Identification of Pathogenic Islands using Comparative Genomics Based Tools

By Kathy Savage and Jo Marie Bacusmo
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 and explore concepts such as benefits and disadvantages of diversifying the genome, relating genome diversity to bacterial survival and fitness. The curriculum tackles several questions: What are the modes of gene transfer, how do bacteria gain or lose traits, and what is the driving force behind genome diversity? Students will become familiar with common pathogenic factors and the significance of these genes to pathogenesis and determine what characteristics make up an effective pathogen. Additionally, students will also 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. Finally, this curriculum opens discussion about natural products, cancer research, pharmaceutical synthesis, and ethics.
# LESSON SEQUENCING GUIDE AND SUMMARIES:

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

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<tr>
<th>Day</th>
<th>Lesson</th>
<th>Description</th>
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<tr>
<td>Day 1</td>
<td><strong>Lesson #1:</strong> Bad Bacteria</td>
<td>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.</td>
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<tr>
<td>Day 2</td>
<td><strong>Lesson #2:</strong> Pathogen Prototype</td>
<td>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.</td>
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<tr>
<td>Day 3</td>
<td><strong>Lesson #3: Activity #1:</strong> Video ‘Gene Transfer’</td>
<td>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.</td>
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<td><strong>Lesson #3: Activity #2:</strong> Activity ‘Hunger Games: Pathogen Edition’</td>
<td>Students team up and play a teacher directed game demonstrating genome diversification via gene transfer, highlighting its impact on bacterial fitness and survival.</td>
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<tr>
<td>Day 4</td>
<td><strong>Lesson #4: Activity #1:</strong> Video ‘The Power of Comparative Genomics (7:07)’ <a href="https://www.youtube.com/watch?v=mU9ROpm6d70">https://www.youtube.com/watch?v=mU9ROpm6d70</a></td>
<td>This video introduces comparative genomics as a tool to help scientists focus their research.</td>
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<td></td>
<td><strong>Lesson #4: Activity #2</strong> Video ‘Comparison of Genomes of Eight Enteroaggregative E.coli O104:H4 Isolates’ (2:07) <a href="https://youtu.be/6VTxmnZQXgU">https://youtu.be/6VTxmnZQXgU</a></td>
<td>This video shows how comparative genomics facilitates identification genomic islands contributing to pathogenesis of disease outbreak strains.</td>
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<td></td>
<td><strong>Lesson #4: Activity #3:</strong> Video Tutorials on Pathosystems Resource Integration Center (PATRIC)</td>
<td>Tutorial of PATRIC, a comparative genomics web based tool.</td>
</tr>
<tr>
<td>Day 5</td>
<td><strong>Lesson #5a:</strong> Student group research on specific pathogenic bacteria species</td>
<td>Students work in groups using PATRIC to research virulent genes and disease outbreaks for an assigned bacteria species.</td>
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<tr>
<td>Day 6</td>
<td><strong>Lesson #5b:</strong> Student presentations</td>
<td>Students present their research to the class and are graded according to a rubric (provided).</td>
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</table>
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 or cilium: 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.
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<tr>
<th>BENCHMARK</th>
<th>LESSON</th>
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<tr>
<td>SC.912.L.14.1</td>
<td>X</td>
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<tr>
<td>Describe the scientific theory of cells (cell theory) and relate the history of its discovery to the process of science</td>
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<tr>
<td>SC.912.L.14.2</td>
<td>X X X</td>
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<tr>
<td>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)</td>
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<tr>
<td>SC.912.L.14.6</td>
<td>X X X</td>
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<tr>
<td>Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.</td>
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<tr>
<td>SC.912.L.14.52</td>
<td>X X X</td>
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<tr>
<td>Explain the basic functions of the immune system, including specific and nonspecific immune response, vaccines, and antibiotics.</td>
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<tr>
<td>SC.912.L.15.1</td>
<td>X X X</td>
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<tr>
<td>Explain how the scientific theory of evolution is supported by the fossil record, comparative anatomy, comparative embryology, biogeography, molecular biology, and observed evolutionary change.</td>
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<tr>
<td>SC.912.L.15.13</td>
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<td>Describe the conditions required for natural selection, including: overproduction of offspring, inherited variation, and the struggle to survive, which result in differential reproductive success.</td>
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<tr>
<td>SC.912.L.15.15</td>
<td>X X</td>
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<tr>
<td>Describe how mutation and genetic recombination increase genetic variation.</td>
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<tr>
<td>SC.912.L.16.10</td>
<td>X</td>
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<tr>
<td>Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.</td>
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<tr>
<td>SC.912.L.16.7</td>
<td>X</td>
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<tr>
<td>Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.</td>
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<tr>
<td>SC.912.L.17.4</td>
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<td>Assess the need for adequate waste management strategies.</td>
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<tr>
<td>SC.912.L.17.6</td>
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<tr>
<td>Compare and contrast the relationships among organisms, including predation, parasitism, competition, commensalism, and mutualism.</td>
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<tr>
<td>SC.912.L.18.4</td>
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<tr>
<td>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.</td>
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<tr>
<td>SC.912.L.18.11</td>
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<tr>
<td>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.</td>
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<td>SC.912.N.1.1</td>
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<td>Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:</td>
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<tr>
<td>1. Pose questions about the natural world</td>
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<td>2. Conduct systematic observations.</td>
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<td>3. Examine books and other sources of information to see what is already known.</td>
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<td>4. Review what is known in light of empirical evidence.</td>
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<td>5. Plan investigations.</td>
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<td>6. Use tools to gather, analyze, and interpret data.</td>
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<td>7. Pose answers, explanations, or descriptions of events.</td>
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<td>8. Generate explanations that explicate or describe natural phenomena.</td>
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<td>9. Use appropriate evidence and reasoning to justify these explanations to others.</td>
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<tr>
<td>10. Communicate results of scientific investigations, and</td>
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<td>11. Evaluate the merits of the explanations produced by others.</td>
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# NEXT GENERATION SUNSHINE STATE STANDARDS - SCIENCE

