Air War — Biological Weapons

Secrets of the Sequence Video Series on the Life Sciences • Grades 9 — 12

Teaching materials developed by **VCU** Life Sciences

Virginia Commonwealth University

Classroom Tested Lesson

Video Description

"Secrets of the Sequence," Show 121, Episode 2
"Air War – Biological Weapons" – approximately 9 minutes viewing time

During the Cold War, Soviet Bloc scientists apparently developed an aerosol disbursed biological weapon using the pneumonic plague virus.

Ward Television
Producer: Dale Minor
Featuring: Dr. Ken Alibek, Center for Biodefense, Ge0rge Mason University, Julian Parkhill, Wellcome Trust Sanger Center, Brendan Wren, Microbial Pathogenesis, London School of Hygiene and Tropical Diseases
Lesson Author; Reviewers: Peggy Deichstetter; Catherine Dahl, Dick Rezba, and Kieron Torres
Trial Testing Teachers: Pam Sparks

National and State Science Standards of Learning

National Science Education Standards Connection

Science as Inquiry
Content Standard A: As a result of activities in grades 9-12, all students should develop

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Life Science
Content Standard C: As a result of their activities in grades 9-12, all students should develop understanding of

- Molecular basis of heredity
- Biological evolution
- Interdependence of organisms
- Behavior of organisms

Science and Technology
Content Standard E: As a result of activities in grades 9-12, all students should develop

- Abilities of technological design

Science in Personal and Social Perspectives
Content Standard F: As a result of activities in grades 9-12, all students should develop understanding of

- Personal and community health

- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

History and Nature of Science
Content Standard G: As a result of activities in grades 9-12, all students should develop understanding of
- Science as a human endeavor

Selected State Science Standards Connections
Use http://www.eduhound.com (click on "Standards by State") or a search engine to access additional state science standards

Virginia
BIO 2. The student will investigate and understand the history of biological concepts. Key concepts include
a) evidence supporting the cell theory;
b) scientific explanations of the development of organisms through time;
c) causative agents of disease; and
d) the evolution of the DNA model.
e) the collaborative efforts of scientists, past and present.

BIO 5. The student will investigate and understand life functions of monerans, protists, fungi, plants, and animals, including humans. Key concepts include
c) analyses of their responses to the environment;
e) human health issues, human anatomy, body systems, and life functions;

BIO 6. The student will investigate and understand common mechanisms of inheritance and protein synthesis. Key concepts include
e) effects of genetic recombination and mutation;
g) exploration of the impact of DNA technologies.

Illinois
Standard 11A. Know and apply the concepts, principles and processes of scientific inquiry
Standard 12.A Know and apply concepts that explain how living things function, adapt and change.
Standard 12.B Know and apply concepts that describe how living things interact with each other and with their environment.
Standard 13. B Know and apply concepts that describe the interaction between science, technology and society.

Testing: A sample related multiple choice item from State Standardized Exams
Measles is an extremely contagious viral infection spread by the respiratory route. Figure 1 shows the course of measles from time of exposure to recovery from the infection.

After recovery from measles, the infected individual develops immunity or resistance to reinfection. Figure 2 shows the development of immunity indicated by the antibody level.

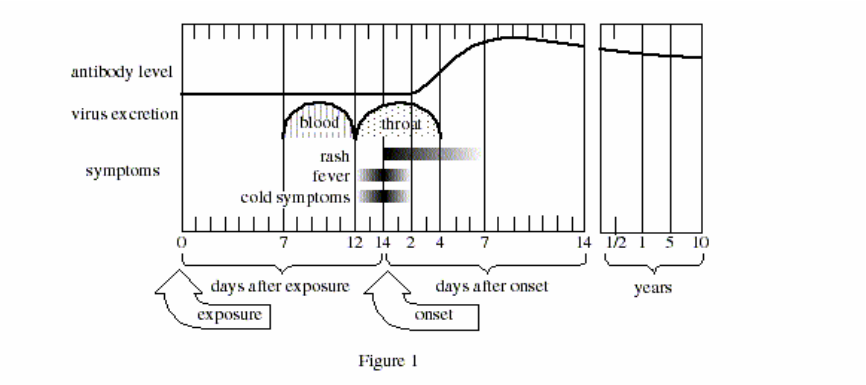


Figure 1

Figure 1 adapted from D. M. McLean, Virology in Health Care. ©1980 by Williams & Wilkins.
The number of reported cases of measles from 1950 through 1987 is depicted in Figure 2.

