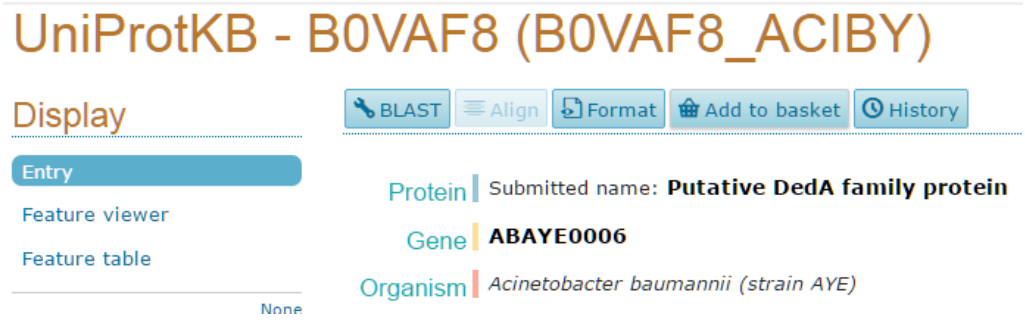
1. Once you identify one or more genomic islands in your excel spreadsheet, look at the 'reference genome function' column (H). Identify a protein within a genomic island (orange or black) that you suspect might be virulence factor. Identify the reference genome locus tag for that protein and copy it.
2. Paste your reference genome locus tag (i.e. ABAYE0006) and 'Uniprot' into a Google search.



3. You should be able to identify a link that looks something like this:

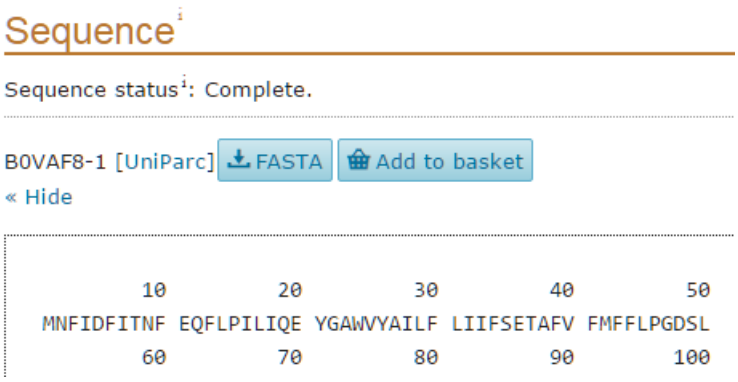
If you do not, in the interest of time, give up and try another gene.

4. Click on the link. It should take you to the Uniprot website and display something like this:



Make sure that you see your locus tag and your reference organism listed.

5. Scroll down until you see the ‘Sequence’ section and click on the link that says ‘FASTA’.



6. You should see protein sequence in FASTA format. It looks something like this.

```
>tr|B0VAF8|B0VAF8_ACIBY Putative DedA family protein OS=Acinetobacter baumannii (strain AYE) GN=ABAYE0006 PE=4 SV=1
MNFIDFITNFEQFLPILIQEYGAWVYAILFLIIFSETAFVFMFFLPGDSLLLTVGALCSV
VELMHLGYMITLLTVAATLGYIVNYSIGRHFGNRIFEAKSRFIKKEYLNKTNRYFLQHGG
KTILLARFIPFARSFAPLAAGSSNMSYGKFLIYNVAGAILWICILLTAGYLFGHALIQVT
DFVEN
```

7. Copy the entire sequence. Then open another tab for Virulent Pred (http://203.92.44.117/virulent/index.html) and click on the ‘Submit’ tab.
8. Paste your FASTA sequence for the protein into the search box. Scroll down and, without checking any boxes, click on ‘submit’. The search may take a minute or two depending on usage. When the search is complete, Virulent Pred will return results that look something like this: From these results we can see that this protein is not virulent.