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<tr>
<td>SC.912.N.2</td>
<td>Describe and explain what characterizes science and its methods.</td>
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<tr>
<td>SC.912.N.1.3</td>
<td>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.</td>
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<tr>
<td>SC.912.N.1.4</td>
<td>Identify sources of information and assess their reliability according to the strict standards of scientific investigation.</td>
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<tr>
<td>SC.912.N.1.5</td>
<td>Describe and provide examples of how similar investigations conducted in many parts of the world result in the same outcome.</td>
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<tr>
<td>SC.912.N.1.6</td>
<td>Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.</td>
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<tr>
<td>SC.912.N.1.7</td>
<td>Recognize the role of creativity in constructing scientific questions, methods and explanations.</td>
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<tr>
<td>SC.912.N.2.4</td>
<td>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.</td>
</tr>
<tr>
<td>SC.912.N.3.5</td>
<td>Describe the function of models in science, and identify the wide range of models used in science.</td>
</tr>
<tr>
<td>SC.912.N.4.1</td>
<td>Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society’s decision making.</td>
</tr>
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</table>


LITERATURE:

Medical Terminology Prefixes and Suffixes

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

Chapter 7: Bacterial Pathogenesis – Virulence Factors

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

Definitions of homolog, analog, ortholog & paralog

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

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

Article: Massive Antibiotic Resistance in China’s Rivers “Fueled by Abuse”

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?

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](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](https://www.youtube.com/watch?v=3UAJS9jzyV4) (3:40) and
   [https://www.youtube.com/watch?v=q5-sxUbEu5M](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.
<table>
<thead>
<tr>
<th>Offensive</th>
<th>Defensive</th>
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<tr>
<td>• Flagella or ability to ‘swim’</td>
<td>• Acid resistance</td>
</tr>
<tr>
<td>• Attachment</td>
<td>• Antibiotic resistance</td>
</tr>
<tr>
<td>• Injection</td>
<td>• Capsule or protective coating</td>
</tr>
<tr>
<td>• Toxins</td>
<td></td>
</tr>
<tr>
<td>• Secretion system</td>
<td></td>
</tr>
</tbody>
</table>

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
- 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
- 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  _______

Using your notes, Google and the Virulence Factors of Pathogenic Bacteria website http://www.mgc.ac.cn/VFs/, 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
1. The ability of a microorganism to cause disease is known as ____.
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   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?
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   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**
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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 Post-It Easel sheet per student group
- 1 set of multi-colored markers 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 easel sheets and markers and place them in the tables where each group will be working.
3. Decide where students will display their easel sheets 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 pathogen.
4. (5 minutes) Have the students display their easel sheets and, as a group, evaluate the various models by filling out the voting cards and taping them to pathogens 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
- Bacterial Virulence and Secretion Systems (VetSci Science & Learning)
- Micro Exam I – Bacterial Virulence Factors
- The Virulence Factor Database
  [http://www.mgc.ac.cn/VFs/](http://www.mgc.ac.cn/VFs/)
- Immune Evasion by Bacteria
- How microbes cause disease

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.

TEACHER’S 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 pathogen 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.

