ViraPalooza
NIH Curriculum: ViraPalooza!
Author: Laura Busta

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Additional information regarding the Bench to Bedside project is available at http://www.cpet.ufl.edu/bench.

Please direct inquiries to the Center for Precollegiate Education and Training at cpet@cpet.ufl.edu.

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Author’s Note
Introduction
**Tips about this Curriculum**

**Lesson Plan Format:** All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

**KEY QUESTION(S):** Identifies key questions the lesson will explore.

**OVERALL TIME ESTIMATE:** Indicates total amount of time needed for the lesson, including advanced preparation.

**LEARNING STYLES:** Visual, auditory, and/or kinesthetic.

**VOCABULARY:** Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

**LESSON SUMMARY:** Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

**STUDENT LEARNING OBJECTIVES:** Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

**STANDARDS:** Specific state benchmarks addressed in the lesson. Also collected in one list.

**MATERIALS:** Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

**BACKGROUND INFORMATION:** Provides accurate, up-to-date information from reliable sources about the lesson topic.

**ADVANCE PREPARATION:** This section explains what needs to be done to get ready for the lesson.

**PROCEDURE WITH TIME ESTIMATES:** The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

**ASSESSMENT SUGGESTIONS:** Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.

**EXTENSIONS:** (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

**RESOURCES/REFERENCES:** This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.

**STUDENT PAGES:** Worksheets and handouts to be copied and distributed to the students.

**TEACHER PAGES:** Versions of the student pages with answers or the activity materials for preparation.

**Science Subject:** Zoology and Biology

**Grade and ability level:** High School Honors

**Science concepts:** virology, characteristics of living things, eukaryotic cells, prokaryotic cells, transmission, public health, classification, genetic material, capsid symmetry, ELISA test, antigen, antibody
Lesson Summaries

LESSON ONE: World Gone Viral
Pictures of living and nonliving things will be displayed throughout the room and students will go from picture to picture and define the thing as living or nonliving. The seven characteristics of a living thing will be reviewed and student answers will be discussed. Students will review the characteristics of eukaryotic and prokaryotic cells by reviewing cell structures using pictures and diagrams. Viruses will be further explained using a web quest in which students will visit different websites to answer questions about viruses. Students will construct a comparison diagram to compare viruses, prokaryotic and eukaryotic cells.

LESSON TWO: Project Virus
Students will be assigned a particular virus and will conduct web-based research on the virus. Students will then create posters with information on the virus and create models of their virus. A virus gallery will be created with the students’ posters and virus models; students will then view each other’s projects and answer basic questions about the viruses.

LESSON THREE: Classify your Viruses!!
There are two classification systems utilized to classify viruses students will explore and compare both systems in this lesson. As a whole class activity, students will use classification criteria to produce a viral classification chart according to the hierarchical classification system. The students will then use the Baltimore classification system to classify the virus they used for the virus and project.

LESSON FOUR: Retroviral Trees
Students will review the process of reverse transcription, transcription and translation using a lentivirus model. With the use of simulated nucleic acid sequences students will construct a phylogenetic tree/cladogram that depicts the relationship between HIV, SIV, FIV, BIV and EIA

LESSON FIVE: Strains and Lions and Cat Oh My!!
Students will read and discuss a case study dealing with FIV in domesticated cats and in lions and answer discussion questions. As part of the case study students will also do a mini web quest to understand ELISA (Enzyme Linked Immunosorbent Assay) testing and run a simulated ELISA test.
Vocabulary

Antibody: is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses

Antigen: is a substance that evokes the production of one or more antibodies

Biological Classification: a method of separating organisms into groups

BIV: bovine immunodeficiency virus

Branches: the lines in a phylogenetic tree that connect terminals to nodes or one node to another

Capsid: the shell of protein that protects the nucleic acid of a virus; it is composed of structural units, or capsomers.

Cladistics: is a method of classifying species of organisms into groups called clades, which consist of an ancestor organism and all its descendants

Cladogram: is a diagram used in cladistics, which shows relations among organisms

Common Ancestor: the most recent ancestral form of a species or entity from which two or more species or entities evolved

DNA virus: a virus that contains DNA as its genetic material

EIA: equine infectious anemia

ELISA Test: enzyme-linked immunosorbent assay; a sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein, especially an antigen or antibody.

Eukaryotic cells: characterized by having a distinct membrane bound nucleus

FIV: feline immunodeficiency virus

Hierarchical: a system of things arranged into ranks

HIV: human immunodeficiency virus

Lentivirus: is a genus of viruses of the Retroviridae family, characterized by a long incubation period

Lysogenic: is complementary to the lytic cycle for viral entry and reproduction within cells

Lytic: Infection of a bacterium by a bacteriophage with subsequent production of more phage particles and lysis, or dissolution, of the cell.

Nodes: points in a tree where branches intersect; they represent ancestors of all species, genes, or proteins that descend from them.

Phylogenetic Tree: a diagram that illustrates the evolutionary relationships among species, genes, or proteins. It is made of branches, nodes, terminals, and a root
**Prokaryotic cells**: an organism of the kingdom Monera (or Prokaryotae), comprising the bacteria and cyanobacteria, characterized by the absence of a distinct, membrane-bound nucleus or membrane-bound organelles, and by DNA that is not organized into chromosomes

**Replication**: the formation of biological viruses during the infection process in the target host cells

**Retrovirus**: is an RNA virus that is duplicated in a host cell using the reverse transcriptase enzyme to produce DNA from its RNA genome.

**RNA virus**: a virus that contains RNA as its genetic material

**SIV**: simian immunodeficiency virus

**Transmission**: the passage of a disease from one individual to another

**Vector**: a carrier that transfers an infective agent from one host to another

**Viral Envelope**: the outer structure that encloses the nucleocapsids of some viruses.

**Virology**: the scientific study of viruses and the diseases they cause

**Virus**: microscopic infectious agents; packets of RNA or DNA that infect cells of other organisms
### Next Generation Sunshine State Standards – Science

<table>
<thead>
<tr>
<th>Benchmark</th>
<th>Lesson</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC.912.L.14.2</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.14.3</td>
<td></td>
</tr>
<tr>
<td>SC.912.L.15.4</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.15.5</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.16.7</td>
<td>X</td>
</tr>
<tr>
<td>Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.</td>
<td></td>
</tr>
<tr>
<td>SC.912.N.1.4</td>
<td>X</td>
</tr>
<tr>
<td>Identify sources of information and assess their reliability according to the strict standards of scientific investigation.</td>
<td></td>
</tr>
<tr>
<td>SC.912.N.1.6</td>
<td>X</td>
</tr>
<tr>
<td>Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.</td>
<td></td>
</tr>
<tr>
<td>SC.912.N.3.5</td>
<td>X</td>
</tr>
<tr>
<td>Describe the function of models in science, and identify the wide range of models used in science.</td>
<td></td>
</tr>
</tbody>
</table>
Background Information
LESSON ONE: World gone Viral

KEY QUESTIONS: What is a virus? Is a virus considered living? What are the similarities and differences between viruses, eukaryotic cells and prokaryotic cells?

KEY SCIENCE CONCEPTS: virology, characteristics of living things, eukaryotic cells, prokaryotic cells

OVERALL TIME ESTIMATE: 2 - 45-minute class periods

LEARNING STYLES: Visual, auditory and kinesthetic

VOCABULARY:
Eukaryotic cells: characterized by having a distinct membrane bound nucleus

Lysogenic: is complementary to the lytic cycle for viral entry and reproduction within cells

Lytic: Infection of a bacterium by a bacteriophage with subsequent production of more phage particles and lysis, or dissolution, of the cell.

Prokaryotic cells: an organism of the kingdom Monera (or Prokaryotae), comprising the bacteria and cyanobacteria, characterized by the absence of a distinct, membrane-bound nucleus or membrane-bound organelles, and by DNA that is not organized into chromosomes

Replication: the formation of biological viruses during the infection process in the target host cells

Virus: microscopic infectious agents; packets of RNA or DNA that infect cells of other organisms

Virology: the scientific study of viruses and the diseases they cause

LESSON SUMMARY: Pictures of living and nonliving things will be displayed throughout the room and students will go from picture to picture and define the thing as living or nonliving. The seven characteristics of a living thing will be reviewed and student answers will be discussed. Students will review the characteristics of eukaryotic and prokaryotic cells by reviewing cell structures using pictures and diagrams. Viruses will be further explained using a web quest in which students will visit different websites to answer questions about viruses. Students will construct a comparison diagram to compare viruses, prokaryotic and eukaryotic cells.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Explain the seven characteristics of living thing.
2. Compare and contrast prokaryotic cells, eukaryotic cells and viruses.
3. Explain the basic anatomy of a virus, how a virus replicates and how viruses affect cells.