S.No.	Protein Name	Prediction results	Predicted Scores
Cascade of SVMs and PSI-BLAST			
1.	>tr|B0VAF8|B0VAF8_ACIBY Putative DedA family protein OS=Acinetobacter baumannii (strain AYE) GN=ABAYE0006 PE=4 SV=1	Non-Virulent	-1.002

9. In your excel spreadsheet, insert a column to the right of your ‘reference genome locus tag’ column (F) and type NONVIRULENT for that gene.
10. Repeat steps #1-9 (you do not need to insert a new column each time (#9) until you have found three virulent proteins.

Once completed, this table should be stapled to the back of the poster and presentation rubric.

PATRIC
https://www.patricbrc.org/portal/portal/patric/Home

Use PATRIC to research your assigned bacteria strain. You must find three virulent genes and two non-virulent genes and fill in the information in the data table below.

Virulence Factors within Pathogenicity Islands

Student Names:
_____

Bacterial Species Name:
_____

(Suspected Virulence Factor) Reference Gene Locus Tag	Gene Function (Protein)	Virulent or Nonvirulent (VirulentPred)	In what part of the cell does this protein function?	Predicted Virulence Factor Category and Offensive or Defensive

Poster and Presentation Rubric Bacteria Name_____

Group Members Names _____

All points earned according to this rubric assume accuracy of content and can be verified by the teacher.

Criteria	Complete Plus (3)	Complete	Incomplete	Missing (0)
Poster (6 points)	☐ Organization ☐ Excellent balance of text, graphics and color	☐ Organization ☐ Good balance of text, graphics and color	☐ Organization ☐ Fair balance of text, graphics and color	☐ Organization ☐ Fair balance of text, graphics and color
Bacteria (6 points)	☐ Morphology ☐ Photo	☐ Morphology ☐ Photo	☐ Morphology ☐ Photo	☐ Morphology ☐ Photo
Disease (6 points)	☐ Pathology ☐ Epidemiology	☐ Pathology ☐ Epidemiology	☐ Pathology ☐ Epidemiology	☐ Pathology Epidemiology
Story (4 points)	☐ Historical Timeline ☐ Outbreaks	☐ Historical Timeline ☐ Outbreaks	☐ Historical Timeline ☐ Outbreaks	☐ Historical Timeline Outbreaks
Genomics (6 points)	☐ Complete genomic information	☐	☐	☐
Virulence Factors (9 points)	☐ What ☐ Acquired ☐ Evidence	☐ What ☐ Acquired ☐ Evidence	☐ What ☐ Acquired ☐ Evidence	☐ What ☐ Acquired ☐ Evidence
Resources (3 points)	☐ Typed bibliography in APA format attached	☐ Typed; APA	☐ Typed; APA	☐ Typed; APA
Oral Presentation (9 points)	☐ Roles are clearly defined ☐ Each student presents and seems confident and knowledgeable and correctly pronounce all words ☐ Time limit is met, but not exceeded	☐ Roles mostly well defined ☐ Three students seem confident and knowledgeable ☐ Time is within 2 minute window	☐ Roles are not clearly defined ☐ Two students seem confident and knowledgeable ☐ Time is within 4 minute window	☐ Students do not seem to know their role ☐ An obvious and heavy reliance on one student for knowledge ☐ Time is within 5 minute window
Total Points from each column				
			Points Earned:	/49

Bacteria Cards		
Reference: Salmonella enterica subsp. enterica serovar Enteritidis str. EC20110223 (1412451.3) vs *Salmonella enterica subsp. enterica serovar Typhi strain E99-6478 (90370.563)*	Reference: Escherichia coli O104:H4 str. C227-11 (1038927.9) vs *Escherichia coli 55989 (585055.8)*	Reference: Staphylococcus aureus MRSA 252 vs *Staphylococcus aureus MSSA 476*
Reference: Vibrio cholera O1 biovar El Tor str N16961 (243277.26) vs *Vibrio cholera O1 str. Amazonia*		

LESSON SIX: STUDENT PRESENTATIONS

KEY QUESTION(S): How are genomic islands related to the pathogenicity of specific bacteria? Are these genomic islands similar between different pathogenic species of bacteria?