**Bacterial Virulence Factors**

![Image Source:](http://www.geocities.ws/micro2052000/disease.htm)
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 may 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
<table>
<thead>
<tr>
<th>Voting Card</th>
<th>Name of Group Members</th>
<th>Most Successful Bacteria Name</th>
<th>Justification</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Voting Card</th>
<th>Name of Group Members</th>
<th>Least Successful Bacteria Name</th>
<th>Justification</th>
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<thead>
<tr>
<th>Voting Card</th>
<th>Name of Group Members</th>
<th>Least Successful Bacteria Name</th>
</tr>
</thead>
</table>
LESSON THREE: HUNGER GAMES: PATHOGEN EDITION

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 Hunger Games: Pathogen Edition 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. Familiarize the various modes of gene transfer
4. List some selective pressures acting on a population leading to diversification and evolution
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:**
- 1 set of Game Cards (attached powerpoint file)
- Worksheet: Hunger Games: Pathogen Edition (1 copy per group)
- Hunger Games: Pathogen Edition 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 reading through the steps of the game to get a good understanding before playing with the class.
- (2 minutes) Print off the ‘Hunger Games: Pathogen Edition’ worksheet (1 per student group)
- (2-10 minutes) Print the Game Cards from the attached powerpoint file. This provides enough cards for 6-8 groups. 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. Alternatively, cards may be printed in colored card stock paper.
- (1 minute) Print the Hunger Games: Pathogen Edition Reference Sheets and distribute one copy per group.
- (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. (1 minute) Set up groups of desks or lab stations as each of the four environments (water, soil, insect, human).
3. (2 minutes) Deal the cards to each group and distribute worksheets and Hunger Games: Pathogen Edition Reference Sheet.
4. (5 minutes) Go over the rules of the game with the students using the Hunger Games: Pathogen Edition Reference Sheet.
5. (30 minutes) Hunger Games: Pathogen Edition
6. (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?
HUNGER GAMES: PATHOGEN EDITION

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 in preparation for gene diversification. The ability is dependent on the fitness of the bacteria in its environment.

The second phase is Gene Transfer. The bacteria diversify its genome in response to selective pressures and the environment. Groups will go around the classroom and use their Gene exchange cards to 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 to maintain a reasonable 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).

Objective of the Game: Evolve and SURVIVE!

Activity Set-up:

1. Divide the class into 6-8 groups consisting of 3-5 students per group.
2. Divide the classroom into 4 locations (Human, Bug, Water, Dirt/Soil) and mark each locations using the printed location markers
3. Build 6-8 “Starter Decks” containing the following cards:
   - Two (2) random Gene Transfer (red) cards (consists of Conjugation, Transformation, Phage Transduction, Phage Resistance)
   - Three (3) random Gene Pool (yellow) cards (consists of Antibiotic Resistance, Toxin Production, Secretion System, Metal resistance, Cell Surface Remodeling, Adhesion, Anti-toxin)
4. Each deck should contain a total of eight (8) cards: three (3) Essential Genes, two (2) Gene Transfer cards, and three (3) Gene Pool cards.
5. 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.
   - Place the Event Cards (pink) in one pile. The event phase is skipped in Round 1. Start drawing Event Cards at Round 2.
   - Designate a space on the table as the “Transformation Deck”. Randomly select any 3 cards and place it in this space. This will be available for students to pick up in the Gene transfer phase.
   - Take a 6-sided dice and designate this section for the Phage Dice. Here, students with the Phage Transduction card may roll the dice where their fate will be decided.
   - On another section of the table, segregate the cards into individual piles for easy access as the game goes along.
6. Supplementary PowerPoint presentation is provided to aid in facilitating activity.
7. Distribute worksheets to each of the groups.
8. Each group does the following:
   - Give their bacterial species a name.
   - 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.

<table>
<thead>
<tr>
<th>Water</th>
<th>Dirt/Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required genes:</td>
<td></td>
</tr>
<tr>
<td>• Gene Replication</td>
<td>• Gene Replication</td>
</tr>
<tr>
<td>• Antibiotic resistance</td>
<td>• Secretion System</td>
</tr>
<tr>
<td>• Metal resistance</td>
<td>• Metal resistance</td>
</tr>
<tr>
<td>• Omnitoxin production</td>
<td>• Omnitoxin production</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human</th>
<th>Bug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required genes:</td>
<td></td>
</tr>
<tr>
<td>• Gene Replication</td>
<td>• Gene Replication</td>
</tr>
<tr>
<td>• Antibiotic resistance</td>
<td>• Omnitoxin production</td>
</tr>
<tr>
<td>• Metal resistance</td>
<td>• Cell surface remodelling</td>
</tr>
<tr>
<td>• Omnitoxin production</td>
<td>• Adhesion proteins</td>
</tr>
<tr>
<td>• Cell surface remodelling</td>
<td>• Omnitoxin resistance</td>
</tr>
<tr>
<td>• Adhesion proteins</td>
<td>• Secretion system</td>
</tr>
<tr>
<td>• Omnitoxin resistance</td>
<td></td>
</tr>
<tr>
<td>• Secretion system</td>
<td></td>
</tr>
</tbody>
</table>

When a Bacteria satisfies the list of required genes in a given location (see diagram above), it is deemed fit. As an example, let us compare Bacteria A and B located in Water.

<table>
<thead>
<tr>
<th>Location: Water</th>
<th>Bacteria A</th>
<th>Bacteria B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required genes:</td>
<td>✅ Antibiotic Resistance</td>
<td>❌ Omnitoxin Resistance</td>
</tr>
<tr>
<td></td>
<td>✅ Omnitoxin Production</td>
<td>❌ Adhesion</td>
</tr>
<tr>
<td></td>
<td>✅ Metal Resistance</td>
<td>❌ Cell Surface Remodelling</td>
</tr>
<tr>
<td></td>
<td>❌ Conjugation</td>
<td>❌ Phage Transduction</td>
</tr>
<tr>
<td></td>
<td>❌ Transformation</td>
<td>❌ Conjugation</td>
</tr>
<tr>
<td></td>
<td>❌ Metabolism</td>
<td>❌ Metabolism</td>
</tr>
<tr>
<td></td>
<td>❌ Resource Gathering</td>
<td>❌ Resource Gathering</td>
</tr>
<tr>
<td></td>
<td>✅ Gene Replication</td>
<td>✅ Gene Replication</td>
</tr>
</tbody>
</table>

Can replicate any 4 genes  Can replicate any 1 gene

Bacteria A’s genome contains Antibiotic Resistance, Omnitoxin Production, Metal Resistance and Gene Replication, which satisfies the entire list of required genes in Water, while Bacteria B only satisfies one out of the 4 required genes. Thus, Bacteria A is more fit and can replicate any four (4) genes while Bacteria B can only replicate any one (1) gene.

Alternative: In lieu of time constraints, the teacher may opt to disregard fitness requirements in this phase and standardize replication to three (3) genes.
Phase II: Gene exchange (RED CARDS)

Gene transfer only occurs between bacteria within the same location. Ignore this rule if locations are disregarded. Students gain or lose genes (exchange cards) as indicated in their Gene Transfer cards.

a. Conjugation – Any bacteria containing this gene can exchange one gene card with 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 on the Teacher’s Table
c. Phage transduction – Any bacteria holding this gene can:
   - pick a random gene from other bacteria except from those holding phage resistance.
   - Roll the Phage Dice on the Teacher’s Table

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 Table).
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)
Event Cards.

Skip Events in Round 1.

Event Phase takes effect beginning at Round 2. At the end of Phase III: Gene Conservation, 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.
Hunger Games: Pathogen Edition Reference Sheet

Shorthand Notation for Gene Cards

**Essential Genes - Phase I (BLUE)**
- ResG - Resource Gathering
- GenR - Gene Replication
- Met - Metabolism

**Gene Transfer - Phase II (RED)**
- Conj - Conjugation
- Tran - Transformation
- PTran - Phage Transduction
- PRes - Phage Resistance

**Gene Pool - Used in the Event Phase (YELLOW)**
- AntR - Antibiotic Resistance
- Omn - Omnitoxin
- AntO - Anti-Omnitoxin
- CSR - Cell Surface Remodelling
- SeSy - 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

Gene Shorthand Notation

**PHAGE TRANSDUCTION**
- Type: GENE TRANSFER
- Action: Take one (1) gene card from any bacteria
- OR Roll the 'Phage Dice'
- Use once per round: Keep card after use.

**Gene Name**

**Gene Type**

Indicates the phase when the card is used

Describes the action(s) allowed by this gene

AntR - Antibiotic Resistance

**ANTIBIOTIC RESISTANCE**
- Action: Resistance to antibiotics only
- Event: Antibiotics have been administered

Additional clauses on using this card

Indicates the event this card is used in
Required Genes:

- Gene Replication
- Antibiotic Resistance
- Metal Resistance
- Omnitoxin Production
Required Genes:

- Gene Replication
- Omnitoxin Production
- Metal Resistance
- Secretion System
Required Genes:

- Gene Replication
- Antibiotic Resistance
- Metal Resistance
- Omnitoxin Production
- Cell Surface Remodeling
- Adhesion Proteins
- Omnitoxin Resistance
- Secretion System
Required Genes:

- Gene Replication
- Omnitoxin Production
- Cell Surface Remodeling
- Adhesion Proteins
- Omnitoxin Resistance
- Secretion System
Hunger Games: Pathogen Edition

Bacterial Species: __________________________________________

Group Members: ______________________________________________

Locations:
Round 0: ________________
Round 1: ________________
Round 2: ________________
Round 3: ________________
Round 4: ________________

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