STANDARDS:
SC.912.L.14.2
SC.912.L.14.3
SC.912.L.16.7

MATERIALS:
• 10 pictures of living and nonliving things (be sure to include: bacteria, viruses, and eukaryotic cells)
• One computer per student (for the web quest)
• One copy of the “Viruses” web quest and “Comparing Prokaryotic and Eukaryotic Cells” worksheet per student
• One microscope for every two students
• Prepared plant cell, animal cell and bacteria slides one set per microscope or one set per two students.

BACKGROUND INFORMATION: High school biology teaches that there are seven basic characteristics of a living thing and by applying that principle students can differentiate between living and nonliving things. The seven characteristics of a living thing are: that they are made up of at least one cell, they have different levels of organization, are able to obtain and use energy, grow and develop, reproduce, respond to their environment, and adapt to their environment. Viruses are classified as non-living even though they meet most of the criteria that defines a living thing but students must understand to be considered living, a thing must display all of the characteristics.

Eukaryotic cells are defined as either animal or plant cells that contain different types of organelles that work together to carry out all the functions needed to maintain life. Eukaryotic cells are defined by the way they store their genetic material - a membrane bound nucleus (center of the cell). Prokaryotic cells are basic cells and are classified as bacteria or cyanobacteria. Prokaryotic cells lack a membrane bound nucleus and their genetic material floats freely. Prokaryotes lack many of the organelles that eukaryotes possess and instead of containing chromosomal DNA as genetic material they contain a plasmid. A plasmid is a circular strand of genetic material.

Viruses are microscopic infectious agents; packets of RNA or DNA that infect cells of other organisms. Viruses infect eukaryotic cells and bacteriophages infect prokaryotic cells. Viruses infect cells because they do not have the biochemical machinery to reproduce on their own. Viruses have the ability to take over their host's system in order to make copies of its self and once they are done using the cell’s machinery they destroy the cell.

Viruses use lytic or lysogenic cycles to reproduce in the host cell. The lysogenic cycle occurs first, new genetic material (a prophage) is formed due to the coalescence between the nucleic acid in the bacteriophage and the host genome; the new genetic material is then transmitted to the other daughter cells. The lysogenic cycle also occurs in eukaryotic cells but is not fully understood. During the lytic cycle the virus will attach to the outside of the host cell and inject its genetic material; the newly injected genetic material tells the host cell to replicate the virus parts; the cell then assembles the virus parts into progeny and then the progeny lyse the cell and infect other cells.

ADVANCE PREPARATION:
Day 1
Before class:
1. Make sure to have 10 pictures of living and nonliving things; be sure to include bacteria, viruses and cells.
2. Number the pictures 1-10 and place throughout the classroom.
3. Set up microscope stations with prepared slides of plant cells, animal cells, and bacteria; one station for every two students; each station should include one microscope and all three slides.

Day 2
Before class:
1. Make sure that all websites can be accessed at your school.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES
Day 1
During class:
1. (~2 minutes) Have students come into the classroom and instruct them to take out of sheet of paper and to number it 1-10.
2. (~10 minutes) Divide the students into ten groups and explain to them that they will be rotating to every picture and deciding if the picture they are looking at is a living or nonliving thing. They must write “living” or “nonliving” next to the number on their paper that corresponds to the number of the picture they are looking at and let them know they will have one minute at each poster.

3. Have the students return to their seats with their answer paper.

4. Go to each picture and explain to the students what the picture is and poll the class to see how many thought it was a living thing and how many thought it was nonliving.

5. (~10 minutes) Explain the seven characteristics of a living thing to the class and have them write them down on their papers for later reference.

The seven characteristics of a living thing are:

1. that they are made up of at least one cell,
2. have different levels of organization
3. are able to obtain and use energy,
4. grow and develop,
5. reproduce,
6. respond to their environment,
7. adapt to their environment.

6. Handout worksheet “Comparing prokaryotic and eukaryotic cells” one per student.

7. (~15 minutes) Have students go to microscope stations and instruct them to read the instructions and questions on the worksheet and use the microscope and slides to answer the questions.

8. Collect worksheet “Comparing Prokaryotic and Eukaryotic Cells” as the students are dismissed.

Day 2
During class:

1. Explain to students that they will be completing a web quest today and they must follow the order in which the web quest is written. Take them to the computer lab or make sure that students have a computer with Internet access.

2. (~35 minutes) Handout the web quest and have the students work independently to complete the web quest.

3. Have students draw a Venn diagram to compare and contrast viruses, prokaryotes and eukaryotes. If there is not sufficient class time assign as homework.

ASSESSMENT SUGGESTIONS:

<table>
<thead>
<tr>
<th>Learning Objective</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explain the seven characteristics of living thing.</td>
<td>Give students a quick assessment on day 2 and have them either list or explain each of the seven characteristics</td>
</tr>
<tr>
<td>Compare and contrast prokaryotic cells, eukaryotic cells and viruses.</td>
<td>Use a Venn diagram or other organizer to compare and contrast prokaryotes, eukaryotes and viruses.</td>
</tr>
<tr>
<td>Explain the basic anatomy of a virus, how a virus replicates and how viruses affect cells.</td>
<td>The web quest will cover all this information in detail. Assess with the following questions: 1.</td>
</tr>
<tr>
<td>Name and explain the three shapes that viruses can be? 2. Explain the lytic and lysogenic cycles?</td>
<td></td>
</tr>
</tbody>
</table>

**RESOURCES/REFERENCES:**

- Characteristics of Living Things: [http://infohost.nmt.edu/~klathrop/7characterisitcs_of_life.htm](http://infohost.nmt.edu/~klathrop/7characterisitcs_of_life.htm)
The world of cells is divided into two main designations prokaryotes and eukaryotes.

**Prokaryotes**
Prokaryotes are simple cells and are usually unicellular organisms; they lack a nucleus to protect their genetic information and lack any membrane-encased organelles. Prokaryotes are either bacteria or archea and their DNA is a single supercoiled circular chromosome.

1. Looking at the graphic above, what structure does a prokaryote utilize for movement?
   *Flagellum*

2. Find and list two structures that are in both prokaryote and eukaryotic cells.
   *Cytoplasm and cell membrane*
3. Look at the slide labeled prokaryote under the light microscope. First under low magnification and then with high magnification and draw the cells in the area provided below label the magnification used underneath the drawing.

Low magnification:___________________ High magnification:___________________

Answers and drawings will vary

**Eukaryotes**
Eukaryotic cells have a membrane bound nucleus; where they store their DNA. Eukaryotic cells are further divided into designations: plant and animal cells. They contain many organelles that carry out specific functions and are usually enclosed in their own membrane within the cell.

1. Looking at the picture above, what cellular components do plant cells contain that animal cells lack?
   *Chloroplasts and cell wall, large central vacuole*

2. How do plant cells get their energy? How do animal cells get their energy?
   *Plants = sun
   Animal = through the food consumed by the animal*

3. Look at the slide labeled plant cell under the light microscope. Draw and label what you see in the area provided; switch from low to high power.

   **Low magnification:**________________  **High magnification:**________________

4. Look at the slide labeled animal cell under the light microscope. Draw and label what you see in the area provided; switch from low to high power.

   **Low magnification:**________________  **High magnification:**________________

5. Compare the sizes of prokaryotic, plant and animal cells; place them in order from smallest to largest.
   *Prokaryotic – animal - plant*
6. Draw a Venn diagram to compare prokaryotic, plant and animal cells. Look at the pictures included and utilize your textbook for additional information.
Go to the websites listed and read the information provided. Answer the questions in a clear and concise way.

1. Go to the following website: http://www.odec.ca/projects/2004/lijja4j0/public_html/disease.htm and click on the link that reads “What is a Virus?” read the information and answer the following questions:
   - What does the word “virus” mean? 
     Poisonous fluid in Latin
   - Place the following in order from smallest to largest: animal cell, virus, plant cell, bacteria and viroid. 
     Viroid-virus-bacteria-animal cell- plant cell
   - What type of microscope is used to see viruses? 
     Electron microscopes
   - Are viruses considered living or non-living? Explain why. 
     Non-living because they utilize hosts to replicate and cannot reproduce on their own.
   - What is a host and why do viruses need them? 
     A host is an organism that a virus infects a plant, animal, bacteria or human. Viruses need hosts in order to survive
   - Describe the types of genetic information a virus can contain. 
     DNA and RNA double or single stranded.
   - Describe the capsid and what it is made of? 
     The capsid is a protective shell made of proteins.