SCIENCE SUBJECT: Bioscience, Biotechnology, Biology

GRADE AND ABILITY LEVEL: This is designed for an honors or other upper level chemistry course, though it could be easily modified for lower levels.

SCIENCE CONCEPTS: Genomic Islands, Pathogenicity, and PATRIC

OVERALL TIME ESTIMATE: 1- 50 minute class period

LEARNING STYLES: Visual, auditory, and or kinesthetic.

LESSON SUMMARY: Groups will present their research from Lesson #5A (PATRIC Research).

Materials:

- Clock or timer

Procedure with Time Estimate

1. (<1 minute) Have groups staple their PATRIC Table and their typed list of resources to the back of their presentation rubric.
2. (<1 minute) Collect these packets from each group.
3. (<1 minute) Pass out six copies of the rubric to each student.
4. (<1 minute) Tell the students that they will be responsible for assigning a grade and writing comments on the presentation and the content presented. These will be collected at the end of the period.
5. (<1 minute) Assign each packet a number from 1 -6 and write it at the top.
6. (<1 minute) Roll a dice to see which group will present first.
7. (36 – 50 minutes) Allow each group to present attempting to keep them within the six minute window.

Discussion Questions:

If there is any time remaining after the presentations, the teacher may wish to ask the following discussion questions. Alternatively, the teacher may ask students to write down their answers to these two questions as an exit ticket.

1. Were there virulence factors that all species of bacteria had in common? List these virulence factors, if any.
2. Were there significant differences in the genomic islands of each species? List the differences, if any.

Poster and Presentation Rubric Bacteria Name _____

Group Members Names _____

All points earned according to this rubric assume accuracy of content and can be verified by the teacher.

Criteria	Complete Plus (3)	Complete	Incomplete	Missing (0)
Poster (6 points)	☐ Organization ☐ Excellent balance of text, graphics and color	☐ Organization ☐ Good balance of text, graphics and color	☐ Organization ☐ Fair balance of text, graphics and color	☐ Organization ☐ Fair balance of text, graphics and color
Bacteria (6 points)	☐ Morphology ☐ Photo	☐ Morphology ☐ Photo	☐ Morphology ☐ Photo	☐ Morphology ☐ Photo
Disease (6 points)	☐ Pathology ☐ Epidemiology	☐ Pathology ☐ Epidemiology	☐ Pathology ☐ Epidemiology	☐ Pathology Epidemiology
Story (4 points)	☐ Historical Timeline ☐ Outbreaks	☐ Historical Timeline ☐ Outbreaks	☐ Historical Timeline ☐ Outbreaks	☐ Historical Timeline Outbreaks
Genomics (6 points)	☐ Genomic information	☐ Genomic Information	☐ Genomic Information	☐ Genomic Information
Virulence Factors (9 points)	☐ What ☐ Acquired ☐ Evidence	☐ What ☐ Acquired ☐ Evidence	☐ What ☐ Acquired ☐ Evidence	☐ What ☐ Acquired ☐ Evidence
Resources (3 points)	☐ Typed bibliography in APA format attached	☐ Typed; APA	☐ Typed; APA	☐ Typed; APA
Oral Presentation (9 points)	☐ Roles are clearly defined ☐ Each student presents and seems confident and knowledgeable and correctly pronounce all words ☐ Time limit	☐ Roles mostly well defined ☐ Three students seem confident and knowledgeable ☐ Time Limit	☐ Roles are not clearly defined ☐ Two students seem confident and knowledgeable ☐ Time Limit	☐ Students do not seem to know their role ☐ An obvious and heavy reliance on one student for knowledge ☐ Time Limit
Total Points from each column				
			Points Earned:	/49

Comments: