Masters

Lesson 1—Ideas about the Role of Evolution in Medicine

Activity 1: Outbreak!

Master 1.1, *Information about MRSA* ..... student copies

Activity 2: Models and Medicine

Master 1.2, *Aniridia: An Eye Disease*..... transparency
Master 1.3, *Learning about Human Health from Other Organisms* ..... student copies
Master 1.4, *Photographs of Normal Eyes and Eyes with Pax6 Mutations* ..... color transparency
Master 1.5, *An Evolutionary Tree* ..... student copies and transparency

Lesson 2—Investigating Lactose Intolerance and Evolution

Master 2.1, *Lactase Investigation*..... student copies
Master 2.2, *Lactase Results from Other Researchers*..... transparency
Master 2.3, *Investigating Patterns in Lactase Persistence* ..... student copies**
Master 2.4, *Questions about the Genetic Basis for Lactase Persistence* ..... transparency
Master 2.5, *Lactase and Human Evolution*..... transparency
Master 2.6, *Explaining the Evolution of Lactase*..... student copies
Master 2.7, *Data from Africa*..... transparency
Master 2.8, *Data from Europe, Part A*..... student copies for half of the class
Master 2.9, *Data from Europe, Part B*..... student copies for half of the class
Master 2.10, *Summing Up Lactase Persistence and Nonpersistence*..... student copies
Master 2.11, *Map of Lactase Test Results*..... transparency*
Master 2.12, *Map of Complete Lactase Test Results* ..... transparency*
Master 2.13, *Exploring Patterns of Lactase Persistence*..... student copies*
Master 2.14, *Lactase Persistence and Mutation*..... transparency*

Lesson 3—Evolutionary Processes and Patterns Inform Medicine

Activity 1: Investigating a Mystery Disease

Master 3.1, *A Medical Mystery*..... transparency
Master 3.2, *Investigating a Medical Mystery*..... student copies
Master 3.3, *Blood Test Data*..... 2–3 copies*
Master 3.4, *Reference Manual*..... team copies*
Master 3.5, *Summarizing the Mystery Disease Data*..... transparency
Master 3.6, *Map of the Distribution of Thalassemia across the Eastern Hemisphere*..... transparency
Master 3.7, *The Alpha-Globin Gene and Alpha-Thalassemia* ..... transparency
Master 3.8, *Alpha-Globin and Variation.* ..... student copies
Master 3.9, *Map of the Incidence of Malaria across the Eastern Hemisphere*..... transparency

*Print version only **Web version only

Master 3.10, *Alpha-Thalassemia and Malaria in Papua New Guinea* ..... student copies for half of the class
Master 3.11, *Alpha-Thalassemia and Malaria in Kenya* ..... student copies for half of the class
Master 3.12, *Summing Up Thalassemia, Malaria, and Evolution* ..... student copies
Master 3.13, *Inheriting Thalassemia* ..... student copies (optional)

Activity 2: Using Evolution to Guide Research

Master 3.14, *Cleft Lip and Palate* ..... student copies
Master 3.15, *Calculating Times for an Evoprint* ..... transparency
Master 3.16, *Interpreting Evoprints* ..... transparency
Master 3.17, Irf6 *Evoprint Comparison* ..... student copies**
Master 3.18, *Evoprint Introduction* ..... transparency*
Master 3.19, *Investigating* Irf6 *Evoprints* ..... student copies*
Master 3.20, Irf6 *Evoprints* ..... team copies*

Lesson 4—Using Evolution to Understand Influenza

Master 4.1, *Email from Viroformatics* ..... transparency
Master 4.2, *Notes about Influenza and Evolution* ..... student copies
Master 4.3, *Introduction to Influenza* ..... student copies
Master 4.4, *Influenza Sequences* ..... team copies
Master 4.5, *Aligned Influenza Sequences* ..... transparency
Master 4.6, *Exploring a Genetic Database* ..... student copies**
Master 4.7, *Viroformatics Virus Database* ..... transparency*
Master 4.8, *Influenza Hemagglutinin Sequence* ..... transparency*
Master 4.9, *Influenza-Over-Time Alignment* ..... team copies*
Master 4.10, *Relationships among Influenza Viruses* ..... transparency*
Master 4.11, *Influenza and the Immune System* ..... student copies

Lesson 5—Evaluating Evolutionary Explanations

Master 5.1, *Editing an Article about Vitamin C and Evolution* ..... student copies
Master 5.2, *Editing an Article about Vitamin C and Evolution, Answer Key* ..... transparency

*Print version only **Web version only

Information about MRSA

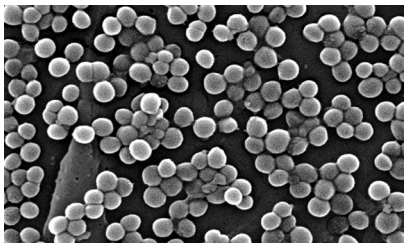
Name: _____

Read the following information about methicillin-resistant *Staphylococcus aureus* (MRSA). Then answer the questions at the end of this handout on your own. It's okay not to know the answers to these questions at this point. You will revise your answers later. Recording your ideas now will make it easier to learn this material. If you have questions, ask them during a class discussion.

What is *Staphylococcus aureus?*

Staphylococcus aureus is a species of bacterium. This species is often called "staph." These bacteria are spherical in shape and sometimes form clusters that look like grapes (see Figure 1). These bacteria are commonly found on the skin or in the nose of healthy people. They are also found on other animals, such as cows, pigs, and chickens. About one in three people have populations of *S. aureus* in their nose. In most cases, this does not cause any illness. But sometimes, *S. aureus* does cause infections. While most are minor skin infections, some are serious, especially if the bacteria get into the body through a wound, such as a cut. They are frequent causes of infections after surgery, bloodstream infections, and pneumonia.

Figure 1. Staphylococcus aureus. *These bacteria are about 1/1,000th millimeter in diameter. If lined up in single file, more than 25,000 of them would be 2.5 centimeters (1 inch) long.*



(CDC, Janice Haney Carr; Jeff Hageman, MHS)

Like other bacteria, *S. aureus* reproduce by binary fission. When an *S. aureus* cell divides, it produces two daughter cells that have the same genetic instructions. However, mutation introduces genetic variation into a population of bacteria.

What is MRSA (pronounced "mer-sah")?

Methicillin is a type of drug called an antibiotic because it can kill bacteria. Methicillin belongs to a group of antibiotics that includes others that may sound familiar to you: penicillin and amoxicillin. Unfortunately, some populations of *S. aureus* are resistant to the group of antibiotics that include methicillin. Resistant populations of bacteria are not killed by this group of drugs. So, MRSA stands for "methicillin-resistant *Staphylococcus aureus*." Can you see why we simply call it "MRSA"?

People infected with MRSA on their skin usually don't have any health problems. Sometimes, they have red, swollen, and painful patches on their skin that look like pimples or spider bites. If the infection penetrates into the body, it can become more serious and cause pneumonia or infections of the blood or bone. Serious problems usually occur in people who have weakened immune systems.

MRSA spread through skin-to-skin contact. They also spread when a person's skin comes into contact with items or surfaces that touched the open wound of an infected person. Passing MRSA on to others can be stopped by simply washing hands, covering open wounds with bandages, and not sharing personal items (such as towels) that have been contaminated with MRSA.

MRSA populations are resistant to the group of antibiotics that are most often used to treat these infections. However, most people with MRSA infections can be treated successfully with other types of antibiotics.

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Questions

1. Researchers developed the antibiotic methicillin to treat people with infections of *S. aureus* that are resistant to penicillin. Within two years, populations of *S. aureus* that were resistant to methicillin started showing up in hospitals. How would a scientist explain how the change may have occurred in *S. aureus* populations?

2. How can the study of evolution (such as the adaptation of bacterial populations to an antibiotic) help researchers improve people's health? Explain your initial ideas.

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Aniridia: An Eye Disease

A husband and wife are expecting a baby. They hope their baby will be healthy. One reason they are concerned is that the father has the disease aniridia (pronounced "an-eye-rid-ee-ah"). A person with aniridia is missing part of the iris, or colored part of the eye. The husband's mother, who is the baby's grandmother, also has the disease.

During a doctor visit, the parents ask what is known about the disease. The doctor tells them that aniridia is a rare disease. Worldwide, it occurs in 1 out of 50,000–100,000 newborns. In addition to missing part of the colored part of the eye, people with aniridia often have pupils that are misshapen or abnormal. They may also have other eye problems, such as glaucoma (increased pressure in the eye), cataracts (clouding of the lens of the eye), or abnormalities in the optic nerve. Most people with aniridia have poor vision. Sometimes, special glasses, medications, or surgery can help, but individuals with aniridia run the risk of losing their eyesight.

Questions

1. Do you think the parents' concern for their baby's health is justified? Why?

2. From the information provided, would you expect aniridia to be caused by an infection, an environmental factor, or a genetic factor? Explain your answer.

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Learning about Human Health from Other Organisms

Name: _____

For each part on this handout, you will analyze a different type of data about the *Pax6* gene.

Part 1: Comparing Amino Acid Sequences

Scientists have purified the protein coded by the *Pax6* gene in humans and other organisms. Look at the sequences of amino acids that make up a portion of the protein from four different species* and answer the questions that follow. Each letter in the sequence represents one amino acid in the protein.

Figure 1. *Amino acids for a portion of the Pax6 protein from humans, mice, zebrafish, and fruit flies.*

```
Human:        LQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE
Mouse:        LQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE
Zebrafish:    LQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE
Fruit fly:    LQRNRTSFTNDQIDSLEKEFERTHYPDVFARERLAGKIGLPEARIQVWFSNRRAKWRREE
```

Questions

1. What do you notice about the amino acid sequences in the different species?

2. What can you infer about the *Pax6* gene from the protein sequences from these four species?

Part 2: What Happens If *Pax6* Does Not Function Normally?

One way that scientists study the function of genes is to find out what happens when the gene is not functioning normally. In other words, what happens if there is a mutation in the gene that affects its function? Use the pictures your teacher will show to answer the following question.

Question

3. On the basis of the pictures, do you think the function of the *Pax6* gene is similar in all four species? Explain your reasoning.

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Part 3: Further Comparing Genes across Species

Scientists now have the ability to isolate DNA sequences that contain the genes they are interested in studying. In some cases, scientists can also insert the genes from one species into another species. The results tell researchers whether the gene functions similarly in the two different species.

Scientists have isolated the *Pax6* gene from a variety of animals, including squid, fruit flies, zebrafish, and mice. Scientists know that fruit flies that have two mutant copies of the *Pax6* gene do not have eyes. When scientists inserted the DNA sequence for the squid *Pax6* gene into these fruit flies, the flies developed eyes. In a second experiment, scientists inserted the DNA sequence for the mouse *Pax6* gene into the fruit flies with two mutant *Pax6* genes. The flies developed eyes in this case, too.

Question

4. What do these experiments suggest about the function of the *Pax6* gene? Explain your thinking.

Summary Question

5. In the sequence data, you saw that the protein coded by the *Pax6* gene is very similar in fruit flies, zebrafish, mice, and humans. In the other experiments, you examined evidence related to the gene's function. Why might many species have almost exactly the same gene that has a similar function?

*Sequences for the fruit fly, mouse, and human are from here: S. Carroll. 2006. *The Making of the Fittest*. New York: W. W. Norton. The zebrafish sequence was downloaded from GenBank, http://www.ncbi.nlm.nih.gov/genbank/.

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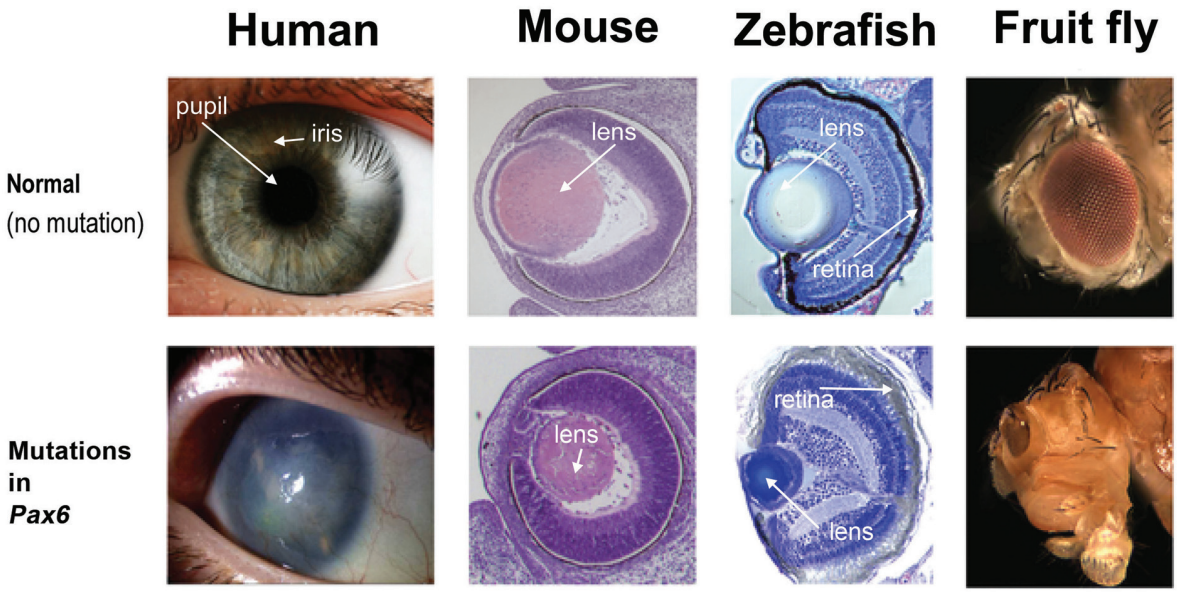
Photographs of Normal Eyes and Eyes with *Pax6* Mutations



Phenotype with *Pax6* mutation	Opaque cornea Iris absent Degenerated retina Opaque lens Pressure on eyeball	Eye small Lens fused to cornea Iris anatomy changed Other eye abnormalities	Eye small Lens small Retina malformed	Eye small or absent

Source: Adapted from N.L. Washington et al. 2009. Linking human diseases to animal models using ontology-based phenotype annotation. *PLoS Biology,* 7(11): e1000247. doi:10.1371/journal.pbio.1000247.

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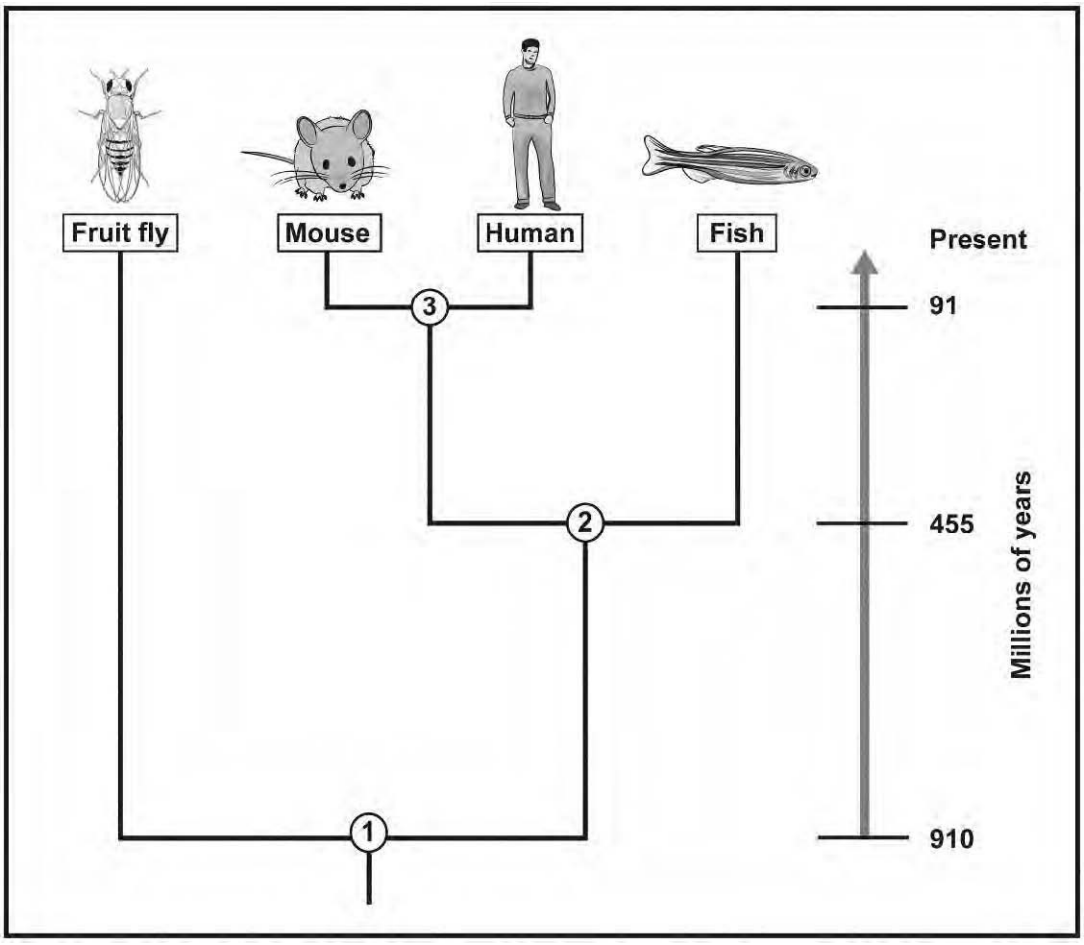
An Evolutionary Tree

Name: _____



Questions

1. What part of the evolutionary tree diagram represents the common ancestor of humans, mice, and zebrafish (but not fruit flies)? Why did you identify this part of the diagram?

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2. Does the evolutionary tree suggest that the mouse is more closely related to the zebrafish or the fruit fly?

3. Is the fruit fly the ancestor of all the other species on the evolutionary tree? Explain your answer.

4. What does the vertical line beneath Point 1 represent?

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Lactase Investigation

Name: _____

Introduction

Lactose is a type of sugar in milk. Other than milk and some dairy products made from milk, lactose is not found naturally in foods humans eat. Lactose is a type of carbohydrate made up of two simple sugars: glucose and galactose. The body digests lactose by breaking it into those simple sugars. The reaction is catalyzed by an enzyme called **lactase**. A summary of the reaction follows:

$$\text{lactose} + \text{water} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$$

If lactase is made by the cells lining the small intestine, lactose gets broken down. The simple sugars are then absorbed into the bloodstream. Individuals who make the lactase enzyme throughout their lives (in other words, it persists) are called **lactase persistent**. People who make lactase are generally **lactose tolerant**.

If someone's small intestine does not make lactase, lactose is not broken down. Then what happens? The lactose passes into the large intestine, or colon. There, two things can happen that can cause problems. First, bacteria in the colon break down the lactose and use the galactose and glucose. In the process, they release hydrogen gas. As this gas builds up, it can cause a bloated feeling. It can also cause increased flatulence. Lactose that is not broken down by bacteria can also change the water balance in the colon. Too much lactose means that more water is drawn into the colon by osmosis, causing diarrhea. People who experience these symptoms when ingesting lactose are called **lactose intolerant**. Because these individuals have usually stopped making the enzyme lactase, they are also sometimes called **lactase nonpersistent**.

In this investigation, you will examine simulated samples from individuals in Europe, Asia, Africa, and the Middle East. You will gather evidence from your samples to determine whether or not the individuals are lactase persistent.

Procedure

1. Record the final color of the glucose test strip after your teacher placed it in the milk.
2. Your teacher will assign you a sample or two and show you information to help you determine the region and country your sample(s) came from. Record all this information in Table 1 (page 2 of this master).
3. Add 2 milliliters of milk to your sample. Hold your sample in your hand for three minutes to keep it near human body temperature.
4. Measure the amount of glucose in the sample using a glucose test strip. Record the result in Table 1.
5. Make a claim about whether or not the individual is lactase persistent. Describe how you used evidence to make that claim.

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Table 1. Lactase Investigation Data

Sample number	Region	Country	Color of glucose test strip when placed in sample *before* the reaction	Color of glucose test strip when placed in sample *after* the reaction	Make a claim about whether or not the individual is lactase persistent. Describe how you used evidence to make this claim.	Were your results confirmed by other researchers?

Source: http://www.medicinenet.com/lactose_intolerance/article.htm

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Lactase Results from Other Researchers

Sample number	Region	Country	Lactase persistence? (Yes/No)
1	East Africa	Kenya	Yes
2	East Africa	Tanzania	Yes
3	Southern Europe	Italy	No
4	West Africa	Nigeria	No
5	East Asia	South Korea	No
6	West Africa	Nigeria	No
7	East Asia	China	No
8	Middle East 2	Saudi Arabia	Yes
9	Northern Europe	Denmark	Yes
10	Southern Europe	Italy	No
11	Middle East 1	Pakistan	No
12	Middle East 2	Saudi Arabia	Yes
13	West Africa	Nigeria	No
14	East Asia	China	No
15	Middle East 2	Saudi Arabia	Yes
16	West Africa	Nigeria	No
17	East Africa	Sudan	Yes
18	Southern Europe	Italy	Yes
19	Middle East 1	Pakistan	No
20	Middle East 2	Saudi Arabia	Yes
21	Middle East 2	Saudi Arabia	Yes
22	Southern Europe	Spain	Yes
23	East Asia	South Korea	No
24	East Africa	Sudan	No
25	West Africa	Nigeria	No
26	Middle East 1	Iran	No
27	Northern Europe	Norway	Yes
28	Northern Europe	Ireland	Yes
29	Middle East 1	Iran	No
30	Northern Europe	United Kingdom	No
31	East Africa	Sudan	No
32	East Africa	Sudan	Yes
33	West Africa	Nigeria	Yes

(*Continued*)

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(*Continued*)

Sample number	Region	Country	Lactase persistence? (Yes/No)
34	Southern Europe	Spain	Yes
35	East Africa	Tanzania	Yes
36	Southern Europe	Greece	No
37	West Africa	Nigeria	No
38	Middle East 2	Saudi Arabia	No
39	Middle East 1	Iran	No
40	East Asia	Japan	No
41	Middle East 1	Afghanistan	No
42	Northern Europe	Norway	Yes
43	East Asia	China	No
44	Northern Europe	Sweden	Yes
45	East Africa	Kenya	No
46	Southern Europe	Greece	No
47	Northern Europe	Sweden	Yes
48	Middle East 2	Saudi Arabia	No
49	East Asia	China	No
50	East Africa	Sudan	Yes
51	East Asia	China	No
52	Middle East 1	Afghanistan	No
53	Middle East 2	Saudi Arabia	No
54	Northern Europe	Sweden	Yes
55	Southern Europe	Spain	No
56	Middle East 2	Saudi Arabia	No
57	East Asia	China	No
58	Middle East 2	Saudi Arabia	Yes
59	West Africa	Nigeria	No
60	West Africa	Nigeria	No
61	Northern Europe	Norway	No
62	Northern Europe	United Kingdom	Yes
63	Middle East 1	Pakistan	Yes
64	East Africa	Tanzania	No
65	Southern Europe	Spain	No
66	West Africa	Nigeria	No
67	Southern Europe	Italy	No
68	Middle East 1	Iran	Yes
69	East Asia	Japan	No
70	Middle East 1	Afghanistan	No

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Investigating Patterns of Lactase Persistence

Name: _____

Work as a group to explore patterns of lactase persistence in people from different parts of the world. Your group will explore three patterns: (1) geographic regions, (2) the role of gender, and (3) differences between adults and infants. After exploring these patterns, work as a group to summarize what you learned. To complete this activity, you will use materials on the Evolution and Medicine Web site:

http://science.education.nih.gov/supplements/evolution/student

Click on "Lesson 2: Investigating Lactose Intolerance and Evolution," then "Fill in samples from other researchers," "View map 2," and finally, "Add samples." After finishing a part, you can click on the "Continue" button to access the map.

Part A: Investigating Geographic Regions

1. Access Map 2, which shows the simulated results from analyses of over 300 people in seven geographic regions.

2. Click on "Explore Lactase Persistence by Geographic Region." Then click on the seven geographic regions to see a summary graph for each region.

3. Estimate the percentage of people who are lactase persistent and lactase nonpersistent in each region. Write that information in Table 1 (below).

Table 1. Investigating Geographic Patterns Summary

Region	Lactase persistent (%)	Lactase nonpersistent (%)
West Africa		
East Africa		
Middle East 1		
Middle East 2		
East Asia		
Northern Europe		
Southern Europe		

4. Make a claim about whether or not lactase persistence varies geographically. Make sure your claim is linked to the evidence in the data table (Table 1).

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Part B: Investigating the Role of Gender

1. Access Map 2, which shows the simulated results from analyses of over 300 people in seven geographic regions.

2. Click on "Explore Lactase Persistence by Gender." Then click on the "Male" and then the "Female" buttons. Record your observations of the patterns you see.

3. Use information on the map to fill in Table 2.

Table 2. Lactase Persistence and Gender Summary

Gender	Lactase persistence	Lactase nonpersistence
Male		
Female		

4. Use the data in the table to make a claim about whether or not lactase persistence is more common in males or females.

Part C: Investigating the Differences between Adults and Infants

1. Access Map 2, which shows the simulated results from analyses of over 300 people in seven geographic regions.

2. The default map that you see shows lactase persistence or lactase nonpersistence for adults. Click on "Explore Lactase Persistence by Age," and then the "Infants" button to see a map of the results for infants sampled for lactase activity. Describe the patterns you see in the results.

3. Use information on the map to fill in Table 3.

Table 3. Lactase Persistence and Age Summary

Age	Lactase activity (for infants) or persistence (for adults)	No lactase activity (for infants) or lactase nonpersistence (for adults)
Infants		
Adults		

4. Why do you think there is a difference between adults and infants for lactase activity?

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The Genetic Basis for Lactase Persistence

Questions

1. Do you think you would find the gene that codes for lactase in both people who are lactase persistent and people who are lactase nonpersistent? Explain your answer.

2. Scientists recognize that two major types of changes in DNA sequences can affect the phenotype of organisms. One type changes the coding sequence of a gene. This can affect the amino acids that form the protein, which can affect the protein's shape and function. The second type of change affects when a gene gets "turned on" or "turned off." What type of change do you think causes the difference between lactase persistence and lactase nonpersistence?

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Lactase and Human Evolution

Questions

1. Nonhuman primates and all other mammals are lactase nonpersistent. Do you think the common ancestor of humans was lactase persistent or lactase nonpersistent? Explain your answer.

2. Genetic studies show that different mutations cause lactase persistence in humans from different geographic regions. Does this evidence suggest that lactase persistence evolved once or more than once in humans? Explain your answer.

3. Did the mutation that causes lactase persistence first come about because people needed the mutation? Explain your answer.

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Explaining the Evolution of Lactase

Scientists have proposed several hypotheses to explain patterns of lactase persistence. Collecting evidence helps them compare the hypotheses. In this portion of the activity, your group will first read descriptions of two of the hypotheses. You will then analyze data and decide whether the data favor one of the hypotheses.

Culture-Historical Hypothesis

Some cultures have a tradition of herding and milking cows, goats, or camels. Cultures that rely on livestock for meat and milk are called "pastoralist." The **culture-historical hypothesis** says that individuals who are lactase persistent had a selective advantage in pastoralist populations. Two scientists separately proposed this hypothesis for the evolution of lactase persistence in humans.

According to this hypothesis, lactase persistent individuals had a higher level of nutrition because they could drink and digest milk. Milk is a high-calorie source of nutrition that is also high in protein. However, some cultures have a large number of individuals who cannot digest lactose. One way to solve this problem is to ferment milk to make cheese. Some cheeses do not have lactose. Yet, the cheese made from fresh milk has about 40 percent fewer calories than fresh milk. So, people who could digest fresh milk could get more calories in their diet than people who ate cheese made from the same amount of milk. Another possibility is that lactase persistent individuals had an advantage because they were able to take in water from the milk. Milk is an important source of liquid, especially in desert regions.

A higher level of nutrition meant that lactase persistent individuals had higher rates of survival and reproduction or had more children who survived and reproduced. Scientists collected data in Africa to test this hypothesis. They examined different groups of people and determined two things. First, they measured the level of lactase persistence in the group. Second, they estimated the level of pastoralism. The culture-historical hypothesis would be supported if populations that have high levels of lactase persistence had high levels of pastoralism historically. Populations that kept livestock but did not milk them would be expected to show low levels of lactase persistence.

Calcium-Absorption Hypothesis

The second hypothesis suggests a different selective advantage for lactase persistent individuals. Understanding this advantage of fresh milk involves knowing something about calcium. This hypothesis is called the **calcium-absorption hypothesis**. Calcium is a vital mineral that supports the structure of our bones and teeth. It's required for muscle contractions and many other functions. Vitamin D helps the body absorb calcium. Today, vitamin D is added to common foods like milk, bread, and cereal because it's so important. However, human diets do not always include foods that are high in vitamin D. Humans *can* make their own vitamin D when ultraviolet (UV) light penetrates their skin.

People who do not get enough calcium or vitamin D can develop rickets, which leads to improper bone growth (Figure 1). Women whose pelvis has been deformed by rickets may not survive the process of childbirth. *This means that women with vitamin D or calcium deficiency may have relatively fewer children.*

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Figure 1. *X-ray showing bone deformities associated with rickets.*

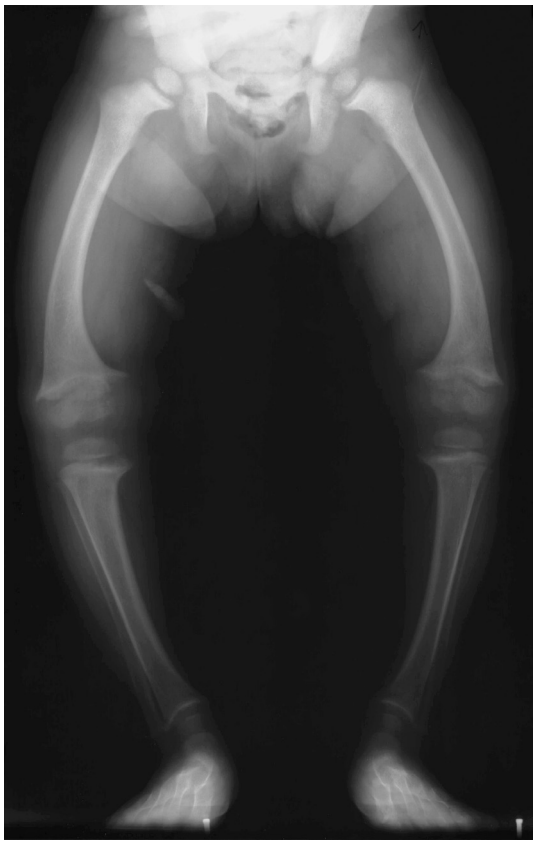

Image courtesy of Michael L. Richardson, M.D.,
University of Washington
Department of Radiology

Fresh milk has a high lactose content. Some studies suggest that lactose also helps the body absorb calcium. Calcium is also present in milk. The calcium-absorption hypothesis suggests that people who could digest lactose had an advantage in environments with limited UV light. Digesting lactose also helped these individuals absorb calcium. Without the lactose, these people would have a problem absorbing calcium because of their low levels of vitamin D. Different types of evidence may support this hypothesis. For example, there should be high levels of lactase persistence in areas that have low amounts of UV at certain times of the year. There should also be evidence showing that rickets was common in the past.

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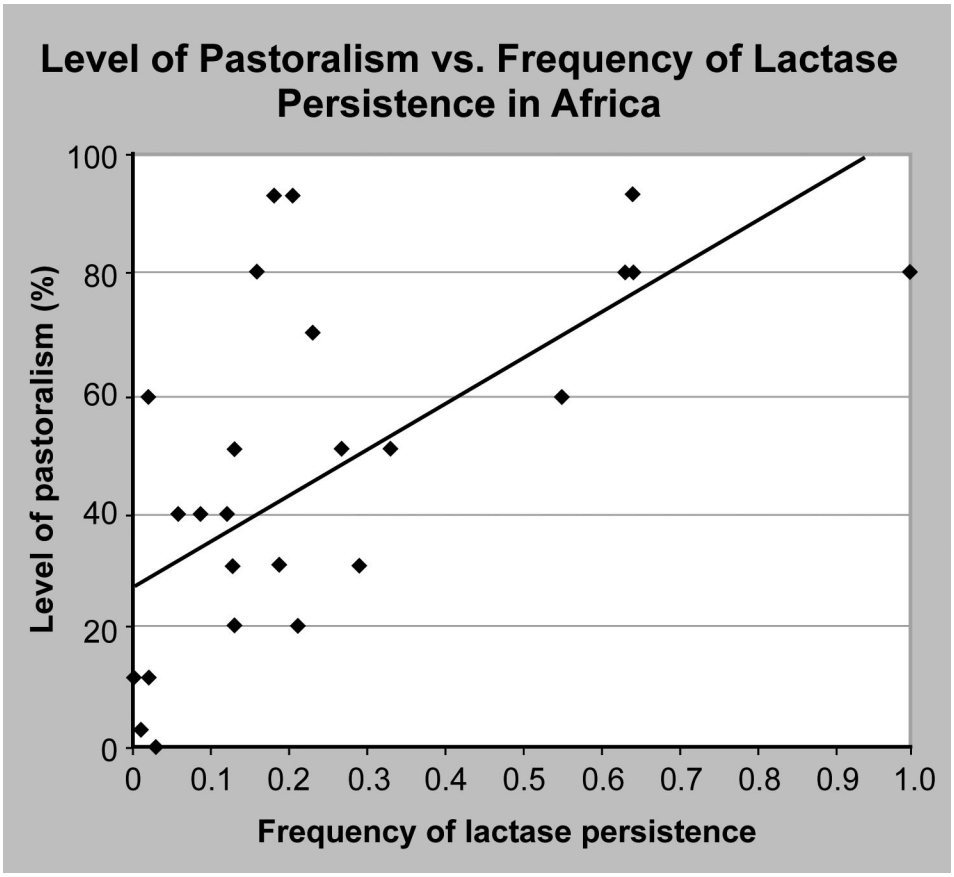
Data from Africa

Question

Do the data in Figure 1 support one of the hypotheses for the evolution of lactase persistence?