2. Click on the link (on the left hand side of the site) “Viruses can cure Disease” and read the different ways a virus can help cure diseases instead of causing disease.
   - How can the virus that causes AIDS (HIV) be used as a cure? 
     HIV can help cure a disease if used as a carrier to insert good genes into people who have diseases such as hemophilia and Alzheimer’s.
   - Explain how Oncolytic viruses function.
They seek to penetrate a host cell, “trick” it into replicating more viruses until the host cell bursts. Oncolytic viruses are viruses that only seek, infect, and replicate in cancer cells.

- How can the virus “Respiratory Enteric Orphan” help patients with brain cancer? *The virus will destroy cancerous brain cells but not effect healthy brain cells.*

3. Go to the following website and read [http://evolution.berkeley.edu/evolibrary/news/071201_adenovirus](http://evolution.berkeley.edu/evolibrary/news/071201_adenovirus) and answer the following questions based on the information provided:

- Fill in the following chart about viral mode of transmission:

<table>
<thead>
<tr>
<th>Mode of Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet Transmission</td>
</tr>
<tr>
<td>Airborne Transmission</td>
</tr>
<tr>
<td>Vector Transmission</td>
</tr>
<tr>
<td>Waterborne Transmission</td>
</tr>
<tr>
<td>Sit and Wait Transmission</td>
</tr>
</tbody>
</table>

- Explain how virulence and transmission affects evolution in viruses? *There is a trade-off between virulence and transmission; a virus that has a high level of virulence may not be transmitted because the virus may kill off the host before it can infect a new host. Viruses are ideally virulent enough to produce many offspring (that are likely to be able to infect a new host if the opportunity arises) but not so virulent that they prevent the current host from presenting them with opportunities for transmission.*

- Why is cholera free to evolve a high level of virulence? *Cholera victims are soon immobilized by the disease, but they are tended by others who carry away their waste, clean their soiled clothes, and, in the process, transmit the bacterium to a water supply where it can be ingested by new hosts.*
In this way, even virulent cholera strains that strike down a host immediately can easily be transmitted to a new host. Accordingly, cholera has evolved a high level of virulence and may kill its host just a few hours after symptoms begin.

4. Go to [http://biology.about.com/od/virology/ss/Virus-Replication.htm](http://biology.about.com/od/virology/ss/Virus-Replication.htm) and read about virus replication to help you answer the following questions.

- Explain the difference between double-stranded DNA and single-stranded RNA in respect to viral replication. *Double-stranded DNA viruses typically must enter the host cell’s nucleus before they can replicate. Single-stranded RNA viruses however, replicate mainly in the host cell’s cytoplasm.*

- How does the “lock and key” mechanism function and limit the host range? *Certain proteins on the virus particle must fit certain receptor sites on the particular host's cell surface and if the virus particles do not fit the receptor sites on the cell the virus will not be able to invade that host. The host range is partially dictated by the “lock and key” mechanism.*

- Explain the six main steps of virus reproduction: label and explain the step and sketch a picture of the step.
5. Go to “you tube” and watch the video “Flu Attack! How A Virus Invades Your Body” and explain how one virus can start a viral infection in a host.
LESSON TWO: Project Virus

KEY QUESTIONS: What are the different structures in a virus? How are viruses transmitted? What are the signs and symptoms of viral infection? How are viruses treated and prevented?

KEY SCIENCE CONCEPTS: virology, transmission, public health

OVERALL TIME ESTIMATE: 3 - 45 minute class periods

LEARNING STYLES: visual and kinesthetic

VOCABULARY:
DNA virus: a virus that contains DNA as its genetic material
RNA virus: a virus that contains RNA as its genetic material
Transmission: the passage of a disease from one individual to another
Vector: a carrier that transfers an infective agent from one host to another

LESSON SUMMARY: Students will be assigned a particular virus and will conduct web-based research on the virus. Students will then create posters with information on the virus and create models of their virus. A virus gallery will be created with the students’ posters and virus models; students will then view each other’s projects and answer basic questions about the viruses.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Explain different modes of virus transmission.
2. Produce a viral model.
3. Conduct research and present findings to peers.

Standards:
SC.912.N.1.4
SC.912.N.3.5

MATERIALS:
- One computer with Internet access per student or group
- “Guided Virus Research” worksheet one copy per student or group
- “Virus Project” rubric one copy per student or group
- Camera- to take pictures of virus models for lesson 3 activity
- “Virus Gallery” data collection chart one per student

BACKGROUND INFORMATION: There are twenty-one virus families and within each family there can be many subfamilies, genus, and species. Picking out which viruses to have students conduct research on can be a very tedious and time consuming process. The table below was created to help in that process. The chosen diseases are ones that students can readily conduct basic research on and that are geared towards a zoology curriculum. For the purpose of this lesson all diseases being studied are ones with animal or human hosts.
<table>
<thead>
<tr>
<th>Virus Family</th>
<th>RNA or DNA</th>
<th>Diseases caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>reovirus</td>
<td>RNA</td>
<td>canine rotavirus</td>
</tr>
<tr>
<td>birnavirus</td>
<td>RNA</td>
<td>infectious bursal disease</td>
</tr>
<tr>
<td>calcivirus</td>
<td>RNA</td>
<td>feline calcivirus</td>
</tr>
<tr>
<td>picorna</td>
<td>RNA</td>
<td>foot &amp;mouth; polio; meningitis</td>
</tr>
<tr>
<td>flavivirus</td>
<td>RNA</td>
<td>west nile; dengue; yellow fever</td>
</tr>
<tr>
<td>togavirus</td>
<td>RNA</td>
<td>eastern equine encephalitis; rubella</td>
</tr>
<tr>
<td>retrovirus</td>
<td>RNA</td>
<td>FIV; SIV; EIA; HIV; BIV</td>
</tr>
<tr>
<td>coronavirus</td>
<td>RNA</td>
<td>canine coronavirus; feline coronavirus; SARS</td>
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<tr>
<td>filovirus</td>
<td>RNA</td>
<td>ebola</td>
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<tr>
<td>rhabdovirus</td>
<td>RNA</td>
<td>rabies</td>
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<td>bunyavirus</td>
<td>RNA</td>
<td>Crimean-Congo hemorrhagic fever; Rift Valley fever</td>
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<td>RNA</td>
<td>influenza</td>
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<td>canine distemper; mumps</td>
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<td>RNA</td>
<td>lassa fever</td>
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<td>parovirus</td>
<td>DNA</td>
<td>canine parvovirus; fifth disease</td>
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<tr>
<td>papovavirus</td>
<td>DNA</td>
<td>human papillomavirus</td>
</tr>
<tr>
<td>adenovirus</td>
<td>DNA</td>
<td>pneumonia; gastroenteritis</td>
</tr>
<tr>
<td>hepadnavirus</td>
<td>DNA</td>
<td>hepatitis B</td>
</tr>
<tr>
<td>herpesvirus</td>
<td>DNA</td>
<td>herpes simian virus B; herpes simplex virus</td>
</tr>
<tr>
<td>iridovirus</td>
<td>DNA</td>
<td>ranavirus</td>
</tr>
<tr>
<td>baculovirus</td>
<td>DNA</td>
<td>nuclear polyhedrosis virus</td>
</tr>
<tr>
<td>poxvirus</td>
<td>DNA</td>
<td>cowpox; monkeypox; smallpox</td>
</tr>
</tbody>
</table>

ADVANCE PREPARATION

Day 1

Before class:
1. Decide if you would like to make this activity a group project or a solo project; group projects usually require more in class time and solo projects can be assigned as take-home projects.
2. Select the viruses you would like to cover based on the number of students you have in the class; try to assign one virus from each family (so that every viral family is represented in lesson 3) but if that is not possible make sure to assign at least one virus from the family retrovirus.
3. Make sure to have copies of the “Guided Virus Research” worksheet, one per student or group.

Day 3

Before class:
1. Make copies of the “Virus Gallery” data collection worksheet, one per student.
2. Make sure you have enough room to hang up all the posters and display the virus models; if not, consider having the RNA viruses set up first and then the DNA viruses.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES

Day 1

During class:
1. Assign each student or student group one of the viruses and handout the “Guided Virus Research” worksheet.
2. (~30 minutes) Provide each student or student group with a computer with internet access and have them conduct their research using the “Guided Virus Research” worksheet.

3. Give each student or student group the rubric for the “Virus Project” and allow students to brainstorm on how they will make their poster board and model. Instruct the student to bring all the materials they will need for the poster board and virus model the following day (poster board, markers, glue, beads, pipe cleaners, etc.)

Day 2
During class:
1. (~45 minutes) Allow students to work on poster boards and virus models; make yourself available to answer student questions and to give advice.

2. At the end of the class period, instruct students to take home their projects and complete them overnight. If working in groups encourage students to get together afterschool or divide up the remainder of the project.