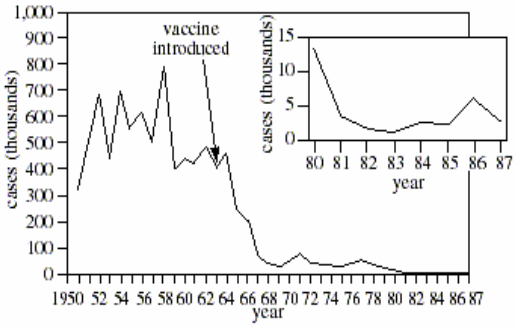


Figure 2

Figure 2 adapted from MMWR, "Summary of Notifiable Diseases." ©1986 Communicable Disease Center.

On Day 10 after exposure to measles, one could conclude that the greatest concentration of the measles virus would most likely be found in which of the following locations?

A. Skin
B. Mouth
C. Blood *
D. Throat

Source: Illinois; ACT Sample Test Question #1

Overview

During the cold war the Soviet Union genetically engineered a deadly disease. Since the break up of the Soviet Union there is serious concern about the lack of security for the laboratories where disease-causing pathogens such as *Yersinia pestis* are stored. Black Plague, the scourge of the Middle Ages, is caused by *Yersinia pestis* and is spread by

fleas from rats to people. Pneumonic plague, also caused by *Yersinia pestis,* is spread person to person and has symptoms similar to pneumonia. Pneumonic *Yersinia pestis* can spread up to six miles and remain infectious for up to an hour. Dr. Ken Alibek, who was a Soviet scientist working on *Y. pestis* as a bioweapon, now heads the U.S. Biodefense effort. He is trying to find a way to stop these pathogens. He doesn't think that vaccines will work because there are too many varieties that are also very resistant to antibiotics. He is studying non-specific immunity to help the body develop the ability to fight invading pathogens by itself.

In England scientists have studied extensively the DNA for *Y. pestis* and among their findings is the genetic history of the pathogen. At one time this microbe was a benign microbe living in our intestines, but sometime between 2,000 and 20,000 years ago the bacteria acquired a gene that made it deadly. The natural form of the plague still kills 3,000 people per year but the greatest danger lies with the genetically modified forms that were previously developed as weapons of bioterrorism.

Video Preparation

Preview the video and make note of the locations you will need later to pause the video for discussion.

Before Viewing

1. Ask the following questions:

    a. What is the Black Death?
    The Black Death is Bubonic Plague
    b. What causes this disease
    It is spread by fleas to rats and then to humans
    c. Can you still get this disease or has it been eliminated?
    It has not been eliminated but the disease is predominantly controlled and less dangerous.

2. Distribute "infected" handouts (Appendix A) of several questions for students to answer while they watch the video. See teacher notes below for directions and possible focus questions. Students should not know at this point that their handouts have been infected.

During Viewing

1. START the video.

2. PAUSE the video (3:52 minutes into the video) after Dr. Alibek says, "…the general idea is to find some ways to modulate, to enhance non-specific immunity of the body. And to induce it in such a way that the immune system would be able to eliminate this invading pathogen by itself."

    Ask:
    a) Why are genetically engineered plague pathogens resistant to many antibiotics?
    The pathogens are engineered to be resistant to known antibiotics – that is what makes them a weapon.
    b) Why does Dr. Alibek think that vaccines won't work in protecting us from these pathogens?

Vaccines will not work because there are too many variations of the pathogens for any single antibiotic to be effective

c) Dr. Alibek is working on an approach to the problem that enhances non-specific immunity in the body. What does non-specific immunity mean?
The immunity is not related to one kind of bacteria in particular.

3. RESUME the video and play to the end

After Viewing

1. Discuss the students' responses to the questions on the handout, *Appendix A: Video questions for Air War – Biological Weapons*

2. Now, tell them that they have been "infected" with a safe material that glows.

3. Walk around the room with the black light showing the students how the material has spread.
They should see it on their hands, faces and anywhere else they touched

4. Have students wash their hands before leaving class.

5. Discuss the following questions with your students:
   - If this glowing powder were really germs, how many of you would be infected?
   - Suppose the powder was a virus and thus very small; do you have any small cuts, abrasions, or chapped skin through which it could pass?
   - Do you have any powder near your nose or mouth where it could be inhaled?
   - How might you spread the "germs" to your family?
   - What could you do to minimize being infected by germs?
   - How could you minimize infecting others?

6. Conduct Student Activity 1: Who's Infected? See teacher notes and student handout.

7. Conduct Student Activity 2: Testing for Antibiotic Resistance in Bacteria – The Kirby-Bauer Method. See teacher notes and student handout.

Teacher Notes for Appendix A: Video questions for Air War – Biological Weapons

Materials

- Handout of focus questions (Appendix A)
- Glo Germ powder to "infect" the handouts (a safe material of inert ingredients that glows under UV light)
- UV light

  Note: Kits containing a UV light and Glo Germ material (from $65) or just Glo Germ powder ($12.95) if you already have a UV light are available from Glo Germ Co., P.O. Box 537, Moab, Utah 84532, 1-800-842-6622, http://www.glogerm.com/

  (Field test teacher commented that some glowing materials are lotions which adhere well to worksheets but do not transfer well to students hands. Therefore, the powder is recommended.)

Procedure

1. Prepare a handout of focus questions for students to answer while they watch the video. (See Appendix A)
2. Lightly dust the handouts with Glo Germ powder *out of sight of your students*. This safe inert material glows in the presence of UV light and graphically illustrates how easily "germs" are spread.
3. Conduct your *Before Viewing* discussion and view the video. During the *After Viewing* part of the lesson, you will tell students that you "infected" their handout sheets; use a UV light to show them how the "germs" have spread.

Answer Key to Appendix A: Video questions for Air War – Biological Weapons

1. What is the difference between bubonic plague and pneumonic plague?
   Bubonic plague is spread by fleas and affects the lymph nodes. The bacteria cause hemorrhaging seen as blackening of the skin, hence the name Black Death.
   Pneumonic plague is spread from person to person through coughing and sneezing. It affects the lungs like pneumonia

2. Why isn't pneumonic plague a problem in its natural (not genetically engineered) state?
   Pneumonic plague in its natural state doesn't live outside the body long enough to infect many people.

3. Why doesn't Dr. Alibek think vaccination is a practical way to deal with bioterrorism?
   There are too many diseases to vaccine against.

4. When did *Yersinia pestis* turn into a killer?
   Between 2,000 and 20,000 years ago Yersinia pestis acquired killer genes.

Teacher Notes for Student Activity 1: Who's Infected?

Materials
Student Handout #1

Procedure

DAY 1
Tell the students the following: "Suppose you are infected with 'Glo Germ Disease' (or any other disease) that is spread by simply touching another person. Keep track of the number of people you touch (infect) between now and tomorrow's class."

DAY 2
Ask: "How many people did you infect (touch) since class yesterday?"

Have students record his or her data in the table on Student Handout #1 identical to the one below.
Tell the students to assume that each person they touched also touched the same number of people as they did. How many people would be infected by tomorrow? In a week?

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total

Give students an example in which someone touched and thus infected 5 people the first day, who in turn each infected 5 more persons. *Do not provide numbers past Day 2* for this example (unless students do not understand the exercise) as it will reduce the impact of the student's recognition of how large these numbers quickly become.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
5	*25*	*125*	*625*	*3125*	*15,625*	*78,125*	*97,655*

Answer Key to Discussion questions:

1. What might happen if one of the infected people boarded a plane and flew to another city? *(The disease would spread beyond the original location.)*

2. Suppose that some of the people on the airplane who were touched by an infected person took connecting flights to other cities. What would happen then? *(The disease could spread to everyplace the passengers flew to.)*

3. How would the numbers be different if you didn't have to touch the person to infect them, but instead only had to be in the same room with them? *(The number of infected persons would be substantially larger.)*

4. If bioengineered bacteria were released, who would be safe from the disease? *(Answers will vary but if the bacteria were unknown, it could spread rapidly with no-one safe*)

Teacher Notes for Student Activity 2: Testing for Antibiotic Resistance in Bacteria - The Kirby-Bauer Method

Materials

- 2 agar plates
- 2 sterile swabs or transfer loops
- antibiotic disks
- marking pencil
- broth cultures of *E. coli* and *S. aureus*
- forceps
- alcohol
- Bunsen burner
- incubator
- metric ruler

Procedure

1. Prepare the necessary materials as listed in the Student Handout # 2

2. Using a set of the materials demonstrate the steps of the activity as students read each step of the procedure set out on Student Handout # 2

3. Remind students of safety procedures.

4. If many of the procedures are new to students, it might be better for them to practice these procedures prior to conducting the activity, such as flaming the mouth of a culture tube, transferring the culture to a Petri dish, or streaking a plate.

Note: A field test teacher commented that the bacterial growth will vary depending on humidity and temperature. After 2 days at room temperature, the zones of inhibition were more clearly defined so you may want to lengthen the time before observation or put in an incubator overnight.

Student Handout # 1: Who's Infected?

Name:_____

Yesterday you were asked to keep track of the number of people you touched between then and today's class. If you really were infected with "Glo Germ Disease" or another disease spread by simply touching another person, how many people did you infect (touch) since class yesterday?

1. Record the number of persons you touched in Day l.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total

2. Now, assume that each person you touched also touched the same number of people as you did. How many people would be infected on Day 2? At the end of the week on Day 7?

   For example, if you touched and infected 5 people the first day, and they in turn each infected 5 more persons, 25 additional persons would be infected by the end of Day 2.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
5	*25*	*?*	*??*	*???*	*????*	*?????*	*??????*

3. Complete the blank table above based on the number of persons you touched on the first day.

4. Discussion questions:

- What might happen if one of the infected people boarded a plane and flew to another city?
- Suppose that some of the people on the airplane who were touched by an infected person took connecting flights to other cities. What would happen then?
- How would the numbers be different if you didn't have to touch the person to infect them, but instead only had to be in the same room with them?
- If bioengineered bacteria were released, who would be safe from the disease?

Student Handout # 2: Testing for Antibiotic Resistance in Bacteria - The Kirby-Bauer Method

Background:

There are many different antibiotics available today. You may even be taking an antibiotic right now. Scientists are working to discover and develop new antibiotics all the time. Scientists need to find out whether different types of bacteria are sensitive or resistant to each antibiotic. If the bacteria are sensitive to an antibiotic, then the antibiotic will either kill the bacteria or prevent its growth. If the bacteria are resistant to an antibiotic, then the bacteria will grow normally.

One method to test for bacterial sensitivity and resistance to a particular antibiotic is a procedure called the Kirby-Bauer Method. In this method an agar plate is covered with a bacterial culture, and then paper disks containing the specific antibiotics are placed on the agar plate. After the agar plate is incubated, the zone of inhibition around each disk is observed and measured. The zone of inhibition is the clear area around the disk in which bacteria were not able to grow. Scientists use the size of the zone of inhibition to determine whether the bacteria are sensitive or resistant to the antibiotic. For example, zones of smaller size or no zone at all show that the bacteria are resistant to the antibiotic. If the bacteria are sensitive, there will be a definite clear zone and the bigger the zone the more sensitive the bacterium is to the antibiotic.

Problem Statement: Are *Staphylococcus aureus* and/or *Escherichia coli* bacteria resistant to various antibiotics?

Hypothesis: Based on what you already may know, write a hypothesis for what you think will happen.

Materials

- 2 agar plates
- 2 sterile swabs or transfer loops
- antibiotic disks
- marking pencil
- broth cultures of *E. coli* and *S. aureus*
- forceps
- alcohol
- Bunsen burner
- incubator
- metric ruler

Procedures - Day One

1. Use the marking pencil to label the top edge of two agar plates with your name and date. Mark one plate S. aureus and the other plate E. coli.
2. Use the marking pencil to draw lines on the bottom of both agar plates dividing the plates into 4 sections. Number the sections 1, 2, 3 and 4 but make the numbers very small. (see Figure 1)
3. Be sure that the alcohol is far away from the Bunsen burner! Carefully light the Bunsen burner. Remove the cap on the S. aureus culture tube and carefully flame the culture tube opening. Insert the sterile swab into the culture of S. aureus. Squeeze the extra fluid off of the swab by gently pressing the swab against the inner side of the culture tube. Remove the swab, flame the opening of your culture tube and recap it.
4. Use the swab to gently streak the entire agar surface of the plate labeled *S. aureus*. Be sure to turn the plate at least three times while streaking to spread the bacteria out evenly. Finally, gently run the swab around the edge of the agar plate. Cover your plate. Discard the swab as directed by your teacher. Allow the covered plate and culture to dry for 5 to 10 minutes at room temperature.
5. Repeat steps 3 and 4 using a new sterile swab and the agar plate labeled *E. coli* and the *E. coli* culture.
6. Turn the Bunsen burner off.
7. Select the four antibiotics that you will be testing. In the Data table provided, record the name of the antibiotic and the section (1-4) in which you will place it. To avoid confusion place the same antibiotic in the same section on both plates.

8. Sterilize the forceps by dipping them in alcohol and holding them to air dry. Use the sterile forceps to pick up one disk of antibiotic number 1 and place it in the center of section 1 on the *S. aureus* plate. IMPORTANT: Do not touch the agar surface with forceps or place the forceps on the lab table or you will need to sterilize them again! Do not press the antibiotic disks into the agar but be sure that contact is made between the disks and the surface of the plate. Do not move the disk once it is placed on the agar.
9. Repeat for disks 2, 3 and 4 on the *S. aureus* plate.
10. Repeat steps 7, 8 and 9 for the *E. coli* culture.
11. Incubate the plates at 35 degrees Celsius until the next class. Do not invert the agar plates when incubating. This will prevent the antibiotic disks from falling off overnight.
12. Clean the lab area and wash your hands with soap and water.

S. aureus *E .coli*

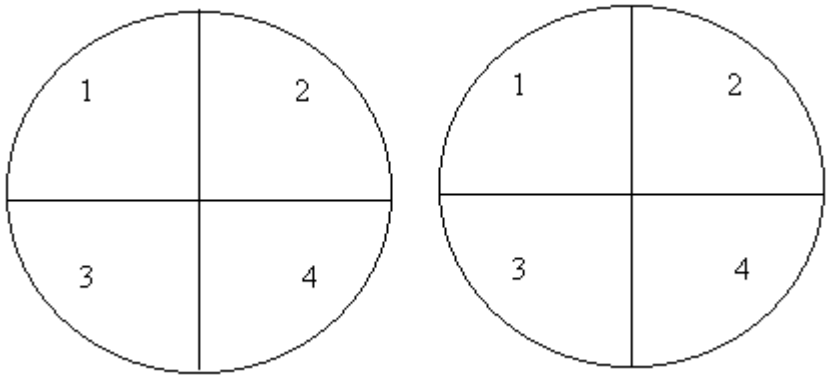


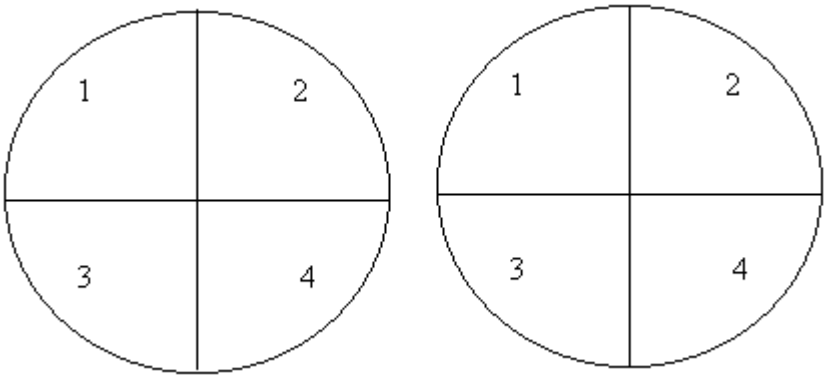
Figure 1

Procedure - Day Two

1. Observe the plates for zones of inhibition. Remember that the zone of inhibition is the clear area around the paper disk in which bacteria have not grown.
2. Draw your results in the Data section. In the Descriptive Column, note whether the zone of inhibition is completely clear of bacterial growth or not.
3. Use a metric ruler to measure the zone of inhibition in millimeters. Measure from the antibiotic disk to the edge of the clear area. Record the measurements in the Data section.
4. Use your observations and measurements to determine whether the bacteria are resistant or sensitive to each antibiotic. The bacteria are sensitive if there is a clear zone of inhibition around the disk where no bacteria have grown. The bacteria are resistant if there is no clear zone of inhibition around the disk or the bacteria have grown all around it.
5. Dispose of the agar plates as directed by your teacher. Wash the lab table and your hands.

Data Collection

S. aureus *E. coli*



Antibiotic Disk-Number and Name					
S .aureus Descriptive	S. *aureus* Zone in mm		*E. coli* Descriptive		*E .coli* Zone in mm

Conclusion

Please write a concluding paragraph based on your hypothesis, what you tested, and your results.

Summary Questions

1. Did you see any differences between *S. aureus* and *E.coli* in their resistance to any of the antibiotics? If yes, describe the differences. _____
2. Can you tell by using this test whether the sensitive bacteria were killed or just not able to grow? _____
3. Did you observe any zones with a few colonies of bacteria within the zone of inhibition? If yes, what is a possible explanation for this occurrence?

   _____

   _____
4. List some factors that a physician would need to consider other than zone of inhibition size before prescribing an antibiotic. _____

ADAPTED FROM: Hudson, Barbara K. Microbiology in Today's World Second Edition. Kendall / Hunt Publishing Company, 1998 and Harley, John P. and Lansing M. Prescott. Laboratory Exercises in Microbiology Fourth Edition. WCB McGraw-Hill, 1999.

Appendix A: Video questions for Air War – Biological Weapons

Name: _____

Answer the following questions while you watch the video:

1. What is the difference between bubonic plague and pneumonic plague?

2. Why isn't pneumonic plague a problem in its natural (not genetically engineered) state?

3. Why doesn't Dr. Alibek think vaccination is a practical way to deal with bioterrorism?

4. When did *Yersinia pestis* turn into a killer?

Additional Resources

Because Web sites frequently change, some of these resources may no longer be available. Use a search engine and related key words to generate new Web sites.

Emerging and Re-emerging Infectious Disease The National Institutes of Health offers this free high-school curriculum supplement. Student activities include lab experiments with bacteria, accompanied by online video support for the experiments. http://science.education.nih.gov

Chemicals, The Environment, and You: Explorations in Science and Human Health
The National Institutes of Health offers this free middle-school curriculum supplement. Students, Grades 7- 8, explore the relationship between chemicals in the environment and human health, utilizing basic concepts in the science of toxicology. http://science.education.nih.gov

Video: Intimate Strangers: Unseen Life on Earth This PBS documentary provides an overview of the microbial world and offers a clear and exciting picture of the field of microbiology. Meet scientists across the globe working to investigate the microbial world in diverse locations from a termite's stomach to a hospital operating room to an African village — and even outer space. These programs increase the microbial literacy of students, the general public, and biotechnology workers. http://www.learner.org/resources/series147.html

Center for Disease Control Website CDC provides a wealth of links to Agents, Diseases, & Threats from Bioterrorism and Chemical Agents, as well as Mass Trauma Related to Catastrophic Events. Also on this website is a NEW Video: "The History of Bioterrorism." Provides current news from the Center for Disease Control and Prevention agency. http://www.bt.cdc.gov/agent/plague/index.asp

Biodefense and Bioterrorism A more hopeful view of biodefense from the National Institute of Allergy and Infectious Diseases. This is the primary NIH organization for research on Biodefense and Bioterrorism at the National Institutes of Health. www.nlm.nih.gov/medlineplus/biodefenseandbioterrorism.html

The National Institutes of Health website on biodefense Discover which diseases are used for bioterrorism, how they are spread and how you can prevent becoming infected. http://health.nih.gov/result.asp?disease_id=72

Website of "Useless Information" The website of "useless information" compares the Bubonic Plague to the Influenza Epidemic of 1918. URL http://home.nycap.rr.com/useless/bubonic_plague/

Play a computer simulation of the Bubonic Plague at: http://www.mcn.org/ed/cur/cw/Plague/Plague_Sim.html

Genomic Revolution The Web site to the government-funded Human Genome Project with links about genomics, the history of the project, and more.
http://www.ornl.gov/sci/techresources/Human_Genome/education/education.shtml

Secrets of the Sequence Videos and Lessons
This video and 49 others with their accompanying lessons are available *at no charge* from www.vcu.edu/lifesci/sosq