Day 3
During class:
1. (~5 minutes) Instruct students to hang up their virus poster and display their virus models.

2. (~42 minutes) Handout the data collection page and have half of the students present their posters as the other half rotates around the “virus gallery”

3. Use the camera to take pictures of the student’s virus projects because they will be useful for lesson 3. If this is not possible have the students use a picture from their poster board.

ASSESSMENT SUGGESTIONS:

<table>
<thead>
<tr>
<th>Lesson Objective</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explain different modes of virus transmission.</td>
<td>Have students explain the following: 1. feco-oral transmission 2. Vector transmission 3. Airborne transmission 4. Bodily excretion transmission</td>
</tr>
<tr>
<td>Produce a viral model.</td>
<td>Assess the quality of the models using the provided rubric</td>
</tr>
<tr>
<td>Conduct research and present findings to peers.</td>
<td>Collect and assess poster boards and “Guided Virus research” worksheet. Poster rounds in which half of the class rotates around the “virus gallery” and the other half presents their virus posters to those rotating.</td>
</tr>
</tbody>
</table>

RESOURCES/REFERENCES:

- Canine Rotavirus (reovirus)
- Infectious Bursal Disease (Birnavirus)
  [http://www.worldpoultry.net/diseases/gumboro-d99.html](http://www.worldpoultry.net/diseases/gumboro-d99.html)
- Feline Calicivirus (Calicivirus)
- Foot and Mouth Disease (Picornavirus)
Polio (Picornavirus)

Viral Meningitis (Picornavirus)
http://www.cdc.gov/meningitis/viral.html

Togviruses

Feline Coronavirus

Canine Coronavirus
http://bakerinstitute.vet.cornell.edu/animalhealth/page.php?id=1087

SARS (Coronavirus)

Filoviruses
http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/filoviruses.htm

Influenza Virus (Orthomyxovirus)
http://www.ncbi.nlm.nih.gov/books/NBK8611/

Canine Distemper (Paramyxovirus)
http://www.vetmed.auburn.edu/canine_distemper_virus3

 Arenaviruses
http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/arena.htm

Papoaviruses

Adenovirus
http://virus.stanford.edu/adeno/adeno.html

Hepadnavirus

Herpes Simian Virus B
http://www.cdc.gov/herpesbvirus/index.html

Ranavirus (Iridovirus)
http://www.nwhc.usgs.gov/disease_information/other_diseases/ranavirus.jsp

Baculovirus
http://www.inhs.uiuc.edu/research/biocontrol/pathogens/typesofpathogens/baculoviruses.html#npv

Baculovirus
http://www.biocontrol.entomology.cornell.edu/pathogens/baculoviruses.html

Poxvirus
http://emedicine.medscape.com/article/226239-overview
Today, you will be conducting some Internet based research on your assigned virus. It is important that you try to get all the information you will need for both your virus model and your poster board.

**Basic Information:**
1. What family does your virus belong to?

2. What type of genetic material does your virus utilize? Be specific (single or double stranded).
   - Does it utilize transcription or reverse transcription?
   - If it is RNA single stranded, is considered a (+) or (-) strand?

3. What type of capsid does your virus have?

4. Is your virus a naked or enveloped?

**Effects of Virus:**
5. What disease does your virus cause?

6. What is the host animal for your virus?

7. Explain how your virus is transmitted to the host animal.

8. Is there a vector species that transmits your virus? List the species.
9. Explain how this virus affects the host animal’s health.

**Diagnosis and Treatment:**
10. How is this viral infection diagnosed?

11. What is the treatment for this viral disease?

12. How can this viral disease be prevented?

13. Is there a vaccine for this viral infection?

**Project Planning:**
14. Draw a sketch of your virus.

15. Brainstorm: How could you make a model of your virus? What materials do you need?

16. Find pictures for your poster board and save the web links to a word document and email yourself the document or print them out (if a printer is available). Pictures to find: the virus, the host species infected, any treatments or vaccines, vector species, life cycle of the virus.

17. Brainstorm; how will I incorporate all the information and pictures onto my poster board? Sketch the layout below.
Virus Project Rubric

NAME: ______________________
DATE: ______________________

Utilize this rubric to guide your poster board and model making.

**Poster Board:** (2 grades)

1. Information needed
   • Name of virus and disease; include the family it belongs to (5pts)
   • Explain transmission method and include vector species (15pts)
   • Type of genetic material virus contains (5pts)
   • Affects on host species (15pts)
   • Diagnosis, treatment and prevention of viral infection (15pts)
   • Shape of capsid and if it is enveloped or naked (5pts)

2. Poster pictures needed
   • Picture of virus (5pts)
   • Picture of host species and vector (if applicable) (5pts)
   • Lifecycle of virus (25pts)
   • Pictures of vaccines, treatments or test used to diagnose (if applicable) (5pts)

   Grade: _______________________/100

**Virus Model:** (2 grades)

Criteria used to grade model:
   • Model resembles pictures of virus (45pts)
   • Creative use of materials (10pts)
   • Model must be able to stand or hang (20pts)

   Grade: _______________________/75
Student Pages: Virus Gallery Data Collection Sheet

NAME: ______________________
DATE: ______________________

Fill out this collection sheet for every peer’s virus project you visit.

Name of Virus: ______________________

Host species: ______________________

General affects on host: ______________________

RNA or DNA virus: ______________________

Prevention: ______________________

Treatment: ______________________

Name of Virus: ______________________

Host species: ______________________

General affects on host: ______________________

RNA or DNA virus: ______________________

Prevention: ______________________

Treatment: ______________________

Name of Virus: ______________________

Host species: ______________________

General affects on host: ______________________

RNA or DNA virus: ______________________

Prevention: ______________________

Treatment: ______________________
LESSON THREE: Classify your Viruses!!

KEY QUESTION(S): How are viruses classified? What is the Baltimore classification system?

KEY SCIENCE CONCEPTS: viruses, classification, genetic material, capsid symmetry

OVERALL TIME ESTIMATE: 2 - 45 minute class periods

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY:
Biological Classification: a method of separating organisms into groups
Hierarchical: a system of things arranged into ranks
Capsid: the shell of protein that protects the nucleic acid of a virus; it is composed of structural units, or capsomers.
Viral Envelope: the outer structure that encloses the nucleocapsids of some viruses.

LESSON SUMMARY: There are two classification systems utilized to classify viruses students will explore and compare both systems in this lesson. As a whole class activity, students will use classification criteria to produce a viral classification chart according to the hierarchical classification system. The students will then use the Baltimore classification system to classify the virus they used for the virus and project.

STUDENT LEARNING OBJECTIVES
The student will be able to...
1. Utilize and explain the Baltimore virus classification system.
2. Classify virus using the hierarchical system of virus classification.
3. Compare and contrast the Baltimore and hierarchical systems of virus classification

STANDARDS:
SC.912.L.15.4
SC.912.L.15.5
SC.912.L.16.7
SC.912.N.1.6

MATERIALS:
- Peel and stick magnets or putty/ tape
- Pictures of student virus models taken from lesson 2
- “Baltimore Classification System Chart” worksheet one per student

BACKGROUND INFORMATION: Hierarchical classification of viruses is based general viral characteristics: genetic material that the virus contains, the structure/ symmetry of the capsid and whether the viruses in enveloped or naked. The virus will either have RNA (double or single stranded) or DNA (double or single stranded) as its nucleic acid. A viral envelope is a structure composed of lipids, proteins and carbohydrates and lies on the outside of the capsid and a naked virus lacks an envelope. There are three basic capsid structures: icosahedral, helical and complex. The viral capsid is a protein coat that gives the virus its form.

The Baltimore system of virus classification is based on the various mechanisms of viral genome replication. This system will lead the students to not only understand how viruses are classified but also to understand how viruses replicate their genome once inside the host cell. The mechanism that a virus utilizes to
copy its genome is family specific and therefore fits into the hierarchical classification of viruses. According to this system, there are seven types of genetic material present in the virus. The genetic material in the virus must be transcribed into mRNA and from mRNA translated into proteins. The following chart is a visual representation of the seven classes.

![Diagram of genetic material present in the virion]

This graphic was sourced from [http://viralzone.expasy.org/all_by_species/254.html](http://viralzone.expasy.org/all_by_species/254.html).

**ADVANCE PREPARATION**

*Before class:*
1. Print out pictures of student virus models taken during lesson
2. Add magnets (or putty or tape if you are not using a magnet surface) to each picture.

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

*During class:*
1. (5 minutes) As students walk into the class hand them their virus model picture and ask them to take a seat.
2. Explain to students that today they will be making a system to classify all the virus models.
3. (30 minutes – steps 3-15) Ask the students “How could we divide all these viruses into two groups?” allow students to come up with an answer and use prompting techniques (such as asking “Think of a characteristic that all viruses have and that there are only two different types.” …Etc.) to help them to get to the answer DNA and RNA.
4. Write DNA on the right side of the board and RNA on the left side of the board.

5. Instruct the students that have RNA viruses to sit on the left side of the room and those with DNA viruses to sit on the left side of the room.

6. Ask the students “How can we further divide these two groups?” allow students to come up with an answer and use prompting (such as “What characteristic can be used that has different character states?”) to help them to get to the answer capsid symmetry/shape.

7. On the board under the word RNA draw a line and then write icosahedral then draw another line originating from the word RNA and write helical.

8. On the board under the word DNA draw three lines and write icosahedral at the end of one line, helical at the end of the next line and complex at the end of the third line.

9. Have the students sitting on the right side of the class divide into two groups- icosahedral and helical (based on their virus).

10. Have the students sitting on the left side of the class divide into three groups- icosahedral, helical and complex (based on their virus).
11. Ask the students “how can we further divide these groups?” allow students to come up with an answer and use prompting such as to help them to get to the answer enveloped and naked.

12. Add the following labels and lines so the board looks like the following:

13. Have the students on the right side of the room that have identified their virus as icosahedral to further divide into two groups: naked and enveloped. The students that have identified their virus as helical do not further divide since all helical viruses are enveloped.

14. The students sitting on the left side of the class that have identified their virus as icosahedral will further divide into two groups naked and enveloped. The ones that identified their virus as helical and complex do not need to further divide since both groups only contain viruses that are enveloped.

15. Have the students place their viruses on the board under the correct heading.

16. (10 minutes) Handout the “Baltimore Classification System Chart” worksheet and have the students read and complete the worksheet for the remainder of the class period and if needed for homework.

**ASSESSMENT SUGGESTIONS:**

<table>
<thead>
<tr>
<th>Learning objective</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utilize and explain the Baltimore virus classification system. Compare and contrast the Baltimore and hierarchical systems of virus classification</td>
<td>Have students classify the virus they used in the virus project utilizing the Baltimore system.</td>
</tr>
<tr>
<td>Classify virus using the hierarchical system of virus classification.</td>
<td>Have students complete the “Hierarchical Virus Classification System” worksheet and collect and grade.</td>
</tr>
</tbody>
</table>

**RESOURCES/REFERENCES:**

- Baltimore Viral Classification  
- Virus Classification  
  [http://www.nlm.ch/Virologytutorials/Classification.htm](http://www.nlm.ch/Virologytutorials/Classification.htm)
- Replication and Baltimore Classification  
  [http://virology-online.com/general/Replication.htm](http://virology-online.com/general/Replication.htm)
Student Pages: Baltimore Virus Classification System

NAME: __________________________
DATE: __________________________

David Baltimore is an American biologist that proposed the Baltimore classification system of viruses. This system groups viruses into one of seven classes depending on their type of genome (DNA, RNA, single-stranded (ss), double-stranded (ds), etc.) and their method of replication. The Baltimore system is based on relationship between viral genetic material and mRNA.

In order to classify your virus according to the Baltimore classification system you will need to know the form and type of the genetic material in your virus (ex. Double stranded DNA) and the way that the genetic material arrives to the mRNA. Utilize question #2 on worksheet “Guided Virus Research” to aid you in using the following dichotomous key.

_Baltimore Virus Classification Dichotomous Key:_

1. Is your virus a DNA virus?.................................................................Yes go to #2 / No go to #5
2. Is the DNA single stranded?.........................................................Yes go to #3/ No go to #4
3. This virus is class I in the Baltimore system
4. This virus is class II in the Baltimore system
5. Is the RNA double stranded?..........................................................Yes go to #6/ No go to #7
6. This virus is class III in the Baltimore system
7. Does the virus use reverse transcription?.................................Yes go to #8/ No go to #9
8. This virus is class VI in the Baltimore system
9. Is the RNA a (+) strand?.................................................................Yes go to #10/ No go to #11
10. This virus is class IV in the Baltimore system
11. This virus is class V in the Baltimore system

You have learned the family name of your virus when we used the “Hierarchical System of Virus Classification” and now you should have learned what class your virus belongs to according to the “Baltimore System of Virus Classification”. Now you will put the two together fill out the chart by placing the names of the families that belong in each of the
Baltimore classes. Work with your classmates to get their virus family name and Baltimore class.

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
<th>Class V</th>
<th>Class VI</th>
</tr>
</thead>
</table>

Questions:

1. Which system gives more information about the virus structure?

2. Which system gives more information about how the virus functions?

3. Which system do you think will be the most useful when studying viruses explain your answer.
LESSON FOUR: Retroviral Trees

KEY QUESTIONS: What is a lentivirus? How do lentiviruses make proteins? What is a phylogenetic tree? How do you construct and interpret a phylogenetic tree? How are all lentiviruses related?

KEY SCIENCE CONCEPTS: virology, taxonomy, phylogenetic trees

OVERALL TIME ESTIMATE: 2 - 45 minute class periods

VOCABULARY:
BIV: bovine immunodeficiency virus

Branches: the lines in a phylogenetic tree that connect terminals to nodes or one node to another

Cladistics: is a method of classifying species of organisms into groups called clades, which consist of an ancestor organism and all its descendants

Cladogram: is a diagram used in cladistics, which shows relations among organisms

Common Ancestor: the most recent ancestral form of a species or entity from which two or more species or entities evolved

EIA: equine infectious anemia

FIV: feline immunodeficiency virus

HIV: human immunodeficiency virus

Lentivirus: is a genus of viruses of the Retroviridae family, characterized by a long incubation period

Nodes: points in a tree where branches intersect; they the represent ancestors of all species, genes, or proteins that descend from them.

Phylogenetic Tree: a diagram that illustrates the evolutionary relationships among species, genes, or proteins. It is made of branches, nodes, terminals, and a root

Retrovirus: is an RNA virus that is duplicated in a host cell using the reverse transcriptase enzyme to produce DNA from its RNA genome.

SIV: simian immunodeficiency virus

LESSON SUMMARY: Students will review the process of reverse transcription, transcription and translation using a lentivirus model. With the use of simulated nucleic acid sequences students will construct a phylogenetic tree/cladogram that depicts the relationship between HIV, SIV, FIV, BIV and EIA.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Construct and interpret a phylogenetic tree of select lentiviruses.
2. Transcribe and translate a DNA sequence.

Standards:
SC.912.L.16.7
MATERIALS:

- “Constructing a Phylogenetic Tree” activity packet - one per group of five students
- Rulers and scissors, one of each per group of five students
- Computer lab with a computer for every student with access to YouTube or a LCD projector with internet access
- “DNA, Transcription and Translation” worksheet with questions

BACKGROUND INFORMATION: Retroviruses have been around for thousands of years in a variety of vertebrate hosts. Recently, through the emergence of HIV into the human population, these viruses have gained popularity and research scientists have begun studying them in depth. Viruses that do not commonly affect humans are often overlooked in high school sciences and this is a disadvantage to our students since many emerging pathogens have evolved from strains that commonly affect other animals.

Phylogenetic trees or evolutionary trees are branching diagrams or "trees" showing the inferred evolutionary relationships among various biological species or other entities based upon similarities and differences in their physical and/or genetic characteristics. A phylogenetic tree is useful because it helps students understand the relationships between organisms. Constructing a (molecular based) phylogenetic tree is a process in which genetic material is being compared and the basic assumption is that the greater the amount of similarities between the genetic sequence of two species, the closer the relation. The phylogenetic tree of lentiviruses is a complex tree that shows the relationship between lentiviruses and between their hosts. Note that the close relationship between host species translates to close relationship in corresponding lentivirus.
In this lesson students will be using lentiviruses, RNA viruses that use reverse transcription, to construct a phylogenetic tree based on similarities of amino acid sequences. Students will need to understand the process of reverse transcription, transcription, and translation prior to the start of the activity.

Reverse transcription is a process utilized by RNA viruses to produce cDNA (which is a DNA strand that is complimentary to the viral RNA); it is the opposite of transcription. Transcription is the process in which DNA makes a copy of its sequence using RNA nucleic acid bases (A,U,G,C) the product of this process is termed mRNA. The mRNA then exits the nucleus (where the DNA is stored in the eukaryotic cell) and goes to the ribosome to translate (thus termed “translation”) the sequence into amino acids that will make proteins (every three nucleic acids codes for one amino acid; many amino acids produce a protein). When a virus takes over this cellular mechanism, the mechanism functions to produce viral proteins that will be used to produce progeny viruses. The graphic below depicts the process of a retrovirus invading a eukaryotic cell and utilizing its “machinery” to produce progeny.


ADVANCE PREPARATION

Day 1

Before class.

1. Decide if you would like to review DNA, transcription and translation as an in class activity or as a homework assignment.
2. If you choose to make it a homework assignment please note that students will need access to a computer with Internet access and the ability to watch a “youtube” video.
3. If you choose to review in class you can have the students do the review as a whole class activity in which you project the “youtube” video and then give them the worksheet “DNA Transcription
and Translation”. If you have access to a computer lab with Internet access and “youtube” playing capabilities then you can make it an individual assignment.

4. The “youtube” video that the questions worksheet is based on is “Transcription and Translation”
http://youtu.be/41_Ne5mS2ls

Day 2
Before class:
1. Make enough copies of the “Constructing a Phylogenetic Tree” activity packet- one per group of five students
2. Make sure you have enough rulers and scissors- one of each per group of five.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES

Day 1

Day 2
During class: (estimated time: ~45minutes)
1. Divide the class into groups of five.
2. Hand each group an activity packet, rulers and scissors.
3. Explain the instructions to the groups and allow them to begin.
4. Make sure to walk around the room and help any students that may need assistance.
5. Collect the completed activity packets as the students leave the class.

ASSESSMENT SUGGESTIONS:

<table>
<thead>
<tr>
<th>Learning Objective</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Construct and interpret a phylogenetic tree of select lentiviruses.</td>
<td>Give students a phylogenetic tree and have them answer questions about the tree.</td>
</tr>
<tr>
<td>Transcribe and translate a DNA sequence.</td>
<td>Have students transcribe and translate a DNA sequence using the genetic code.</td>
</tr>
</tbody>
</table>

RESOURCES/REFERENCES:

- How to Read Phylogenetic Trees
  http://www.nescent.org/media/NABT/mega_workshop.php
- Lentiviruses and Molecular Phylogeny
- Viral Cladogram
- Genetic Code
  http://biology.kenyon.edu/courses/biol114/Chap05/Chapter05.html
- Activity
Go to the website “You Tube” and watch the video “Transcription and Translation” (4:06) http://youtu.be/41_Ne5mS2Is. Answer the following questions.

1. What is the central dogma of modern biology?

2. What is the first step of the process of transcription?

3. Explain what the blue molecule is doing to the DNA strand?

4. The RNA molecule that is produced is mRNA, what is the difference between the mRNA molecule and the DNA strand it is being modeled after?

5. After the mRNA is complete it leaves the nucleus and what organelle forms around the strand?

6. What does the process of translation produce?

7. How do the amino acids get to the ribosome?

8. How many different types of amino acids are there and what do they carry according to the video?

9. What are proteins made of?
In this activity you will be working as a group to construct a phylogenetic tree that depicts the evolutionary relationships between lentiviruses: HIV, SIV, FIV, BIV and EIA. Lentiviruses belong to the virus family retroviridae and contain RNA as their genetic material and use reverse transcription to utilize host cell biochemical processes to produce their viral proteins. Nucleic acids and ecological niches define viral species. The ecological niche of a virus is its host cell and most viruses are host-cell specific.

Using RNA nucleotide sequences for the env gene (which is responsible for the viral surface glycoprotein and transmembrane proteins that mediate cellular receptor binding and membrane fusion) you will reverse transcribe the sequence into cDNA, then you will take the cDNA sequence and transcribe it into mRNA and from mRNA you will be able to translate it into amino acids using the genetic code. Once you have the your amino acids you will compare the amino acids of each of the lentiviruses and calculate the percentage of similarities between viruses. Using these calculations you will be able to construct a phylogenetic tree that depicts the evolutionary relationships between the lentiviruses.

An overview of how a lentivirus utilizes reverse transcription, transcription and translation to make viral proteins is depicted below:
Part 1: Getting the Amino Acids
1. Cut out and give one sequence worksheet to each member of your group.
2. Work independently to first reverse transcribe your sequence then to transcribe your sequence into mRNA and lastly use the genetic code to sequence your amino acids.

Part 2: Comparing the Amino Acid Sequences
1. Compare the amino acid sequences and count the similarities in sequence.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Number of sequential Similarities</th>
<th>Percentage (multiply by 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA to HIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIA to FIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIA to BIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIA to SIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIV to SIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIV to FIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIV to HIV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. Construct a phylogenetic tree using the following instructions:
   The length of the horizontal lines corresponds to the percent similarity values. Each branch point, or node, in the cladogram represents an ancestor common to all species beyond that node. Each node is defined by a similarity in amino acids present in all species beyond that branch point. Example below:

![Phylogenetic tree example](image)

3. Use a ruler and area below with % similarities guide to construct your phylogenetic tree.
1. Do all the lentiviruses depicted in your phylogenetic tree share a common ancestor? Explain how the phylogenetic tree supports your answer.

2. Explain how the ecological niche of the lentiviruses helps to define the relationships between the lentiviruses?

3. Hypothesize: How did these viruses evolve from a common ancestor to infect different species?
**Student Pages: HIV, SIV, FIV, BIV, EIA - Transcription, Translation Activity**

Name: ______________________
Date: ______________________

**HIV (Human Immunodeficiency Virus)**
1. Use reverse transcription to convert the viral RNA nucleic acid sequence into DNA.
2. Use transcription to convert the DNA into mRNA.
3. Use the genetic code to convert the mRNA into amino acids.

<table>
<thead>
<tr>
<th>Viral RNA</th>
<th>AGU</th>
<th>GUU</th>
<th>AUU</th>
<th>CUU</th>
<th>UUU</th>
<th>UAC</th>
<th>AAC</th>
<th>CAA</th>
<th>CAU</th>
<th>CUU</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRNA</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Amino Acid</td>
<td></td>
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</tr>
</tbody>
</table>

**SIV (Simian Immunodeficiency Virus)**
1. Use reverse transcription to convert the viral RNA nucleic acid sequence into DNA.
2. Use transcription to convert the DNA into mRNA.
3. Use the genetic code to convert the mRNA into amino acids.

<table>
<thead>
<tr>
<th>Viral RNA</th>
<th>AUG</th>
<th>GUU</th>
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<th>CUU</th>
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FIV (Feline Immunodeficiency Virus)
1. Use reverse transcription to convert the viral RNA nucleic acid sequence into DNA.
2. Use transcription to convert the DNA into mRNA.
3. Use the genetic code to convert the mRNA into amino acids.

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<th>Viral RNA</th>
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BIV (Bovine Immunodeficiency Virus)
1. Use reverse transcription to convert the viral RNA nucleic acid sequence into DNA.
2. Use transcription to convert the DNA into mRNA.
3. Use the genetic code to convert the mRNA into amino acids.

<table>
<thead>
<tr>
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</table>
EIA (Equine Infectious Anemia)

1. Use reverse transcription to convert the viral RNA nucleic acid sequence into DNA.
2. Use transcription to convert the DNA into mRNA.
3. Use the genetic code to convert the mRNA into amino acids.

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LESSON FIVE: Strains and Lions and Cats Oh My!!

KEY QUESTION: How do lentiviruses jump from one species to another? How can we test for the presence of lentiviruses?

KEY SCIENCE CONCEPTS: ELISA test, antigen, antibody

OVERALL TIME ESTIMATE: 2 - 45 minute class periods

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY:
Antibody: a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses

Antigen: a substance that evokes the production of one or more antibodies.

ELISA Test: enzyme-linked immunosorbent assay; a sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein, especially an antigen or antibody.

LESSON SUMMARY: Students will read and discuss a case study dealing with FIV in domesticated cats and in lions and answer discussion questions. As part of the case study students will also do a mini web quest to understand ELISA (Enzyme Linked Immunosorbent Assay) testing and run a simulated ELISA test.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Explain the steps of the ELISA test.
2. Comprehend that not all FIV strands are the same.
3. Analyze test results and apply them to a specific case.

STANDARDS:
SC.912.L.16.7
SC.912.N.1.6

MATERIALS:
- Copies of the FIV case study - one per student
- ELISA test (BioRad’s Biotechnology Explorer ELISA Immunoexplorer Kit Catalog # 166-2400EDU Protocol III – Antibody test) one kit for every 48 students.

BACKGROUND INFORMATION: Feline immunodeficiency virus (FIV) infects domestic cats and at least 20 additional species of non-domestic felids throughout the world. Strains specific to domestic cat FIV-Fca produce AIDS-like disease progression and pathology providing an informative model for HIV infection in humans. Less is known about the immunological and pathological influence of FIV in other felid species although multiple distinct strains of FIV circulate in natural populations. In the Serengeti National Park, Tanzania, three divergent subtypes of lion FIV-Ple are endemic, whereby 100% of adult lions are infected with one or more of these strains. Infected domestic cats may appear normal for years. However, infection eventually leads to a state of immune deficiency that hinders the cat’s ability to protect itself against other infections. The same bacteria, viruses, protozoa, and fungi that may be found in the everyday environment – where they usually do not affect healthy animals -- can cause severe illness in those with weakened immune systems. These secondary infections are responsible for many of the diseases associated with FIV. FIV is mainly passed from cat to cat through deep bite wounds.
“The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

There are several different types of ELISA. For our FIV example, we are indirectly measuring the presence of FIV-Ple or FIV-Fca virus in the feline’s serum by capturing antibodies. The steps of an "indirect" ELISA follow the mechanism below:

1. A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
2. A solution of non-reacting protein, such as bovine serum albumin or casein (non-fat milk powder is sometimes used), is added to block any plastic surface in the well that remains uncoated by the antigen.
3. Next the primary antibody is added, which binds specifically to the test antigen that is coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
4. Afterwards, a secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.
5. A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in R&D.
6. The higher the concentration of the primary antibody that was present in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength.”

Source: “Detecting Dengue in the Lab and Field” by Julie Bokor

ADVANCE PREPARATION

Day 1
Before class:
1. Make sure you have one copy of the case study per student.

Day 2
Before class: (~30 minutes)
1. Read all instructions in the BioRad kit the day before the lab and make sure you have all equipment needed.
2. Set up each lab station according to the directions in the BioRad kit.
3. Add the micro centrifuge labels according to the case study instructions.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Day 1
During class:
1. (~20-30 minutes) As students walk into the classroom hand them a copy of the case study “Neferkitty and the Lion”.
2. Choose students to read out loud the case study or read it to them or have them read independently.
3. Stop at the discussion questions and have them answer the questions as a class.
4. (~ 15-25 minutes) At the end of case study part 3 there is a mini web quest, if time permits provide computers with Internet access and allow students to do in class. If there is not enough time assign as homework.
Day 2:

**During class:**
1. (~40 minutes) Divide students into lab group and place each group at a station; I recommend groups of 2 because in larger groups some students may not get involved in the lab.
2. Have the students accomplish the lab according to the instructions in the case study.
3. Discuss results and conclusion questions.

**ASSESSMENT SUGGESTIONS:**

<table>
<thead>
<tr>
<th>Learning Objective</th>
<th>Assessment</th>
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<tbody>
<tr>
<td>Comprehend that not all FIV strands are the same</td>
<td>Have them explain why they needed to conduct the ELISA Test for both FIV-Ple and FIV-Fca</td>
</tr>
<tr>
<td>Explain the steps of the ELISA test.</td>
<td>Have students write down every step of the ELISA test and explain what is the purpose of each step.</td>
</tr>
<tr>
<td>Analyze test results and apply them to a specific case.</td>
<td>Have students write a lab report and answer the following Discussion questions:</td>
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<tr>
<td></td>
<td>1. Is the FIV strand that infected Neferkitty the same as the domestic cat FIV strand? Explain.</td>
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<td>2. Do you think that the strain of FIV will make a difference in treating Neferkitty? Explain</td>
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<td>3. Can Neferkitty be re-infected with another strain of FIV?</td>
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<td></td>
<td>4. How can a virus that host specific infect another species?</td>
</tr>
</tbody>
</table>

**RESOURCES/REFERENCES:**

- “Patterns of Feline Immunodeficiency Virus Multiple Infection and Genome Divergence in a Free-Ranging Population of African Lions”
  http://www.ncbi.nlm.nih.gov/pmc/articles/PMC371036/-
- “Growth of Lion and Puma Lentiviruses in Domestic Cat Cells and Comparisons with FIV”
- “FIV diversity: FIV Ple subtype composition may influence disease outcome in African lions”
- “Detecting Dengue in the Lab and Field” by Julie Bokor (Background information on ELISA test)
Case Study Part 1
Sunday 5/12 8:00am

It was an unexpectedly cool morning when Dr. Margret Levy landed in the Miami International Airport, and she was wishing she could immediately take a return flight to the beautiful and enchanted land she had just spent a month visiting, Africa. She had fallen in love with beautiful landscape and the endless sunny days, but above all, the cats. These large and majestic felines were all she could think about. The thought of going back to the lab to sequence their genomes did make coming back a little easier. She was excited to see what she would find once the DNA was sequenced.

Sunday 5/12 9:00am

“Hi honey. I am home!” declared Dr. Levy as she walked through the door of her Coral Gables home but there was no response. Suddenly like if on cue Neferkitty appeared and started meowing as he rubbed himself against her legs. “Oh Neferkitty, how’s my boy doing?” She placed the blood samples she collected from the lions in Africa on the dining room table and decided to go look for Adam her husband.

Sunday 5/12 10:15am

Suddenly there was a crash and the sound of glass breaking on the tile floor. “Neferkitty!” screamed Dr. Levy as she ran towards the dining room. Sure enough there was Neferkitty standing on a mess of blood and glass chards. “Oh Neferkitty what have you done?” Neferkitty quickly ran away to his favorite hiding spot under the bed. “Oh babe what happened?” Adam inquired as he saw the bloody mess on the floor. “Well Neferkitty decided to play with my blood samples from Africa and of course now they are ruined,” retorted Dr. Levy as she started cleaning up the mess. As she was cleaning she realized that not all the samples had been destroyed, only four, and she wasn’t sure but she thought they were the extra samples, the ones she had collected twice. All of sudden she realizes Neferkitty must be hurt he was walking all over the glass chards. “Adam we need to see if Neferkitty got cut with these glass chards.”

Sunday 5/12 11:07am

“Meow…meow, meeeow” screamed Neferkitty as Dr. Levy and Adam removed the chards of glass from his pink little paws. “Poor thing!” declared Adam as he held Neferkitty. “He will be fine…won’t you Neferkitty” Dr. Levy stated as she methodically removed all the chards and cleansed his wounds. “Could he have gotten any weird disease from that blood?” demanded Adam. “No, I don’t think so…I mean it would be unlikely for sure” Dr. Levy said.
Discussion Questions:

1. Do you think that Neferkitty could have contracted any virus or disease from the lion blood samples? Explain your answer.

2. What should Dr. Levy and Adam do for Neferkitty, knowing that he has been exposed to lion blood? Explain your answer.
Case Study part 2

Monday 5/13 8:00 am
“I need to get to the lab, storing these samples in the fridge is not the best for them!” declared Dr. Levy as she gathered the blood samples from the fridge. “Should I take Neferkitty to the vet or should we wait and see since he seems to be doing well?” Adam asked. “I don’t know but it wouldn’t hurt to take him and have Dr. Silverstone make sure he is ok.” Dr. Levy stated as she walked out the door. “Oh Neferkitty…I am sure she cares…. she is just in a rush” Adam declared as he looked down at Neferkitty, Neferkitty meowed as if to agree.

Monday 5/13 10:00am
“Good morning Adam what brings you in today?” Dr. Silverstone inquired as he looked at Neferkitty. “Well yesterday Maggie came back from Africa and she placed some lion blood samples on the table and Neferkitty got into them and cut up his paws” Adam explained. “Oh Neferkitty” Dr. Silerstone stated as he looked at Neferkitty`s paws. “Well the cuts don’t seem to be to deep and he seems to be doing ok but we will clean and bandage them and give him an antibiotic shot for good measure” stated Dr. Silverstone as he picked up a syringe and filled it with white antibiotic. “That sounds great Doc but do you think that Neferkitty could have contracted any pathogens from the lion blood?” Adam asked. “Hmm, I doubt it but were those samples of sick lions?” asked Dr. Silverstone. “Well I don’t think so but I would have to ask Maggie to be sure,” stated Adam. “Go ahead and call Maggie and ask her cause I do need to know,” Dr. Silverstone stated as he continued to work on Neferkitty`s paws.

Monday 5/13 10:50am
“Hey Maggie, quick question was the blood you collected from sick cats?” Adam said as Maggie picked up the phone. “No these cats were healthy…I mean Adam you should have seen them they were strong and wild not like the ones you see at the zoo,” declared Dr. Levy. “Great I just wanted to make sure and Dr. Silverstone needed to know,” Adam answered.

“Well nothing to worry about Dr. Silverstone, Maggie is sure that the samples came from healthy wild cats” Adam told Dr. Silverstone. “Well in that case Neferkitty is good to go, it was great seeing you Adam and please send my regards to Maggie” Dr. Silverstone replied.

Tuesday 7/22 4:00pm
“I think Neferkitty doesn’t feel good he might be getting a cold or something” Adam stated. “Let me take a look at him,” stated Dr. Levy. “Well yeah he has a runny nose and I have noticed he is a little lethargic lately,” Dr. Levy stated. “I will take some blood and send it out so we can see if his white blood cell count is elevated” Dr. Levy stated. “Sounds good to me...” declared Adam.
Tuesday 7/29 8:00am

“Adam Neferkitty’s white blood cells are elevated I guess he is sick, I will bring home some antibiotics” the message on Adams phone stated. “Well Neferkitty I guess you’re sick” Adam stated as he looked down at the sulking cat.

Monday 8/11 9:00am

“I just want to run an FIV test because he does not seem to be getting better,” stated Dr. Levy. “Ok you’re the Doctor” Adam stated.

Monday 8/11 11:00am

“Adam I have bad news Neferkitty tested positive for FIV” Dr. Levy explained to Adam. “But how he has never been outdoors and he has never played with another...its impossible you need to run that test again,” retorted Adam.

Discussion Questions

1. What is FIV?

2. How could Neferkitty have contracted FIV?

3. Is it possible that the FIV test was a false positive? Explain.

4. What we would you do next if you were Neferkittys owner?
Case Study Part 3

“Well, I didn’t want to tell you this because I didn’t want to worry you but some large cats can carry a strain of FIV” Dr. Levy admitted to Adam. “What?? So you have known all along that Neferkitty could have gotten kitty AIDS from your African blood samples and you choose not to tell me??” Adam retorted angrily. “No, no, no…I never said that Neferkitty could have contracted the FIV from the blood samples, domestic cats and large cats do not carry the same strain and the cats from Africa looked healthy and their blood, that I have been working with for months, does not show any traces of viruses…” Dr. Levy nervously stated. “Well then what are you saying?” exclaimed Adam. “I am saying just to be sure we should run an ELISA test on all the African cat blood samples and on Neferkittys blood as well” Dr. Levy explained. “A what?? What is an ELISA test?” Adam questioned.

Research Questions:
Use the following web links to answer the corresponding questions:
http://www.biobest.co.uk/diagnostics/techniques/elisa-how-does-the-test-work.html
AND watch the following you tube video “Enzyme Linked Immunosorbent Essay”: http://www.youtube.com/watch?v=RRbuz3VQ100

1. What does ELISA stand for?

2. What does the ELISA test for FIV measure?

3. Numerate and explain all the steps of running an ELISA test.

4. What is needed to run an ELISA test?
Case Study part 4

Today you will be conducting the ELISA test to find out if the wild lion blood is infected with a strain of FIV. The FIV strain that is common in wild lions in Africa is FIV-Ple and the strain that is common among domesticated cats is FIV-Fca. When conducting an ELISA test the antibody will only bind to the antigen that is specific to that antibody. Meaning that if Neferkitty is infected with FIV-Fca then he is producing antibodies that bind to the antigen from the FIV-Fca but if he is infected with FIV-Ple then antigens for FIV-Ple needs to be used in order to get a positive test.

In this ELISA test we will be running the lion samples with both the FIV-Fca antigens and the FIV-Ple antigens. The same will be done for Neferkitty’s blood samples.
Student Pages: ELISA Antibody Test Procedures

1. Place the ELISA microplate strip with 12 wells on the lab bench.
2. Place a paper towel unfolded and flat on the table. Mark it “USED PIPETTES”.
3. Find the centrifuge tube with the purified antigen labeled “FIV-Ple”. Using one disposable pipette, place one drop of antigen into wells 1 through 6.
4. Place the disposable pipette on the towel marked “USED PIPETTES”.
5. Find the centrifuge tube with the purified antigen labeled “FIV-Fca”. Using one disposable pipette, place one drop of antigen into wells 7-12.

What Did We Just Do?
You just placed antigens specific to either FIV-Fca or FIV-Ple into the wells where some attached to the walls of the well.

6. Find the tube labeled “Wash” and using one NEW disposable pipette, place one drop of the Wash solution into wells 1 through 12. Set this pipette aside, you will use it again for the Wash Solution.
7. Take the ELISA tray in the palm of your hand and quickly turn it upside down into a sink or a short stack of paper towels to empty the wells. Tap the tray several times, and then place it back on the table right side up.

What Did We Just Do?
You washed away any antigens that were not attached to the walls of the wells.
8. Find the tube labeled “Blocker” and using one NEW disposable pipette, place one drop of the Blocker Protein solution into wells 1 through 12. Place the pipette tip on the towel marked “USED PIPETTES”.

9. Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink or a short stack of paper towels to empty the wells. Tap the tray several times, and then place it back on the table right side up.

10. Find the tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 thru 12. **Set this pipette aside, you will use it again for the Wash Solution.**

11. Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink or to a short stack of paper towels to empty the wells. Tap the tray several times, and then place it back on the table right side up.

**What Did We Just Do?**

You added a solution containing a blocker protein that will stick to any surface of the well that is not coated with antigen. You then washed away any extra blocker proteins that were not attached.

12. Find the tube with positive control labeled “FIV-Ple +” and using a micropipette, place 20ul of the Positive Control solution into wells 1 and 2. Place the pipette tip on the towel marked “USED PIPETTES”.

13. Find the tube with positive control labeled “FIV-Fca +” and using a micropipette, place 20ul of the Positive Control solution into wells 7 and 8. Place the pipette tip on the towel marked “USED PIPETTES.”

**What Did We Just Do?**

You added serum that we know contains antibodies to either FIV-Ple or FIV-Fca. The antibodies attach to the antigens in the wells.
14. Find the centrifuge tube labeled “Neferkitty” and using a micropipette, place 20 ul of the Subject A serum into wells 3, 4, 9 and 10. Place the pipette tip on the towel marked “USED PIPETTES”.

15. **Using a new pipette tip,** place 20 ul drops of the “Lion” serum into wells 5, 6, 11 and 12. Place the pipette tip on the towel marked “USED PIPETTES”.

16. Remembering to use a new pipette tip for each different serum sample

**What Did We Just Do?**
You added the blood plasma from Neferkitty and the lion into the testing wells. If Neferkitty or the lion have been exposed to FIV-Ple or FIV-Fca, he would have had an immune response to it and antibodies will be in his blood. Antibodies will attach to the antigen in the well. (Left)

If Neferkitty or the lion have not been exposed to FIV-PLe or FIV-Fca, there will not be antibodies. No antibody will attach to the antigen in the well. (Figure 2)
17. Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink or to a short stack of paper towels to empty the wells. Tap the tray several times, and then place it back on the table right side up.

18. Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 through 12. **Set this pipette aside, you will use it again for the Wash Solution.**

19. Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.

**What Did We Just Do?**
You washed away any antibodies that had not attached to antigens.

20. Find the tube labeled “F Tag” and using one NEW disposable pipette, place one drop of the Fluorescent Tagged Antibodies into wells 1 through 12.

21. Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink or on to a short stack of paper towels to empty the wells. Tap the tray several times, and then place it back on the table right side up.

22. Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 through 12.

23. Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink or on a stack of paper towels to empty the wells. Tap the tray several times, and then place it back on the table right side up. Place the pipette on the “USED PIPETTES” towel.

**What Did We Just Do?**
You added antibodies that attach to feline antibodies. These anti-feline antibodies have a fluorescent molecule attached to them. Then you washed away any anti-feline antibodies that did NOT attach to something in the well.
24. Obtain an ultraviolet light and shine it over the ELISA plate.

What Did We Just Do?
The ultraviolet light stimulated the fluorescent molecules attached to the antibodies that were attached to the feline antibodies that were attached to the viral or bacterial antigens in the well. These fluorescent molecules then gave off a visible light.

If no light was seen, there were no antibodies in the wells because there were no feline antibodies for them to attach to.

25. Record which wells fluoresced using a + sign on the ELISA Test Data Sheet. The subjects whose blood serum was placed in these wells have tested positive for the presence of antibodies to that particular disease.

26. Record which wells did not fluoresce with a – sign on the ELISA Test Data Sheet. The subjects whose blood serum was placed in these wells do not have antibodies to those particular diseases.
## Record Your Results:

<table>
<thead>
<tr>
<th>Well #</th>
<th>“+” Control or Patient Name</th>
<th>What is being tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ Control</td>
<td>FIV-Ple</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+ Control</td>
<td>FIV-Ple</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
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<tr>
<td>7</td>
<td>+ Control</td>
<td>FIV-Fca</td>
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</tr>
<tr>
<td>8</td>
<td>+ Control</td>
<td>FIV-Fca</td>
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<tr>
<td>12</td>
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</tr>
</tbody>
</table>

## Discussion Questions

1. Is the FIV strand that infected Neferkitty the same as the domestic cat FIV strand? Explain.

2. Do you think that the strain of FIV will make a difference in treating Neferkitty? Explain.

3. Can Neferkitty be re-infected with another strain of FIV?

4. How can a virus that host specific infect another species?