Figure 1. *Graph showing the relationship between the levels of pastoralism and the frequency of lactase persistence in Africa. A high level of pastoralism means that a culture used livestock for meat and milk.*



Source: Data from P. Gerbault et al. 2009. Impact of selection and demography on the diffusion of lactase persistence. *PLoS ONE, 4*(7): e6369. doi:10.1371/journal.pone.0006369.

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Data from Europe, Part A

Work with your group to decide whether the data in Figures 1 and 2 support or refute one of the hypotheses for the evolution of lactase persistence.

Figure 1. *Map of average daily ultraviolet (UV) exposure across Earth's surface. Darker shading indicates greater UV exposure.*

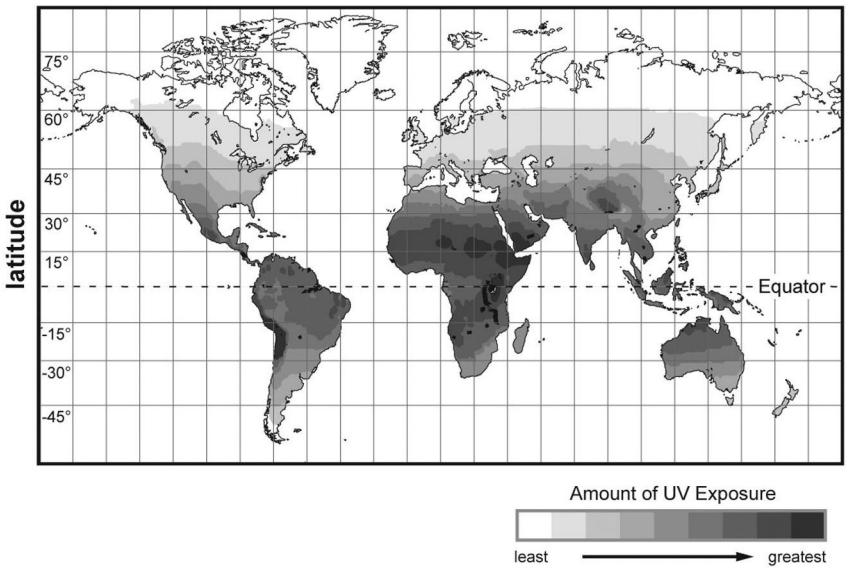
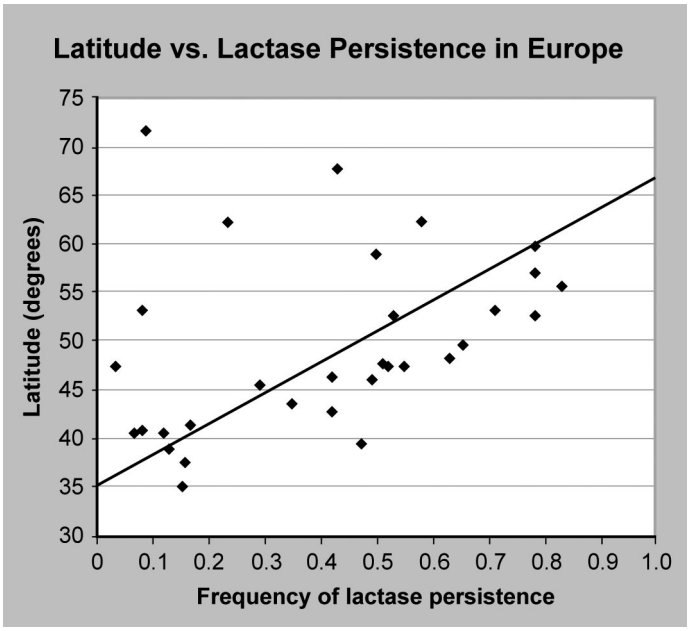


Image copyright George Chaplin. Adapted with permission. *American Journal of Physical Anthropology 107*(2): 221–224, 1998. Copyright 1998 Wiley-Liss, Inc.

Figure 2. *Graph showing latitude and the frequency of lactase persistence in Europe.*



Source: Data from P. Gerbault et al. 2009. Impact of selection and demography on the diffusion of lactase persistence. *PLoS ONE, 4*(7): e6369. doi:10.1371/journal.pone.0006369.

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Data from Europe, Part B

Work with your group to decide whether the data and conclusions described below support or refute one of the hypotheses for the evolution of lactase persistence.

Skeletal evidence

Archaeologists study human remains to infer how frequent a disease might have been in the past. Rickets causes specific changes to bones that allow researchers to determine whether a person had the disease. Use the data in Table 1 to decide whether the frequency of rickets was high in the past.

Table 1. Data about Possible Rickets Cases for Sites in Europe

Approximate time period	Number of skeletons examined	Possible cases of rickets
3000 B.C.E.	616	6*
C.E. 400–1000	635	0
C.E. 800–1100	1,055	6
C.E. 1200	364	1

*Some researchers interpret these skeletons differently and claim they did not have rickets.
Data source: F.J. Simoons. 2001. Persistence of lactase activity among Northern Europeans: A weighing of evidence for the calcium absorption hypothesis. *Ecology of Food and Nutrition 40(5)*: 397–469.

Calcium absorption

Some studies suggest that simple sugars like glucose or galactose can help people absorb calcium, just as lactose does.** When people make yogurt from milk, the lactose gets broken down into simple sugars. This means that people in cultures that made dairy products like yogurt may not have had a problem obtaining enough calcium.

Computer models

Researchers developed a computer model of natural selection and the movement of individuals among populations.† Their results show that the pattern of lactase persistence in Europe could be explained by the culture-historical hypothesis.

** Summarized in F. J. Simoons. 2001. Persistence of lactase activity among Northern Europeans: A weighing of evidence for the calcium absorption hypothesis. *Ecology of Food and Nutrition, 40*(5): 397–469.

† Study by Y. Itan et al. 2009. The origins of lactase persistence in Europe. *PLoS Computational Biology, 5*(8): e1000491. doi:10.1371/journal.pcbi.1000491.

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Summing Up Lactase Persistence and Nonpersistence

Name: _____

In this activity, you learned a lot about lactase persistence and lactase nonpersistence. To help you summarize what you learned, answer each question below.

Questions

1. Do humans vary in their ability to digest lactose? What is the evidence for your answer?

2. Can the ability to digest lactose as an adult be passed from parents to offspring? What is the evidence for your answer?

3. Describe how mutations to DNA are important in lactase persistence and nonpersistence.

4. In certain environments, did digesting lactose seem to affect an individual's ability to survive and reproduce? Explain.

5. What's the evidence that the frequency of the mutation that causes lactase persistence changed in certain groups of people over time?

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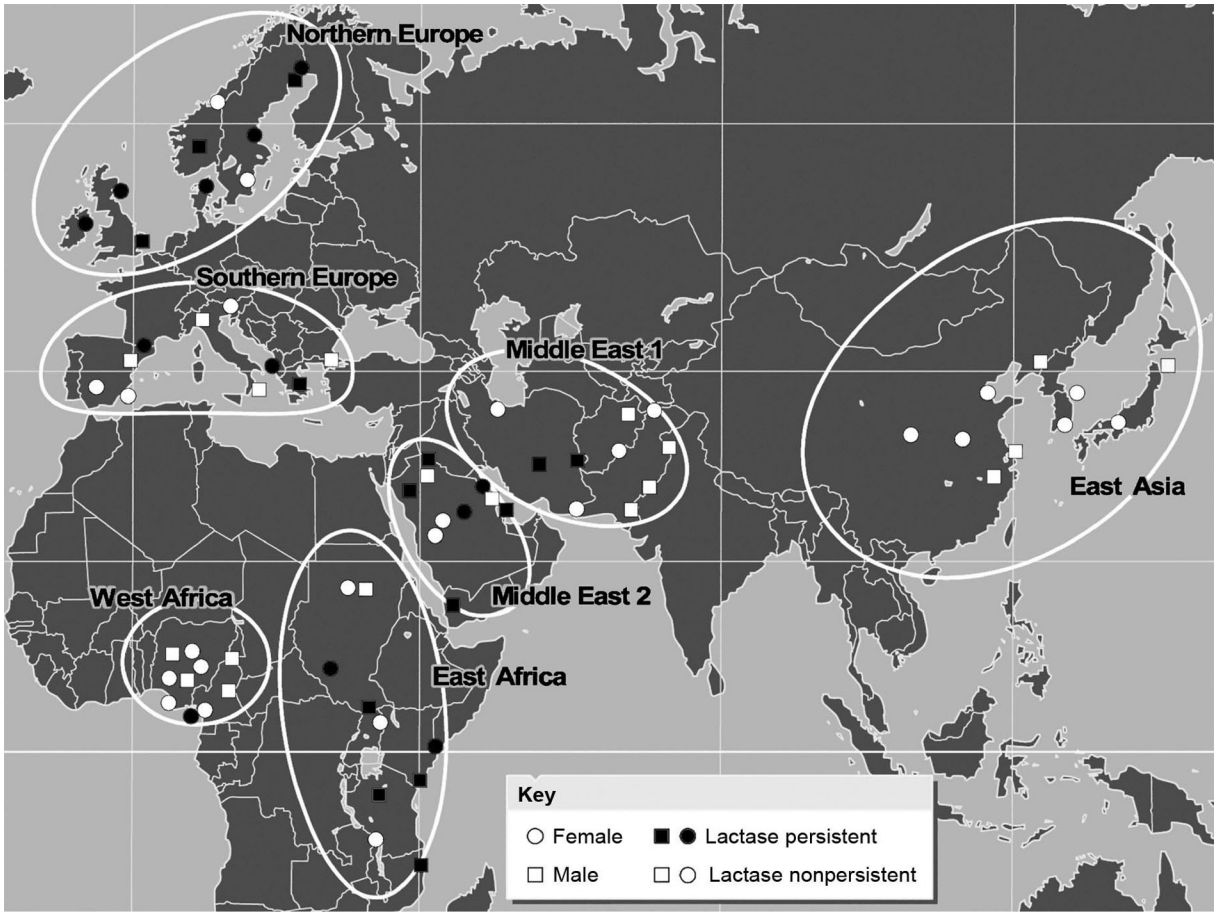
Map of Lactase Test Results



Use the map to answer this question: Does the proportion of people who are lactase persistent vary in different parts of the world?

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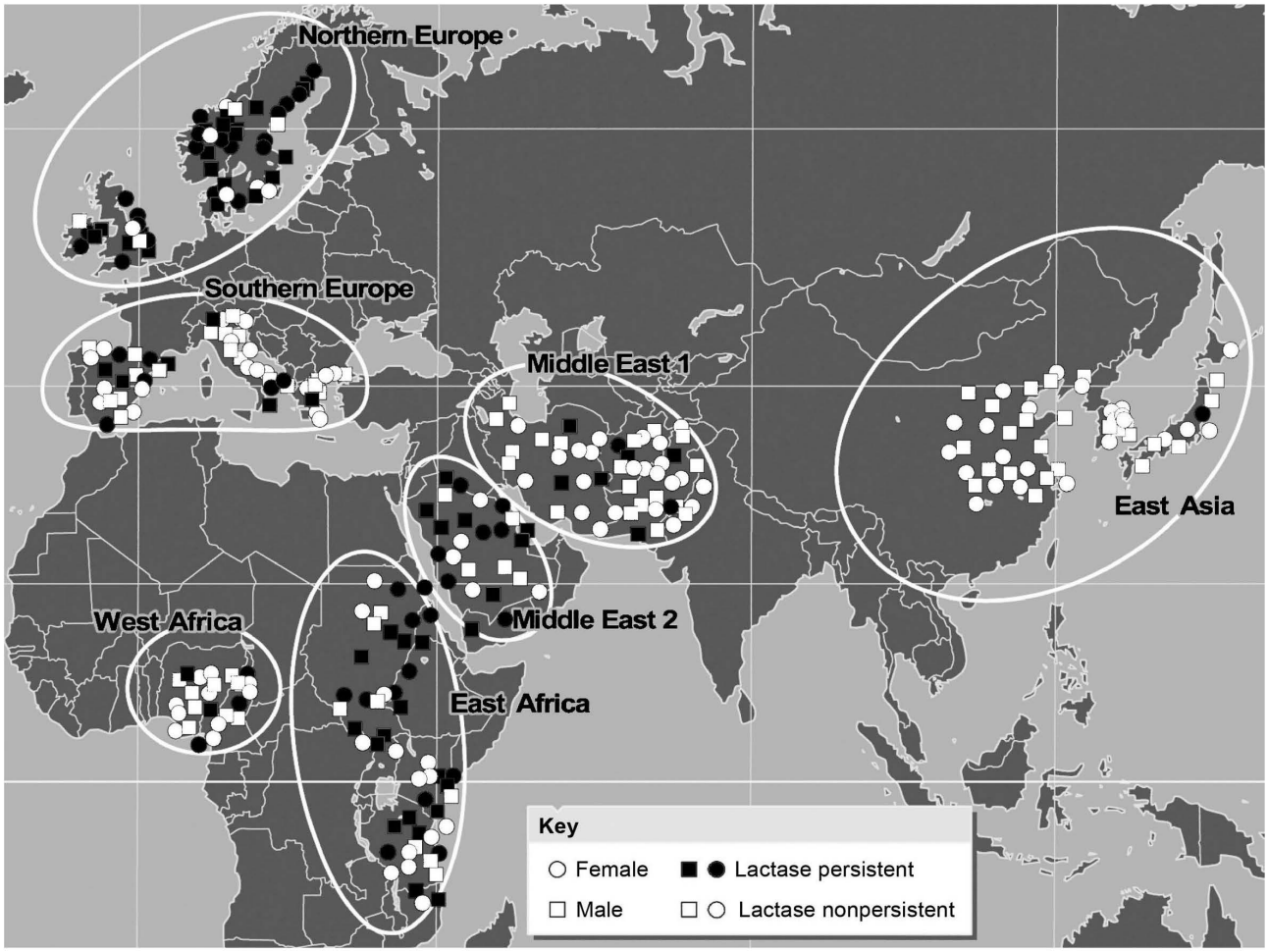
Map of Complete Lactase Test Results



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Exploring Patterns of Lactase Persistence

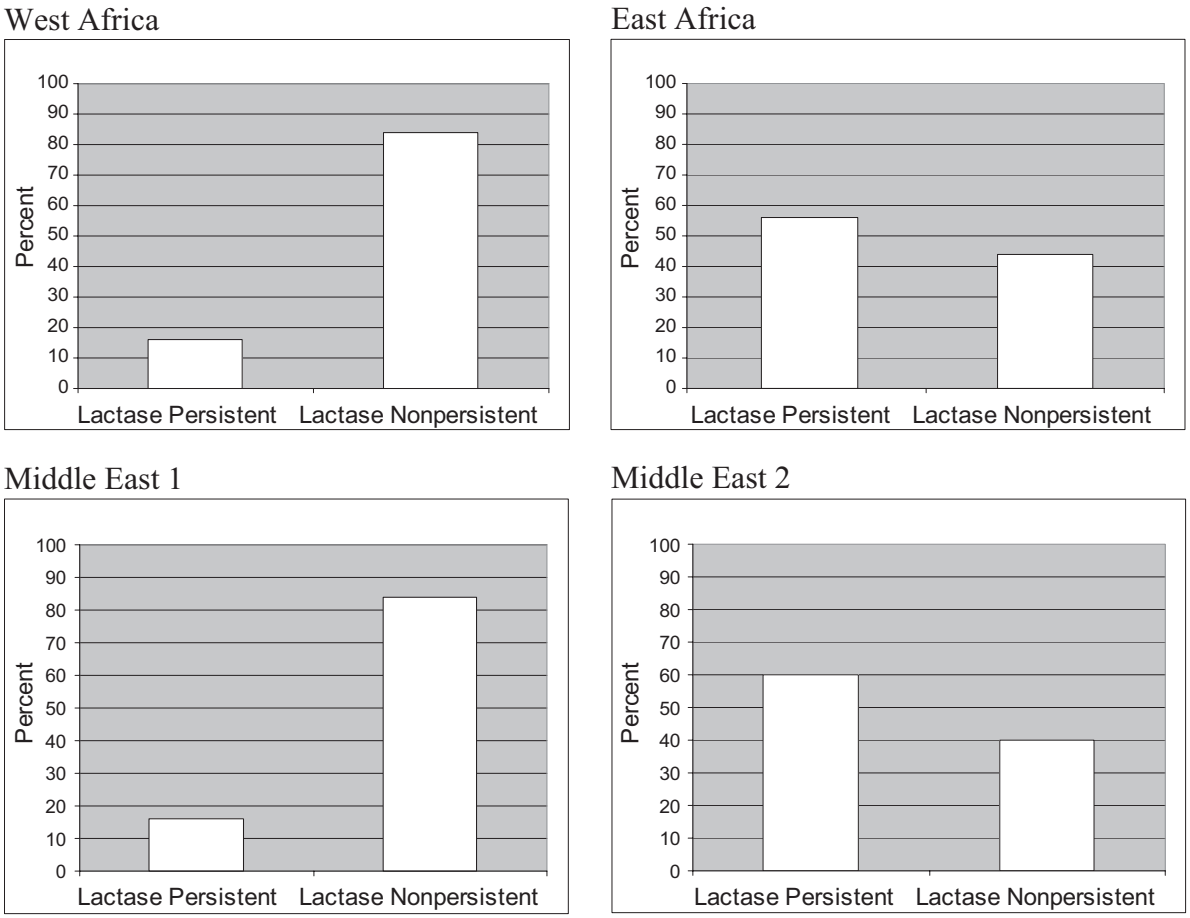
Name: _____

Work as a group to explore patterns of lactase persistence in people from different parts of the world.

Part A: Investigating Geographic Patterns

1. Graphs indicating the percentage of people who are lactase persistent or lactase nonpersistent are in Figure 1. Examine the percentage of people who are lactase nonpersistent and lactase persistent in each region. Write that information in Table 1.

Figure 1. *Graphs of percentage of lactase persistence by region.*



(Continued)

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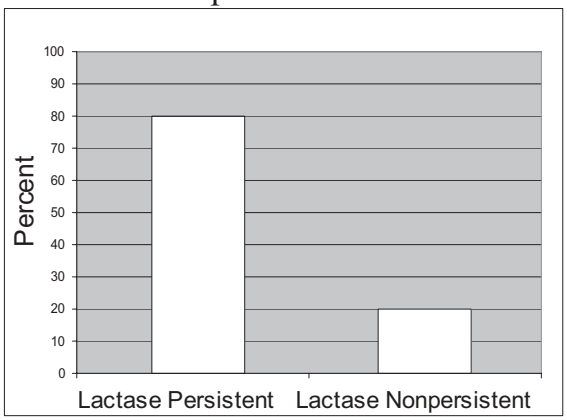
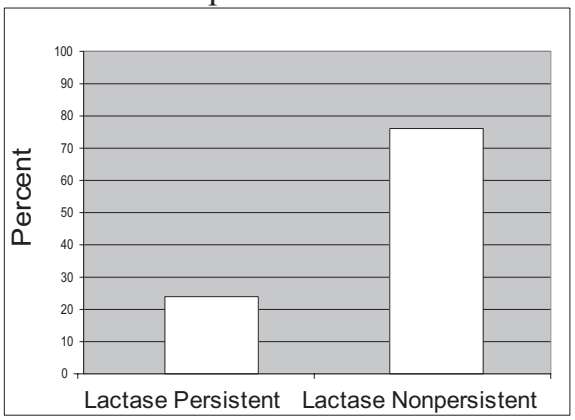
***Figure 1**. (Continued)*

Northern Europe



Southern Europe



East Asia

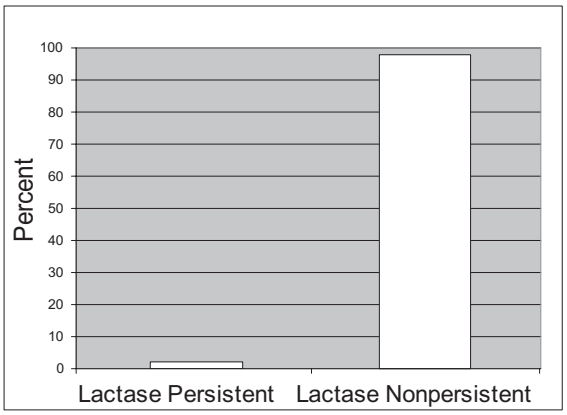


Table 1. Summary of Geographic Patterns of Lactase Persistence

Region	Lactase persistent (%)	Lactase nonpersistent (%)
West Africa		
East Africa		
Middle East 1		
Middle East 2		
Northern Europe		
Southern Europe		
East Asia		

2. Make a claim about whether or not lactase persistence varies geographically. Make sure your claim links to the evidence in Table 1.

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Part B: Investigating the Role of Gender

1. Examine Table 2, which shows the number of males and females who are lactase persistent or lactase nonpersistent for the complete lactase results.

Table 2. Lactase Persistence and Gender Summary

Gender	Lactase persistent	Lactase nonpersistent
Male	56	94
Female	52	98

2. Use the data in the table to make a claim about whether or not lactase persistence is more common in males or females.

Part C: Investigating the Differences Between Adults and Infants

1. Examine the following table, which shows patterns of lactase activity in adults and infants for the complete lactase results.

Table 3. Lactase Persistence and Age Summary Table

Age	Lactase activity (infants) or lactase persistent (adults)	No lactase activity (infants) or lactase nonpersistent (adults)
Infants	299	1
Adults	108	192

2. Why do you think there's a difference between adults and infants for lactase activity?

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Lactase Persistence and Mutation

Table 1. Percentage of Different Mutations for People in Seven Geographic Regions

Geographic Region	Mutation 1 (%)	Mutation 2 (%)	Mutation 3 (%)
West Africa	0	0	0
East Africa	0	26	2
Middle East 1	10	0	0
Middle East 2	0	0	56
Northern Europe	76	0	0
Southern Europe	20	0	0
East Asia	0	0	0

Data source: Enattah et al., 2007; Enattah et al., 2008; Gerbault et al., 2009; Tishkoff et al., 2007.

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A Medical Mystery

High Incidence of Anemia in Papua New Guinea

Health workers are uncovering a health mystery in Papua New Guinea (Figure 1). A high percentage of the citizens on this island in the South Pacific suffer from anemia. Anemia is a medical condition in which a person does not have enough red blood cells to carry oxygen to the cells of the body. People who have anemia can have symptoms ranging from fatigue and shortness of breath to headaches to an irregular heartbeat. Doctors know that many diseases can cause anemia, and they wonder why so many people in Papua New Guinea have it. To find out, researchers are collecting blood samples from many people there who have anemia.

Figure 1. *Map showing the location of Papua New Guinea, one of the countries on the world's second-largest island. Papua New Guinea is north of Australia.*

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Investigating a Medical Mystery

Name: _____

For each patient you investigate, complete the "Microscopic Analysis of Red Blood Cells" and the "Blood Test Data" sections of this master. The Reference Manual (Master 3.4) will help you interpret the information about each patient. Use this information to draw conclusions and then complete the "Making a Diagnosis" section.

When viewing blood samples in the virtual microscope, you will see mostly red blood cells (RBCs). You may also see white blood cells (WBCs). WBCs are usually larger than RBCs and irregular in shape. The nuclei of WBCs are darkly stained in these samples.

Patient _____

Microscopic Analysis of Red Blood Cells

1. Use the measurement tool to measure the diameter of four red blood cells in the field of view of the virtual microscope.

   Cell 1 _____ Cell 2 _____ Cell 3 _____ Cell 4 _____

   Average size of red blood cell _____

2. Observe the red blood cells in the microscope, noting the shape, color, and any irregularities you see. Record your observations here.

Conclusions from the Microscopic Analysis

Did you find any differences between your patient's red blood cells and normal red blood cells that could be important clues to the patient's condition? Explain.

Blood Test Data

Analyze the blood test data provided. Record any test results that are outside the normal range. If values are outside a normal range, be sure to record whether they are above or below normal.

Conclusions from the Blood Test Data

Do the blood test data indicate a specific disease? Explain. Use the information in the Reference Manual to help you determine the patient's problem.

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Making a Diagnosis

Now that you have observations and data to inform you, use the information from both the microscopic analysis and the blood test data to diagnose the patient's condition. Explain how both types of data support your diagnosis.

Patient _____

Microscopic Analysis of Red Blood Cells

1. Use the measurement tool to measure the diameter of four red blood cells in the field of view of the virtual microscope.

   Cell 1 _____ Cell 2 _____ Cell 3 _____ Cell 4 _____

   Average size of red blood cell _____

2. Observe the red blood cells in the microscope, noting the shape, color, and any irregularities you see. Record your observations here.

Conclusions from the Microscopic Analysis

Did you find any differences between your patient's red blood cells and normal red blood cells that could be important clues to the patient's condition? Explain.

Blood Test Data

Analyze the blood test data provided. Record any test results that are outside the normal range. If values are outside a normal range, be sure to record whether they are above or below normal.

Conclusions from the Blood Test Data

Do the blood test data indicate a specific disease? Explain. Use the information in the Reference Manual to help you determine the patient's problem.

Making a Diagnosis

Now that you have observations and data to inform you, use the information from both the microscopic analysis and the blood test data to diagnose the patient's condition. Explain how both types of data support your diagnosis.

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Blood Test Data

Patient 1, Adult Male

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.4 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 1. Complete Blood Count Results for Patient 1

Blood component	Test result	Normal values
White blood cell (WBC) count	5,600 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.7 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	32%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	8.4 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	68 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	280 µg/dL	240–450 µg/dL
Serum ferritin concentration	75 ng/mL	15–306 ng/mL

Patient 2, Adult Female

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.7 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 2. Complete Blood Count Results for Patient 2

Blood component	Test result	Normal values
White blood cell (WBC) count	4,700 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.1 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	29%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	11.5 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	70 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	12	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	320 µg/dL	240–450 µg/dL
Serum ferritin concentration	62 ng/mL	15–306 ng/mL

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Patient 3, Adult Female

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.2 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 3. Complete Blood Count Results for Patient 3

Blood component	Test result	Normal values
White blood cell (WBC) count	8,700 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	3.7 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	25%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	9.4 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	68 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	15	—
RDW (RBC distribution width)	High	—
Total iron-binding capacity (TIBC)	480 µg/dL	240–450 µg/dL
Serum ferritin concentration	11 ng/mL	15–306 ng/mL

Patient 4, Adult Male

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.1 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 4. Complete Blood Count Results for Patient 4

Blood component	Test result	Normal values
White blood cell (WBC) count	6,500 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.2 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	33%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	12.5 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	79 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	12	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	350 µg/dL	240–450 µg/dL
Serum ferritin concentration	81 ng/mL	15–306 ng/mL

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Patient 5, Adult Female

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.8 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 5. Complete Blood Count Results for Patient 5

Blood component	Test result	Normal values
White blood cell (WBC) count	4,800 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	3.9 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	30%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	10.8 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	77 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	320 µg/dL	240–450 µg/dL
Serum ferritin concentration	60 ng/mL	15–306 ng/mL

Patient 6, Adult Female

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.0 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 6. Complete Blood Count Results for Patient 6

Blood component	Test result	Normal values
White blood cell (WBC) count	6,400 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.0 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	32%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	10.8 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	79 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	380 µg/dL	240–450 µg/dL
Serum ferritin concentration	61 ng/mL	15–306 ng/mL

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Patient 7, Adult Male

Microscopic Examination of Blood

- **Average red blood cell diameter: 7.4 µm**
- **Microscopic examination: Red blood cells were normal in color. Some red blood cells had an abnormal, sickle shape.**

Table 7. Complete Blood Count Results for Patient 7

Blood component	Test result	Normal values
White blood cell (WBC) count	12,000 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.2 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	38%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	8.4 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	85 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	Not reported	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	220 µg/dL	240–450 µg/dL
Serum ferritin concentration	148 ng/mL	15–306 ng/mL

Patient 8, Adult Male

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.5 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 8. Complete Blood Count Results for Patient 8

Blood component	Test result	Normal values
White blood cell (WBC) count	4,800 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.5 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	36%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	13 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	79 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	400 µg/dL	240–450 µg/dL
Serum ferritin concentration	78 ng/mL	15–306 ng/mL

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Patient 9, Adult Female

Microscopic Examination of Blood

- **Average red blood cell diameter: 7.8 µm**
- **Microscopic examination: Red blood cells were normal in color. Shape was also normal.**

Table 9. Complete Blood Count Results for Patient 9

Blood component	Test result	Normal values
White blood cell (WBC) count	6,800 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.8 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	42%	Males: 42–50% Females: 36%–45%
Hemoglobin (Hb)	13.3 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	87 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	Not reported	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	400 µg/dL	240–450 µg/dL
Serum ferritin concentration	114 ng/mL	15–306 ng/mL

Patient 10, Adult Male

Microscopic Examination of Blood

- **Average red blood cell diameter: 6.3 µm**
- **Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.**

Table 10. Complete Blood Count Results for Patient 10

Blood component	Test result	Normal values
White blood cell (WBC) count	4,700 cells/µL	4,400–11,300 cells/µL
Red blood cell (RBC) count	4.3 million cells/µL	Males: 4.5 million–5.9 million cells/µL Females: 4.1 million–5.1 million cells/µL
Hematocrit	32%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	12.8 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	74 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	400 µg/dL	240–450 µg/dL
Serum ferritin concentration	76 ng/mL	15–306 ng/mL

Source: Blood values based on data in K. Kaushansky et al. 2010. *William's Hematology* (8th ed.). New York: McGraw-Hill.

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Reference Manual

The Complete Blood Count

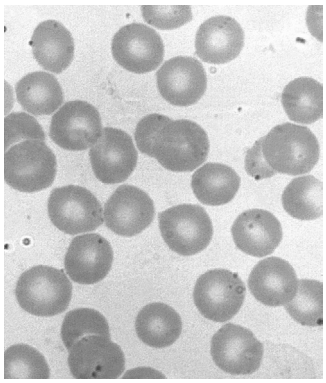
The complete blood count (CBC) is a screening test used to diagnose many diseases. The test can reveal problems with red blood cell (RBC) production and destruction, and it can help diagnose infection, allergies, and problems with blood clotting and fluid volume. It actually consists of several tests that examine different parts of the blood, including the following:

- RBC count is the number of RBCs per volume of blood.
- White blood cell (WBC) count is the number of WBCs per volume of blood.
- Hemoglobin measures the amount of oxygen-carrying protein in the blood.
- Hematocrit measures the percentage of RBCs in a given volume of whole blood.
- Mean corpuscular volume (MCV) is a measurement of the average size of RBCs. MCV increases when the RBCs are larger than normal (macrocytic) and decreases when the RBCs are smaller than normal (microcytic).
- The MCV:RBC ratio is obtained by dividing the mean corpuscular volume by the red blood cell count.
- RBC distribution width (RDW) is a measure of variation in RBC width. Normally, RBCs are fairly uniform in size and shape. In some diseases, a given blood sample may have a high RDW, meaning that there is a great deal of variation in the size of the RBCs in the sample.
- Total iron-binding capacity (TIBC) shows whether there is too much or too little iron in the blood.
- The serum ferritin concentration indicates the body's stores of iron.

Appearance of Normal RBCs

In a normal blood sample prepared for viewing under the microscope, the RBCs are 7–8 micrometers (µm) in diameter. They are very similar in size to each other and have a smooth surface. RBCs are usually dark red. (However, because of their biconcave shape, the center of the cell may look lighter when viewed in the microscope.) Mature RBCs (the kind seen in a blood sample in a microscope) do not have a nucleus. WBCs are often larger than RBCs and have nuclei that are irregularly shaped and darkly stained.

Figure 1. *Normal red blood cells.*



CDC, Dr. Mae Melvin

Abbreviations

The blood test data use the following abbreviations.

- Deciliter (dL) is a unit of volume equivalent to 10^{-1} liter, or 100 milliliters (one-tenth of a liter).
- Milliliter (mL) is a unit of volume equivalent to 10^{-3} liter (one-thousandth of a liter).
- Microliter (µL) is a unit of volume equivalent to 10^{-6} liter (one-millionth of a liter).
- Femtoliter is a unit of volume equivalent to 10^{-15} liter (one-quadrillionth of a liter).
- Nanogram is a unit of mass equal to 10^{-9} grams (one-billionth of a gram).
- Picogram is a unit of mass equal to 10^{-12} grams (one-trillionth of a gram).

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Disease: Alpha-thalassemia

Brief Description: Alpha-thalassemia refers to a disease characterized by reduced or no production of the alpha-globin proteins that form hemoglobin.

Cause: Alpha-thalassemia is caused by changes in the alpha-globin genes on chromosome 16. Each person has four copies of this gene.

Diagnosis: Doctors usually use blood tests to diagnose alpha-thalassemia. Because it is an inherited disease, they will also check family history. In some cases, they may order DNA testing. Thalassemia affects both males and females.

> **Appearance of Red Blood Cells (RBCs):** In patients with alpha-thalassemia, RBCs are often smaller than normal (microcytosis; normal RBCs are 7–8 micrometers (µm) in diameter). The size of the RBCs usually relates to the number of nonfunctional copies of the alpha-globin gene. If a person has one nonfunctional copy, the cells may only be slightly smaller than normal. The RBCs are significantly smaller than normal in people with two or more nonfunctional copies. RBCs in a person with thalassemia may be lighter in color.
>
> The **mean corpuscular volume:RBC ratio (MCV:RBC ratio)** is a way to distinguish thalassemia from other kinds of anemia. In individuals with alpha-thalassemia, the MCV:RBC ratio is less than 13. In iron deficiency anemia, the ratio is above 13.
>
> The **hematocrit** (percentage of blood taken up by RBCs) is usually decreased in people with alpha-thalassemia.
>
> The **total iron-binding capacity (TIBC)** is usually normal in thalassemia.
>
> The **RBC distribution width (RDW)** value is normal in thalassemia. The RBCs in a sample from a patient who has thalassemia may be somewhat smaller than normal, but they are similar in size to the other cells in the sample.

Symptoms: Symptoms of alpha-thalassemia range from no or mild symptoms to severe. One form of alpha-thalassemia is almost always fatal. In general, the symptoms are more serious in patients with more nonfunctional copies of alpha-globin genes.

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Disease: Iron deficiency anemia

Brief Description: Iron deficiency anemia is a common type of anemia—a condition in which the blood lacks adequate healthy red blood cells (RBCs). These cells carry oxygen to the body's tissues.

Cause: Normally, people get iron from the food they eat. In addition, iron can be recycled from old RBCs. Iron deficiency anemia occurs if people do not consume enough iron in the diet, do not produce enough of the iron-containing hemoglobin, or lose too much iron, which occurs most commonly through blood loss.

Diagnosis: Doctors use blood tests to diagnose iron deficiency anemia. Indications of iron deficiency anemia include the following:

- **Abnormal RBCs.** The RBCs in someone with iron deficiency anemia are smaller than normal (microcytosis; normal RBCs are 7–8 µm in diameter) and paler in color than normal (hypochromic). The cells also may be irregular in size and shape. (RBCs within a sample may have different sizes and not be as smooth and round as normal RBCs.)
- **Hemoglobin levels.** Hemoglobin levels in someone with iron deficiency anemia are lower than normal.
- **Hematocrit.** In a person who has iron deficiency anemia, the hematocrit readings are below normal.

Serum Ferritin Concentration: At times, it may be hard to distinguish between iron-deficiency anemia and thalassemia. The serum ferritin concentration is one way that doctors can determine whether a person has alpha-thalassemia or iron deficiency anemia. Iron deficiency anemia is diagnosed when a person's **serum ferritin concentration** is less than 12 ng/mL.

Also, in iron deficiency anemia,

- the **mean corpuscular volume:RBC (MCV:RBC) ratio** is greater than 13,
- the **RBC distribution width (RDW)** is high, indicating a larger variation in size of the RBCs, and
- the **total iron-binding capacity (TIBC)** measurement is above normal.

Symptoms: In mild cases, a person who has iron deficiency anemia may not have any noticeable symptoms. As the deficiency becomes more serious, a person may notice symptoms including the following:

- extreme fatigue
- irregular heartbeat
- pale skin
- shortness of breath
- dizziness
- weakness
- increased number of infections
- headaches

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Disease: Sickle cell disease

Brief Description: Sickle cell disease is an inherited form of anemia. As in other types of anemia, in sickle cell disease there are not enough healthy red blood cells (RBCs) to carry adequate oxygen to all the cells of the body.

Cause: Sickle cell disease is a genetic disease caused by an abnormal type of hemoglobin called hemoglobin S. Hemoglobin S distorts the shape of red blood cells, especially when oxygen levels
are low.

In someone who has sickle cell disease, the RBCs are distorted. Instead of the smooth, circular, biconcave shape of normal RBCs, some of the cells are shaped like crescents. The crescent-shaped cells can clog small blood vessels.

Diagnosis: Sickle cell disease is diagnosed by examining cells under a microscope and with a blood test.

> **Appearance of RBCs:** In sickle cell disease, some of a person's RBCs have a characteristic shape that can be observed under a microscope. Sickle cells are crescent shaped—or shaped like the tool called a "sickle."
>
> In an individual with sickle cell disease,
> - the **total iron-binding capacity (TIBC)** measurement is sometimes below normal.
> - the **white blood cell (WBC) count** is usually somewhat high.

Symptoms: Sickle cells are destroyed rapidly in the body, causing anemia, jaundice, and gallstones.

The sickle cells also block the flow of blood through vessels, resulting in lung tissue damage, pain episodes, and strokes. These cells also cause damage to most organs including the spleen, kidneys, and the liver. Patients with sickle cell disease, especially young children, with damage to the spleen can be easily overwhelmed by certain bacterial infections.

Symptoms may include the following:
- attacks of abdominal pain
- bone pain
- delayed growth and puberty
- jaundice
- rapid heart rate
- chest pain
- poor eyesight/blindness
- strokes
- skin ulcers

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Summarizing the Mystery Disease Data

Table 1. Summary of Mystery Disease Diagnoses and Evidence

Patient	Diagnosis	Key evidence used for diagnosis
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

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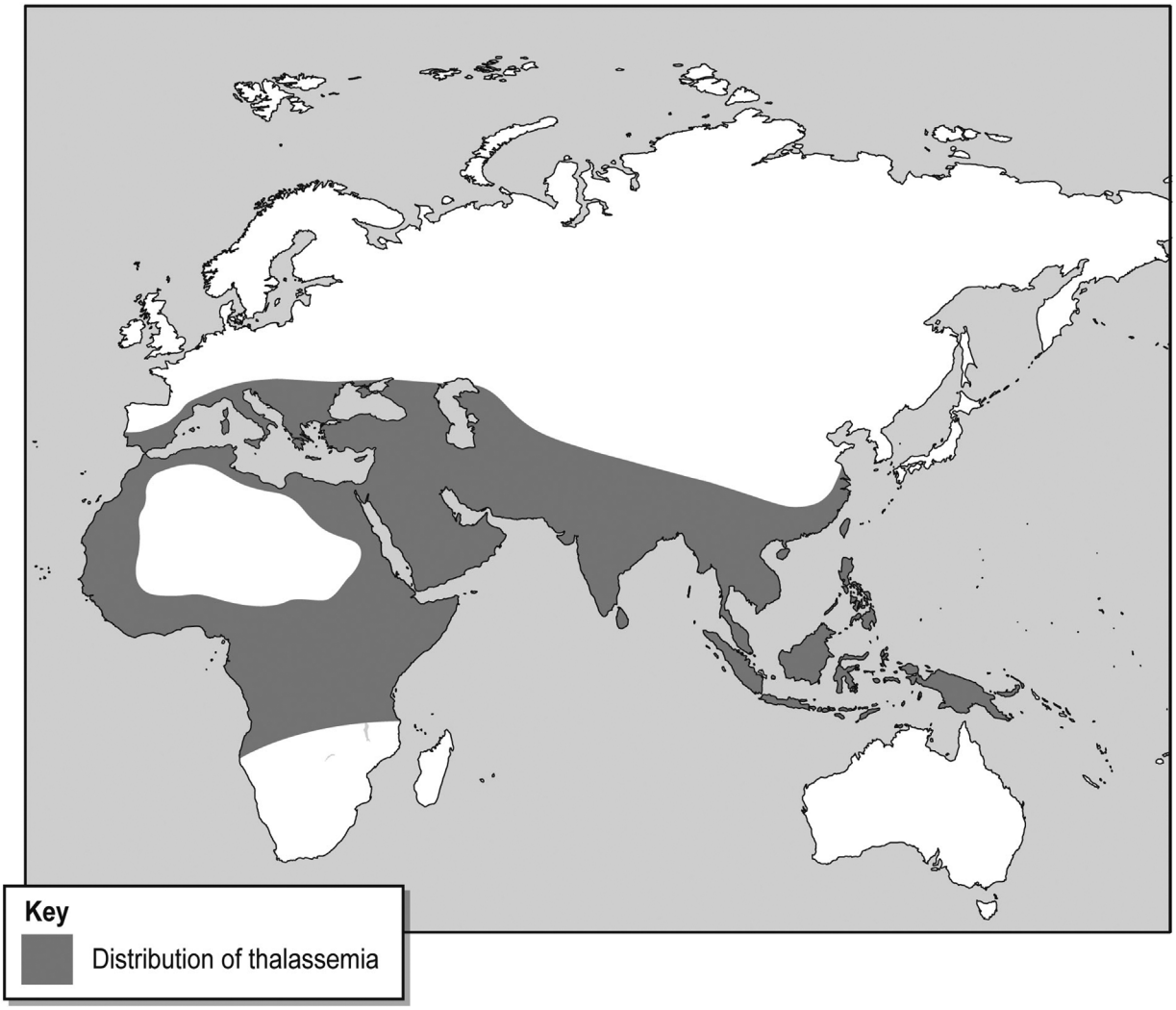
Map of the Distribution of Thalassemia across the Eastern Hemisphere



Map adapted from: http://science.jrank.org/pages/48501/Sickle-Cell-Anemia-Thalassemia.html

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The Alpha-Globin Gene and Alpha-Thalassemia

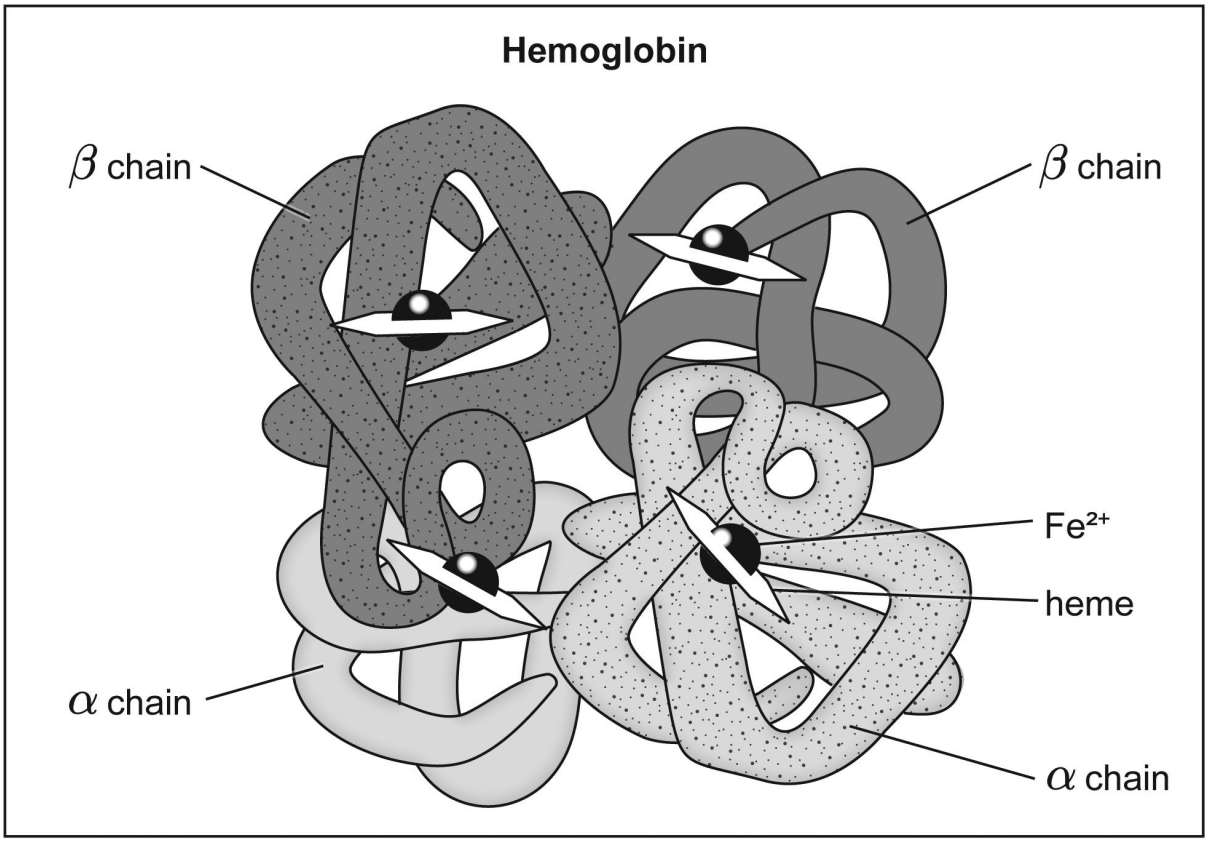
Part 1

Structure of Hemoglobin

Alpha-thalassemia is caused by problems in the production of the alpha-globin protein in hemoglobin.

Hemoglobin is normally made up of two alpha-globin protein chains and two beta-globin protein chains. Different genes code for the alpha- and beta-protein chains.

Figure 1. *Schematic of hemoglobin protein complex.*



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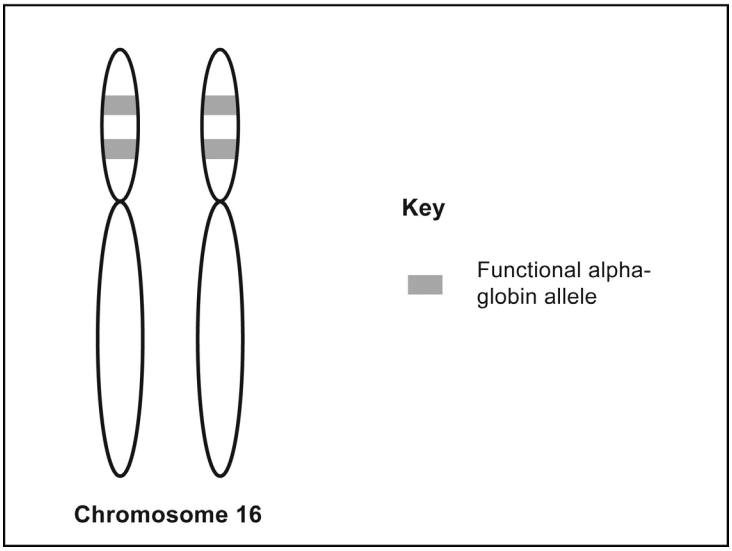
Alpha-Globin Gene

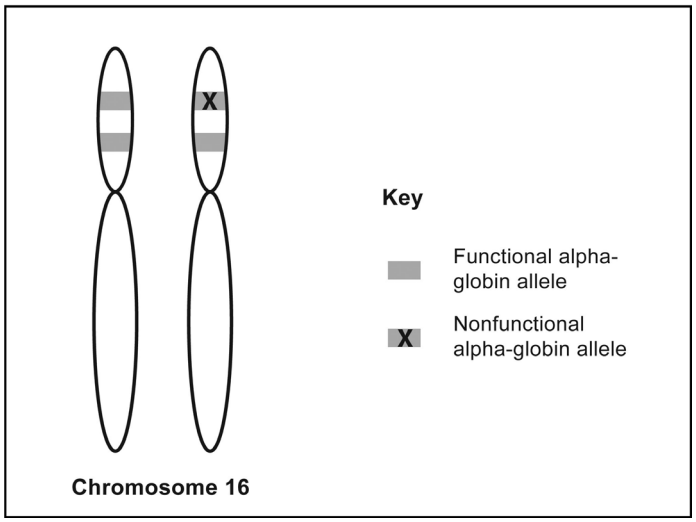
The alpha-globin gene, on chromosome 16 in humans, codes for the alpha-globin protein. Humans normally have four copies of the alpha-globin gene.

Figure 2. *Schematic showing two copies of chromosome 16 and four copies of the alpha-globin gene. All the alleles of the alpha-globin gene are functional in this individual.*



Individuals with alpha-thalassemia have a problem with one or more of their alpha-globin alleles.

Figure 3. *Schematic showing two copies of chromosome 16 and four copies of the alpha-globin gene. One allele of the alpha-globin gene in this individual is nonfunctional.*



Individuals with alpha-thalassemia do not make as much alpha-globin protein as normal individuals do. The amount of protein they make depends on how many working alleles of the alpha-globin gene a person has.

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Part 2

Table 1. Alpha-globin Gene Functional and Nonfunctional Alleles and Related Diseases

Number of functional alleles of the alpha-globin gene	Number of nonfunctional alleles of the alpha-globin gene	Name of disease	Symptoms
4	0	Normal condition (healthy individual)	Not applicable
3	1	Alpha-thalassemia silent carrier	• Usually no symptoms and no anemia • Blood tests usually normal • Hemoglobin normal • Slight changes in size of red blood cells (smaller than normal—microcytic) • Slightly lighter color of red blood cells (hypochromic)
2	2	Alpha-thalassemia trait	• Mild anemia • Small red blood cells (microcytic) • Light, pale color of red blood cells (hypochromic) • Blood tests usually normal • Hemoglobin normal
1	3	Hemoglobin H (HbH) disease	• Moderate to severe anemia • Small red blood cells (microcytic) • Light, pale color of red blood cells (hypochromic) • Fatigue • Mild jaundice • Enlarged spleen • Bone deformities (in some cases)
0	4	Alpha-thalassemia major or hemoglobin Barts hydrops fetalis (Hb Barts) syndrome	• Usually fatal before or shortly after birth

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Alpha-Globin and Variation

Name: _____

Answer the following questions about the alpha-globin gene. Remember to explain your answers.

Questions

1. What might cause the alpha-globin gene not to function?

2. How are differences in the thalassemia trait (phenotype) passed from parents to offspring?

3. Now you know the genetic basis for alpha-thalassemia. What can you add to your description of the variation for thalassemia in humans?

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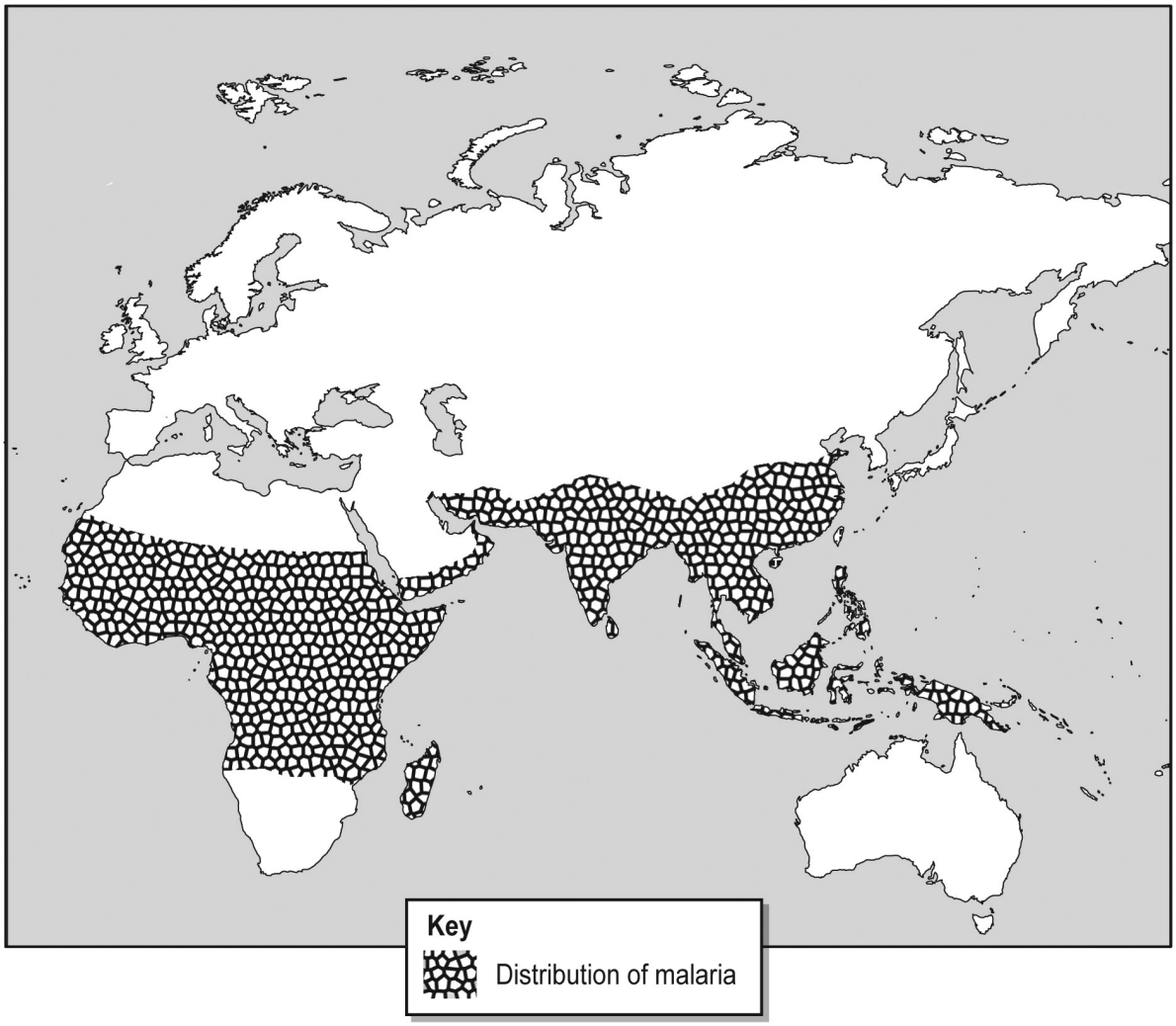
Map of the Incidence of Malaria across the Eastern Hemisphere



Source: Centers for Disease Control and Prevention, Division of Parasitic Diseases, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID).

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Alpha-Thalassemia and Malaria in Papua New Guinea

Name: _____

Scientists studying populations in Papua New Guinea have developed a mathematical model to investigate whether people who have thalassemia have a higher risk of developing severe malarial anemia. Severe malarial anemia is often fatal unless the individual receives a blood transfusion within a short time.

The scientists use a statistic from medical studies called an "odds ratio," which compares the level of risk for different groups. People with thalassemia have at least one nonfunctional copy of the alpha-globin gene.

For this study, an odds ratio

- **equal to 1** means that individuals with thalassemia are as likely to have severe malarial anemia as are normal individuals;
- **greater than 1** means that individuals with thalassemia are **more** likely to have severe malarial anemia than are normal individuals; and
- **less than 1** means that that the individuals with thalassemia are **less** likely to have severe malarial anemia than are normal individuals.

Table 1. Alpha-Thalassemia and Severe Malarial Anemia

Risk factor	One nonfunctional allele of the alpha-globin gene	Two nonfunctional alleles of the alpha-globin gene
Risk of developing severe malarial anemia	0.74	0.52

Source: Data from F.J.I. Fowkes et al. 2008. Increased microerythrocyte count in homozygous α^+-thalassaemia contributes to protection against severe malarial anaemia. *PLoS Medicine, 5*(3): 494-501: e56. doi:10.1371/journal.pmed.0050056

Questions: Fill in the missing word or words for each sentence below.

1. A person who has one nonfunctional allele of the alpha-globin gene is _____ likely to develop severe malarial anemia than a normal individual (all alleles of the gene are functional).

2. A person who has two nonfunctional alleles of the alpha-globin gene is _____ likely to develop severe malarial anemia than a normal individual (all alleles of the gene are functional).

3. After analyzing the data, summarize what you have learned about the relationship between alpha-thalassemia and malaria by completing the following sentence.

   The data suggest that individuals with alpha-thalassemia _____

   _____

   _____

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Alpha-Thalassemia and Malaria in Kenya

Name: _____

Scientists have been studying the relationship between thalassemia and the severity of malarial anemia in a population in Kenya, a country in East Africa. Use the data in the table below to explore the relationship between thalassemia and malaria.

Consider this question: **Is each consequence of malaria more or less frequent in different genotypes?**

To understand the data in the following table, it's important that you make the appropriate comparisons. For each symptom, compare the data for each genotype with each other. In other words, for each row in the table, compare the data in the three columns.

Table 1. Consequences of Malaria by Alpha-thalassemia Genotypes

Consequence of malaria	Four functional alleles of the alpha-globin gene	One nonfunctional allele of the alpha-globin gene	Two nonfunctional alleles of the alpha-globin gene
Coma	45.1%	43.2%	39.8%
Severe anemia (hemoglobin less than 5 g/dL)	25.8%	22.4%	18.1%
Death	12.5%	10.4%	8.4%

Note: Each column shows the percentage of patients within each of three possible genotypes that showed specific symptoms when checking into a hospital due to complications from malaria.

Source: Data from T.N. Williams et al. 2005. Both heterozygous and homozygous α^+ thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood, 106*(1): 368-371.

Question

After analyzing the data, summarize what you have learned about the relationship between alpha-thalassemia and malaria by completing the following sentence.

The data suggest that individuals with alpha-thalassemia _____

_____

_____

_____

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Summing Up Thalassemia, Malaria, and Evolution

Name: _____

Answer the following questions to help you develop an explanation about alpha-thalassemia and its relationship to malaria in humans.

Questions

1. Do the data from the studies in Papua New Guinea and Kenya support the hypothesis that individuals who have thalassemia might have some advantage over other individuals when living in an area where malaria is common? Explain.

2. Depending on their genotype, individuals with nonfunctional alpha-globin alleles may have symptoms that range from mild to more serious, including anemia, fatigue, enlarged spleen, liver problems, or even death. If the alpha-globin mutations are passed from parent to child, and individuals with four nonfunctional alpha-globin alleles die, how is the mutation maintained in the population?

3. The human population shows variation for alpha-thalassemia. How did the variation arise?

4. A common misconception related to evolution is that individuals develop mutations because the mutations fulfill some "need" or the individuals gain some benefit. In this case, this reasoning would suggest that individuals develop a mutation in the alpha-globin gene because they want or need protection from malaria. On the basis of your understanding of evolution and natural selection, explain why this reasoning is faulty.

5. In certain environments, did alpha-thalassemia affect an individual's ability to survive and reproduce? Explain.

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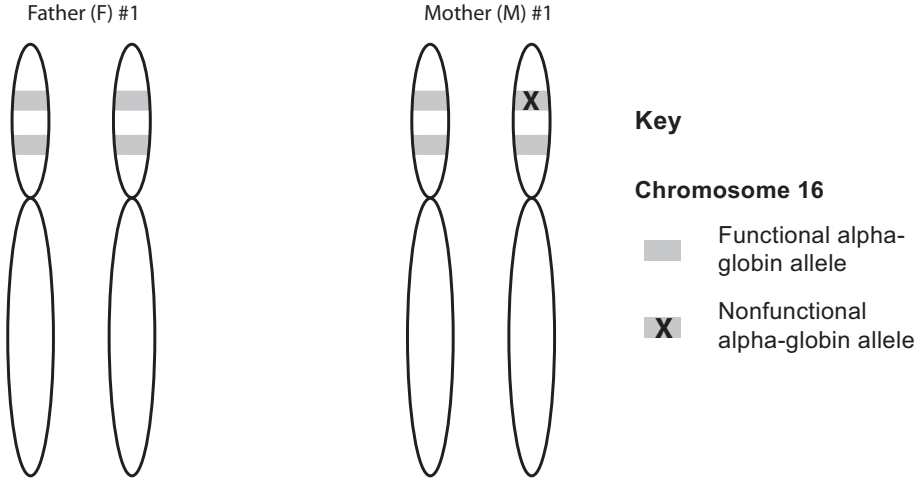
Inheriting Thalassemia

Name: _____

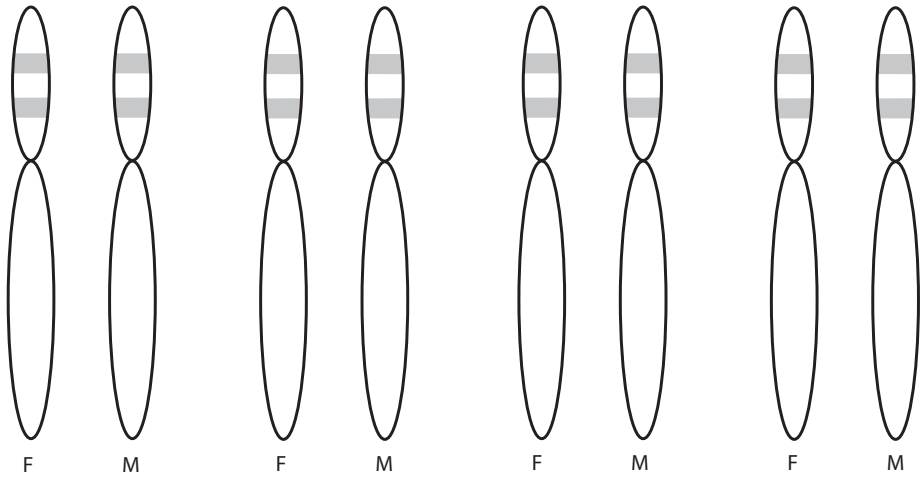
For each problem, use the information provided about the parents to predict the genotypes and phenotypes of their possible children.

Figure 1. *The top part of the diagram shows the genotypes of a mother and a father for the alpha-globin genes. Each chromosome carries two alleles of the gene, so each parent has four alleles of the gene. Nonfunctional alleles are shown with an "X." In the lower part of the diagram, show the different possible offspring if the parents have children. Below the diagrams are blanks to help you summarize the problem.*

Problem 1
Parents



Possible genetic combinations in offspring



Genotype:	_____	_____	_____	_____
Number of nonfunctional alpha-globin genes:	_____	_____	_____	_____
Thalassemia (type?):	_____	_____	_____	_____

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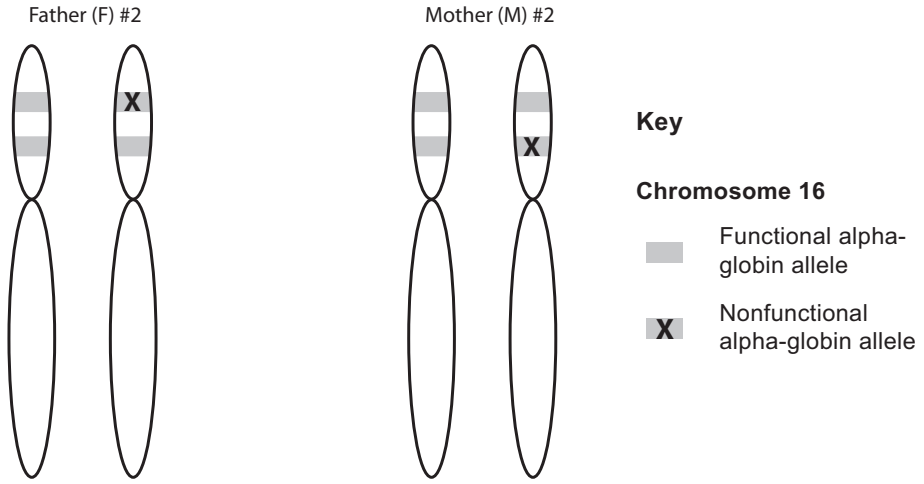
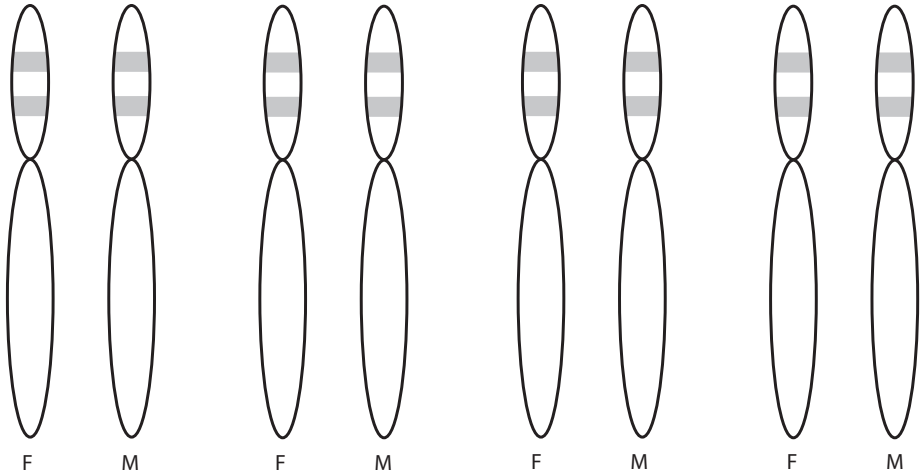
Figure 2. *The top part of the diagram shows the genotypes of a mother and a father for the alpha-globin genes. Each chromosome carries two alleles of the gene, so each parent has four alleles of the gene. Nonfunctional alleles are shown with an "X." In the lower part of the diagram, show the different possible offspring if the parents have children. Below the diagrams are blanks to help you summarize the problem.*

Problem 2
Parents



Possible genetic combinations in offspring



Genotype: _____ _____ _____ _____

Number of nonfunctional alpha-globin genes: _____ _____ _____ _____

Thalassemia (type?): _____ _____ _____ _____

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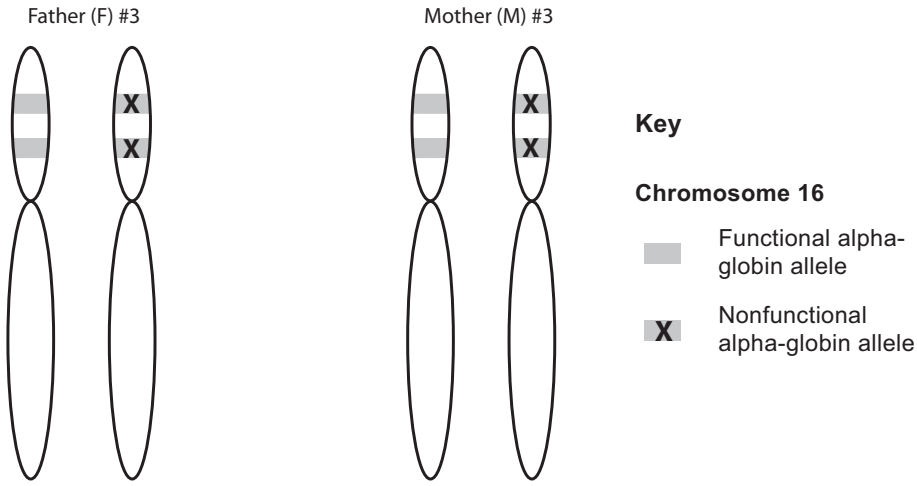
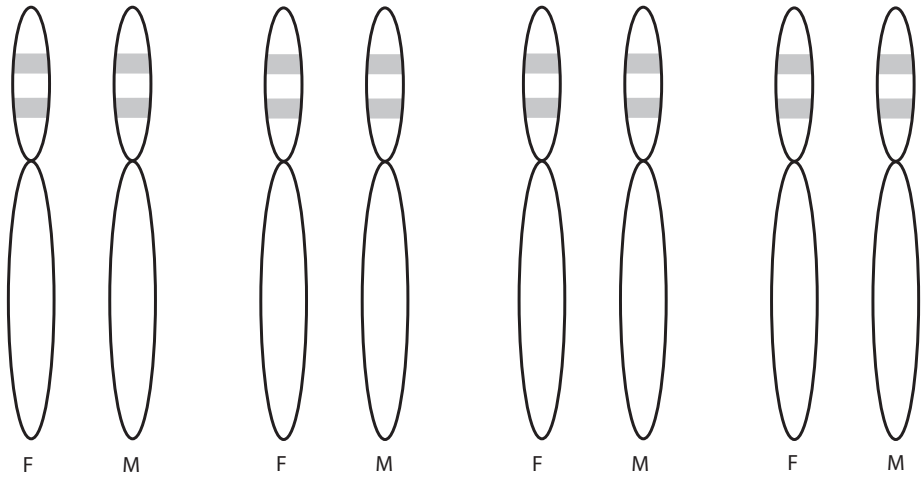
Figure 3. *The top part of the diagram shows the genotypes of a mother and a father for the alpha-globin genes. Each chromosome carries two alleles of the gene, so each parent has four alleles of the gene. Nonfunctional alleles are shown with an "X." In the lower part of the diagram, show the different possible offspring if the parents have children. Below the diagrams are blanks to help you summarize the problem.*

Problem 3
Parents



Possible genetic combinations in offspring



Genotype: _____ _____ _____ _____

Number of nonfunctional alpha-globin genes: _____ _____ _____ _____

Thalassemia (type?): _____ _____ _____ _____

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Cleft Lip and Palate

Name: _____

Cleft lip and palate is a disorder caused by problems during embryonic development. The structures that form the upper lip and the roof of the mouth typically fuse during the first three months of fetal development. In cleft lip and palate, these structures do not join. The result is a gap or a split in the roof of the mouth, the upper lip, or both. The effect can be as simple as a small notch in the lip or as complicated as a large opening that runs into the roof of the mouth and nose (Figure 1). Cleft lip and palate is one of the most common birth defects in the world, occurring in about 14 out of every 10,000 births.

Figure 1. *Cleft lip and palate. This infant has a cleft lip and palate. Multiple surgeries within the first year of life are often required to help children born with this condition.*

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The causes of cleft lip and palate are complex. Many genes play a role in coordinating embryonic development. More than 42 genes are involved in the development of the head and face alone. Genes control the pathway of development that causes the upper lip and roof of the mouth to fuse. Mutations in these genes can cause cleft lip and palate. When genetics is part of the cause of cleft palate, sometimes mutations in many genes are involved, but other times a mutation in only one gene is the cause.

A growing embryo is also very sensitive to changes in its environment. Environmental effects, such as the lack of certain nutrients at specific times during development, can also cause cleft lip and palate. Most cases of cleft lip and palate are the result of interactions between genetics and the environment. In some cases, the exact cause is never determined.

Scientists are making headway in understanding some causes of cleft lip and palate. Some patients who develop a cleft lip and palate have a condition called Van der Woude syndrome. Evidence shows that this syndrome is caused by mutations in just one gene, called the *Irf6* gene. *Irf6* stands for "interferon regulatory factor 6," but it is easier to just call it *Irf6*.

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Irf6 is a gene that codes for a protein that controls other genes. That is, the protein from this gene turns other genes on or off. This protein is required for normal development. In mice, this gene is active in the cells that line the two sides of the forming mouth. The gene is turned on and makes protein just before and during fusion of the two sides of the mouth.

Genetic studies in humans show that some mutations to *Irf6* cause one form of cleft lip and palate (Van der Woude syndrome). Cells with these mutations don't make enough of the protein from this gene. This means that other genes don't get turned on or off at the right time. Mutations to *Irf6* act in many ways like a dominant allele. Only one of the mutated alleles is needed to cause cleft lip and palate.

To better understand this syndrome, scientists would like to know how specific changes to the gene affect the phenotype. However, the *Irf6* gene is very large, more than 18,000 nucleotides long. It would be very helpful to use evidence to identify sections of the gene that may be especially important for gene function.

Questions

Use the information you just read to answer the following questions. Work together as a group to share what you have learned.

1. Calculate the number of babies expected to be born with cleft lip and palate in the United States each year:

    a. The worldwide incidence of cleft lip and palate is 14 out of every 10,000 births. Calculate the frequency of cleft lip and palate by dividing the number of babies with the condition by the number of births.

    b. Assume that there are 4,000,000 births per year in the United States. Multiply the number of births by the frequency of cleft lip and palate you calculated in Question 1a to determine the expected number of babies born with cleft palate each year in the United States.

2. How could a change to a gene cause cleft lip and palate? How might a change in an environmental signal cause cleft lip and palate?

3. Assume that one parent has an allele of the *Irf6* gene with a mutation that causes cleft lip and palate and a second allele that is normal. Also assume that the second parent has two normal alleles for this gene. What is the probability that a child born to this couple will have a cleft lip and palate? Mutated *Irf6* alleles act in a dominant fashion.

4. Explain how studies from mice are helpful to scientists trying to understand cleft lip and palate in humans.

***Sources*:**

http://www.nlm.nih.gov/medlineplus/cleftlipandpalate.html; http://ghr.nlm.nih.gov/condition=vanderwoudesyndrome

http://ghr.nlm.nih.gov/gene=irf6

http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/CraniofacialBirthDefects/PrevalenceCleft+LipCleftPalate.htm

http://www.patient.co.uk/doctor/Cleft-Lip-and-Palate.htm

H.O. Olasoji et al. 2005. Incidence and aetiology of oral clefts: A review. *African Journal of Medicine and Medical Sciences, 34*(1): 1–7.

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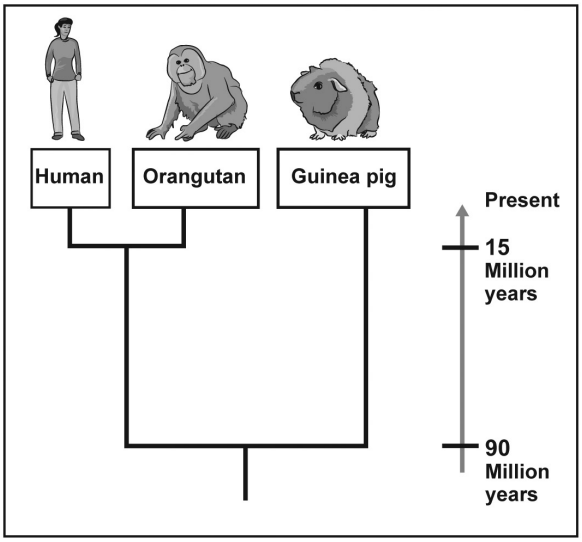
Calculating Times for an Evoprint

Questions

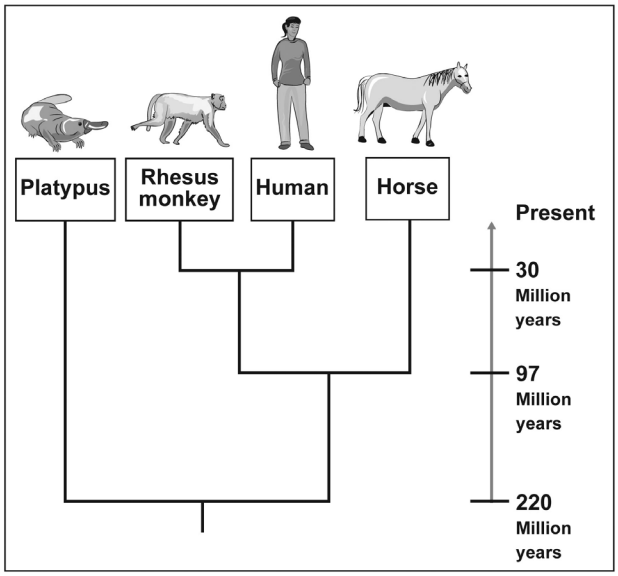
1. How many years are represented in an evoprint constructed from sequences from the three species shown in the evolutionary tree in Figure 1?

Figure 1. *Evolutionary tree of humans, orangutans, and guinea pigs*.



2. If you constructed an evoprint from sequences from the four species represented in the evolutionary tree in Figure 2, how many years would be represented in the evoprint?

Figure 2. *Evolutionary tree of platypuses, rhesus monkeys, humans, and horses*.



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Interpreting Evoprints

Questions

1. How does an evoprint help identify regions of DNA that have not changed over large amounts of time?

2. How does natural selection explain why some sequences of DNA are conserved over large amounts of time?

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Irf6 Evoprint Comparison

Name: _____

In this activity, you will work to identify sections of the *Irf6* gene that may be especially important for the gene to function properly. You will compare DNA sequences for this gene across many different species. The human sequence will be the reference sequence.

Procedure

1. Access the Evolution and Medicine Web site.

   Click on "Lesson 3: Evolutionary Processes and Patterns Inform Medicine," then "Activity 2: Evoprint Comparison." The opening page shows 1,701 nucleotides from the *Irf6* gene in humans.

2. Compare the human sequence with other individual species by checking the button next to the animal of your choice in the "Comparison of two sequences" section. Compare the human sequence with at least two other species. Make a rough estimate of the number of nucleotides that did *not* change. Record the comparisons you made and your estimates here.

3. Describe how the number of changes you observe in the *Irf6* gene relates to the amount of time since the species' common ancestry with humans. Use the comparisons you completed in Step 2 and the data in Table 1 to help you with this task.

Table 1. Time Since Common Ancestry with Humans

Species	Time since common ancestry with humans (millions of years)
Chimpanzee	8
Orangutan	15
Rhesus monkey	30
Dog	97
Horse	97
Cat	97
Cow	97
Rat	91
Mouse	91
Guinea pig	91
Armadillo	105
Opossum	176

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4. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows. Use your observations and data from Tables 1 and 2 to complete the following tasks.

   a. Make a rough estimate of the percentage of nucleotides that did *not* change in this comparison.

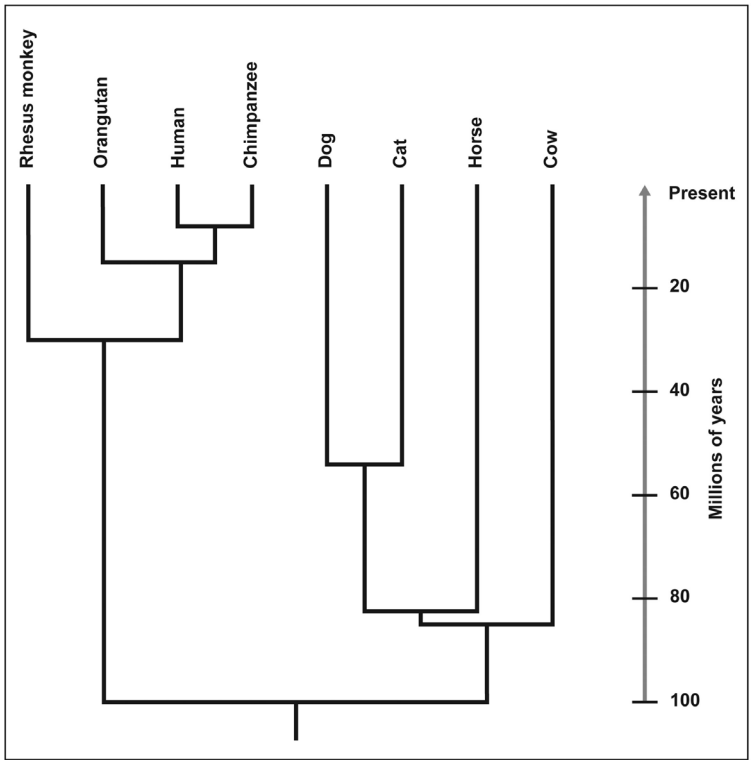
   b. Use the data in Tables 1 and 2 and Figure 1 to calculate the amount of time this evoprint represents.

Part 1: Horse, Cow, Dog, and Cat

Table 2. Time Since Common Ancestry for Additional Species Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Horse	Cow	85
Horse	Dog	83
Horse	Cat	83
Dog	Cat	53

Figure 1. *Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows.*



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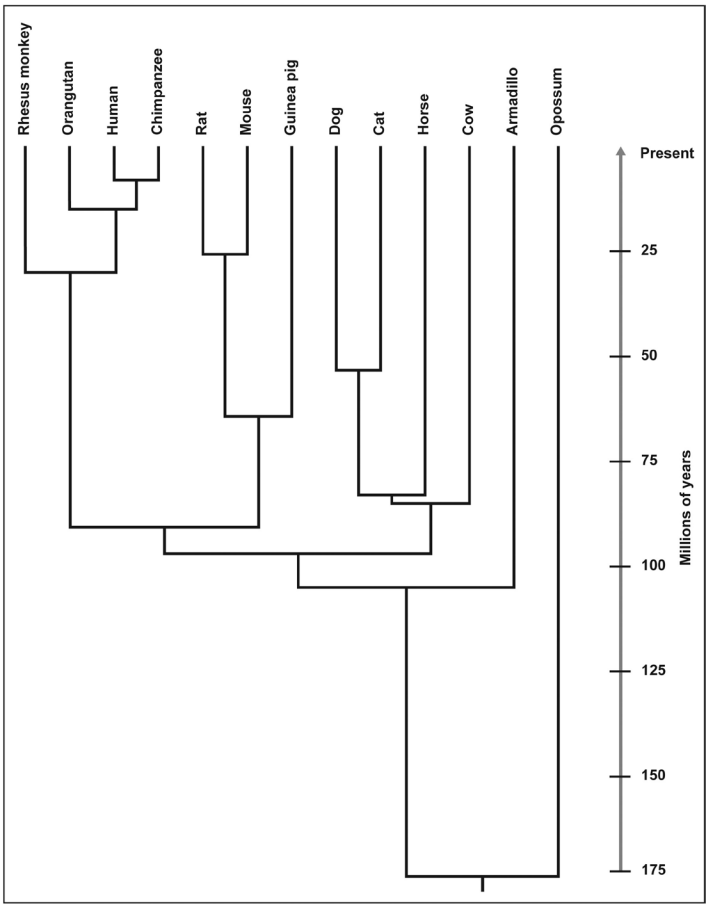
5. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.

Part 2: Guinea Pig, Mouse, and Rat

Table 3. Time Since Common Ancestry for Additional Species Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Guinea pig	Mouse	64
Guinea pig	Rat	64
Rat	Mouse	26

Figure 2. *Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums*.



a. Use the data in Tables 1,2, and 3 and Figure 2 to calculate the amount of time represented in this evoprint.

b. Identify two regions with eight or more nucleotides in a row that have not changed over the amount of time calculated in the previous step. Write out the nucleotides for these regions.

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Evoprint Introduction

Figure 1. *The human reference sequence: the 1,701 nucleotides of the human sequence for the* Irf6 *gene.*

Human Reference Sequence

```
GGTGTGTGCTTATAGTTCCAGCTACGTGGGAGGTTGAGGCGGGAGGATCGCTTGAGC
CTAGGAGGTCAGGGCTGCAGTGAGCTATGATCATGCCACTTGCACTCCAGCCTGGTT
GACAGAGCAAGACCATATCCCACAAAAAAAGAAAAAGAAAGAAAAACTTGCTGAGAG
CCCTCTATACCAATCACATAGGCTATACTGCGTGCCTGCTAACACCTGATAGTTCTG
TCCTGGTCTGAGTATCCATTCCCAGGCCCAAGCAACTCTCTGCATAGCAAGAGCTCT
GAGTTATTGAGGAGGAAGAGTCAACTGCTGACCCTCGCTAGCTTTTGAAACAAACAC
ATCACTACAATCACTAATGAGAGTTTTAGCCAACCCCAGCTGGTATCCTTTGGGATG
TGAGTGAGTACCTTCAATGAGTGGGAAAGAATAAACGGCTTCAACCATTGCAGACAT
GCCCCCAAAAGAGGAATTACTATGCCTGCTGAGTTTGGGCACCCCCATCATAAGCAT
TCTCTCTGTTTCACCAGAGTTTTAGATCTAGTGTATTCCCCATGCCAAAAAAAAAAA
AAAAAAAAAATCCAGAAAGGTCTGATGGTAGAAGAAGTCCTTTACCTTAAAAATGGT
ATTTTCCTCTTCTTGTTGAGGGCTATGCCGGGTGGCATGTTTCCAGGGAATCTGGAA
GCGTTTAGAGTCCCTGTGTAGCCAGATGAGCCCAGGGTAGAGGCCACTATCCACCTG
GGCCACCAGCCAGGGCTTTAGCCGGACTCTGCGGGGGTGGAGGGCCATGATCTGGGG
GGGTCAGAGGGAGAAATGGGAAGAGCAGAAGAATTAGGCCAGCCACTGGGAACCCTT
CCCAGCCACCTTTCCCATCTACTAGAGCTACAGTTTCACTAGATTACAGCAAAGAAA
CTAAATATGGGAATAAGCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTCCTTT
TTATTCTGATTCCAAAGCCATTTACACTTCATGCTCTAGCCAAATCAGACTTATCAG
CTAAGTAGATCACATTTCTTCTATTTAAATCCTGAAGCAGAAAAATAAAAAGGTGAT
GTCATCCTGCTGGAACACAGTCCTTCCTGAAGCACCAAGGGCTTTGGAAGAGAAGGA
AAATTGCTCTTTTCAATCTCATCTCAGGAGAGGGAAGAAAAAAGTTATGGAAACAGC
AACAACTATATAAGTGAGAAGGTGACACATGTTCTCTTTTTAAACAAGACAACTAAA
GGTGAATGGGAATATTTTAGAAATAAATGACACCACCATGATGAGGGAGAAGTAGAT
AAGGGGTAGATCCTGGACTCCAGAGTCCTCACTTACATGGAAGAGCAGGCAGTGTGT
CCACAGGGATCTGAAGAGTCGGCTTGTCTTTCCCTTGACCGCTCAAAGATTCTGTAT
GGAGAAAAGGAGGGGTCTCAGCTGGCTATCCATACAACAATCCAGTGTCTGGGAGAA
GGCTTGGGAACACTGGCGACAAAGGCTGAGAACACGTCTCCACTACTGAGTCTGGTA
AAGCTCAGAAGCCCTAGAATAACCAAATACTAAAGTACTAAGAGTACTTAGAGTACT
AAAAGCAGTCATGAGACAAGCTTTGCATGGCTAGGACAGCCTCCTCCCTTGGGTCTG
TTTATCTCCCAGTGAAGGCTGGAGTGAACCAATTGGTTGCAGGAGCCA
```

Human

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Figure 2. *Evoprint for the comparison of the human and cow sequences for a portion of the* Irf6 *gene. Nucleotides in capital letters are the same between the two species, and nucleotides in lowercase letters differ. The human sequence is the reference sequence*.

Comparison: Human, Cow

```
ggtgtgtgcttatagttccagctacgtgggaggttgaggcgggaggatcgcttgagc
ctaggaggtcagggctgcagtgagctatgatcatgccacttgcactccagcctggtt
gacagagcaagaccatatcccacaaaaaaagaaaaagaaagaaaaacttgctgagag
ccctctataccaatcacataggctatactgcgtgcctgctaacacctgatagttctg
tcctggtctgagtatccattcccaggcccaagcaactctctgcatagcaagagctct
gagttattgaggaggaagagtcaactgctgaccctcgctagcttttgaaacaaacac
atcactacaatcactaatgagagtttTAGCCAacCcCAgcTGgtATCCtTTGGGATG
TGAGTGAGTACCTTcaatgagTGGGAAAGAAtAAACGGCttcaaccattgCAGACAT
GCCCCcAaAAGAGgAATtACTaTGCCTGCTgAGTttgggcaCCCCCATCAtaagCAT
TCTCTctgtttcaccagagttttagatctagtgtattccccatgccaaaaaaaaaaa
aaaaaaaaatccagaaaggtctgatgGTAgAAGAAGtCCTTTACCTTaAAAATGGT
aTTTTCCTCtTCTTGtTGaGGGCTaTGCCGGGTGGCATGTTTCCAGGGaATCTGGAA
GCGTTTaGAGTCCCTGTGTAGCCAGATGAGCCCaGGGTAgAGgCCACTATCCACcTG
GGCCACCAGCCAGGGCTTtAGCCGgACTCTGCGGGGGTGGAGGGCCATGATCTGGGG
GGGtcagAGGGAGAAaTGGGaAGAGcagaAGAATTAGGCCAGCCaCTGGGAAcccTT
CCCAGCCACctTtCCCaTcTacTAGAGCTACAGTTTCAcTAGATTACAGCAAAGAAA
CTAAATATGGGAATAAgCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTcCTTT
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cTAAGTAGATCACATTTCTTCTATTTAAATCCTGAaGCAGAAAAATAAAAAGgTGAT
GTCATCCTGCTGGAACACAGTCCTTCCTGAAGcACCAAGggctttgGAAgAGAAGGa
AAATTGCtCTTTTCAATCTCATCTcAGGAGAGGGAAGAAAAAAGTTATGGAAACAGC
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ggtgaatgggaatattttagaaataAATGACAcCAcCaTGATGAGgGAGAAGtagat
AAGGGGTaGaTCCTGGactccagAGTCCTCACTtACATgGAAGAGCAGGCAGTGTgT
CCACAGGgATCTGAAGagtcggcttgtctttcccttgaccgctcaaagattctgtat
ggagaaaaggaggggtctcagctggcTATCCaTAcAACAATCCAGTGTCTGggagaa
ggcttgggaacactggcgacaaaggctgagaacacgtctccactactgagTCTgGTA
AAGCTCAGAAGCCCtaGAATAAcCAAATACTAAAGTactaagagtacttagagtact
AAAAgCAGTCATGAGACAaGCTttgcAtgGcTAGGACAGCCTCCTcCCTtgGGTCTG
TTTATCTCCCagtgaAGGCTGgAGTGAACcaATTGGtTgCAGGAGCCA
```

Human

Cow

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Figure 3. *Evoprint for the comparison of the human, chimpanzee, orangutan, and rhesus monkey sequences for a portion of the* Irf6 *gene. Nucleotides in capital letters are the same among all the species, and nucleotides in lowercase letters differ. The human sequence is the reference sequence*.

Comparison: Human, Chimpanzee, Orangutan, and Rhesus Monkey

```
GGTGTGTGCTtATAGTTCCAGcTACGTGggAGGTTGAGGcGGGAGgATCGCTTGAGC
CTAGgAGGTCAGGGCTGCAGTGAGCTATGATCATGCCACTTGCACTCCAGCCTGGTT
GACAGAGCAAGACCATATCCCACAAAAAAAGAAAAAGAAAGAAAAACTTGCTGAGAG
cCCTCTATACCAAtCACATAGGCTATACTGcgTGCCTGCTAACACCTGATAGTTCTG
tCCTGGtCTGAGTATCCATTcCCAGGCCCAAGCAaCTCTCTGCATAGCAAgAGCTCT
GAGTtATTGAGgAGGAAGAGTCAACTGCTgACCCTcGCTAGCTTTTGAAACAAACAC
AtCACTACAATCACTAATGAGAGTTTTaGCCAACCCCAGCTGGTATCCTTTGGGATG
TGAgTGAGTACCTTCAAtgAGTGGGAAAGAAtAAAcgGCTTCAaCCATTGCAGAcAT
GCCcCCAAAAGAGGAATTACtATGCCTGCtGAGTTTGGGCACCCCCATCATAAGCAT
TCTCTCTGTTtCACCAGAGTTTTAGATCTaGTgTATTCCCCAtGCCaaaAAAAAaaa
aaaaaaaaaatCCAGAAAGGTCTGATGGTAGAAGAAGTCCTTTACCTTAAAAATGGT
ATTTTCCTCTTCTTGTTGAGGGCTaTGCCGGGTGGCATGTTTCCAGGGAATCTGGAA
GCGTTTAGAGTCCCTGTGTAGCCAGATGAGCCCAGGGTAGAGGCCACTATCCACCTG
GGCCACCAGCCAGGGCTTtAGcCGGACTCTGCGGGGGTGGAGGGCCATGATCTgGGG
GggTCAGAGGGAGAAATGGGAAGAGCAGAAGAATTAGGCCAGCCACTGGGAACCCTT
CCCAGCCACCtTTCCCAtCTACTAGAGCTACAGTTTCACTAGATTACAGCAAAGAAA
CTAAATATGGGAATAAgCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTCCTTT
TTATTCTGATTCCAAAGCCATTTAcACTTCATGCTcTAGCCAAATCAGACTTATCAg
ctAAGTAGATCACATTTCTTCtaTTTAAATCCTGAAGCAGAAAAATAAAAAGgTGAT
GTCATcCTGCTGGAACACAGTCCTTCCTGAAGCACCAAGGgCTTTGGAAGAGAAGGa
AAATTGcTCTTTTCAATCTCATCTCAGGAGAGGGAAGAAAAAAGTTATGGAAACAGC
AACAACTATAtAAGTGAGAAGgTGAcaCAtgTTCTCTTTTTAAACAAGACAACTAAA
gGTGAATGGGAATATTTTAGAAAtAAATGACAcCAcCATGATGAGGGAGAAGTAGaT
aAGGGGTAGATCCTGGACTCCAGAGTCcTCACTTACATGGAAGAGCAGGCAGTGTGT
cCaCAGGGATCTGAAGAGTcGGcTTGTCTTTCCCTTGACCgCTCAAAGaTTCTGTAT
GGAGAAAAGGaGGGGTCTCAGcTGGcTATCCATACAACAATCcAGTGTCTGGGAGAA
GGCTTGGGAACAcTGGCGACAAAGGCTGAGAAcAcGTCTCcaCtAcTGAGTCTGGTA
AAGCTCAGAAGcCCTAGAATAACCAAATACTAAAGTACTAAGAGTACTTAGAGTACt
AAAAgCAGtCATGAGACAAGCTTTGCATGGCTAGGACAGCCTCCTCCCTTGGGTCTG
TTTATCTCCCAgTGAAGgCTGGAGTGAACcaATTGGTTGCaGGAGCCA
```

Human

Chimpanzee

Orangutan

Rhesus Monkey

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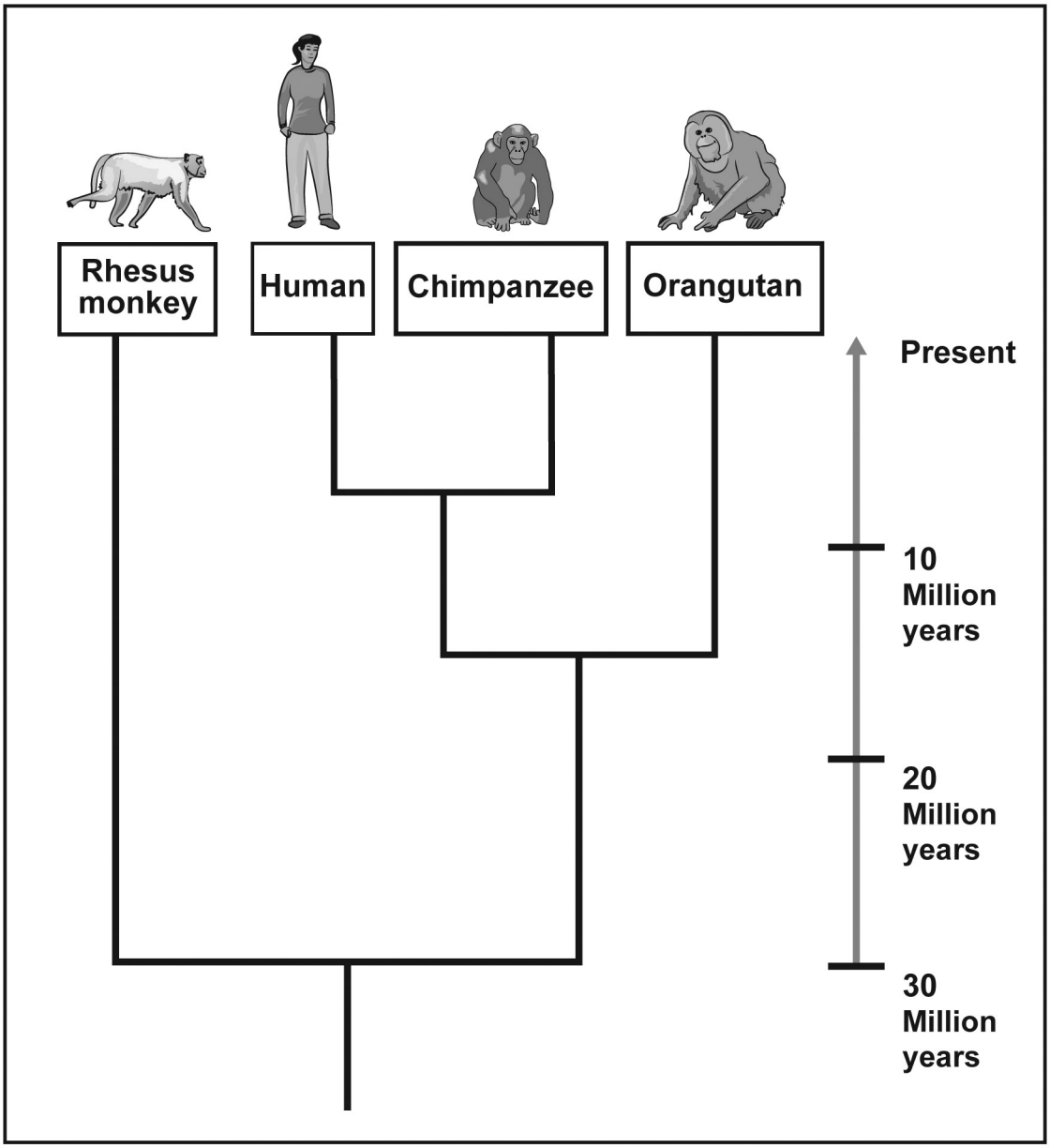
Figure 4. *An evolutionary tree summarizing the relationships among four primate species. How can you use the diagram to help you interpret the time represented in an evoprint for all four species?*



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Investigating *Irf6* Evoprints

Name: _____

In this activity, you will work to identify sections of the *Irf6* gene that may be especially important for the proper functioning of the gene. You will examine evoprints for this gene across many different species. The human sequence is the reference sequence in all cases.

Procedure

1. Work as a group to examine the evoprints in which the human sequence was compared with one other species. For at least three of the comparisons, make a rough estimate of the number of nucleotides that did *not* change. Record the comparisons you made and your estimates below.

2. Describe how the number of changes you observe in the *Irf6* gene relates to the amount of time since the species' common ancestry with humans. Use the comparisons you completed in Step 1 and the data in Table 1 to help you with this task.

Table 1. Time Since Common Ancestry with Humans

Species	Time since common ancestry with humans (millions of years)
Chimpanzee	8
Orangutan	15
Rhesus monkey	30
Dog	97
Horse	97
Cat	97
Cow	97
Rat	91
Mouse	91
Guinea pig	91
Armadillo	105
Opossum	176

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3. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows. Use your observations and data from Table 1 to answer the following questions.

   a. Make a rough estimate of the percentage of nucleotides that did *not* change in this comparison.

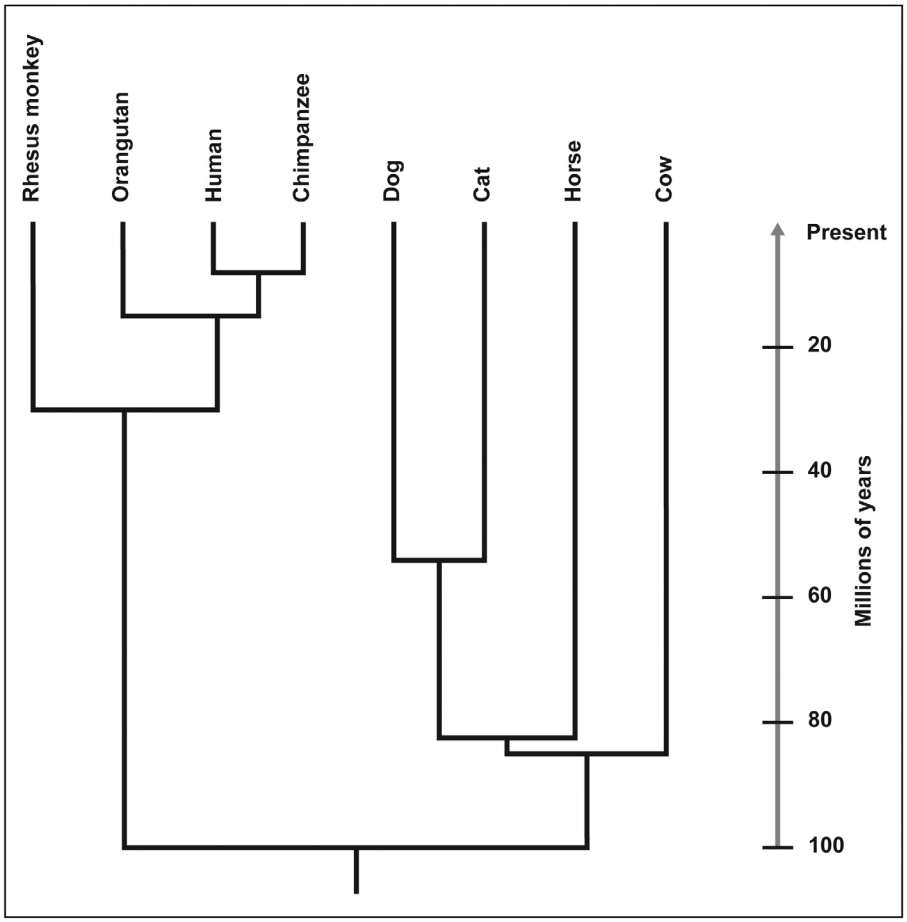
   b. Use the data in Tables 1 and 2 and Figure 1 to calculate the amount of time this evoprint represents.

Part 1: Horse, Cow, Dog, and Cat

Table 2. Time Since Common Ancestry for Additional Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Horse	Cow	85
Horse	Dog	83
Horse	Cat	83
Dog	Cat	53

Figure 1. *Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows.*



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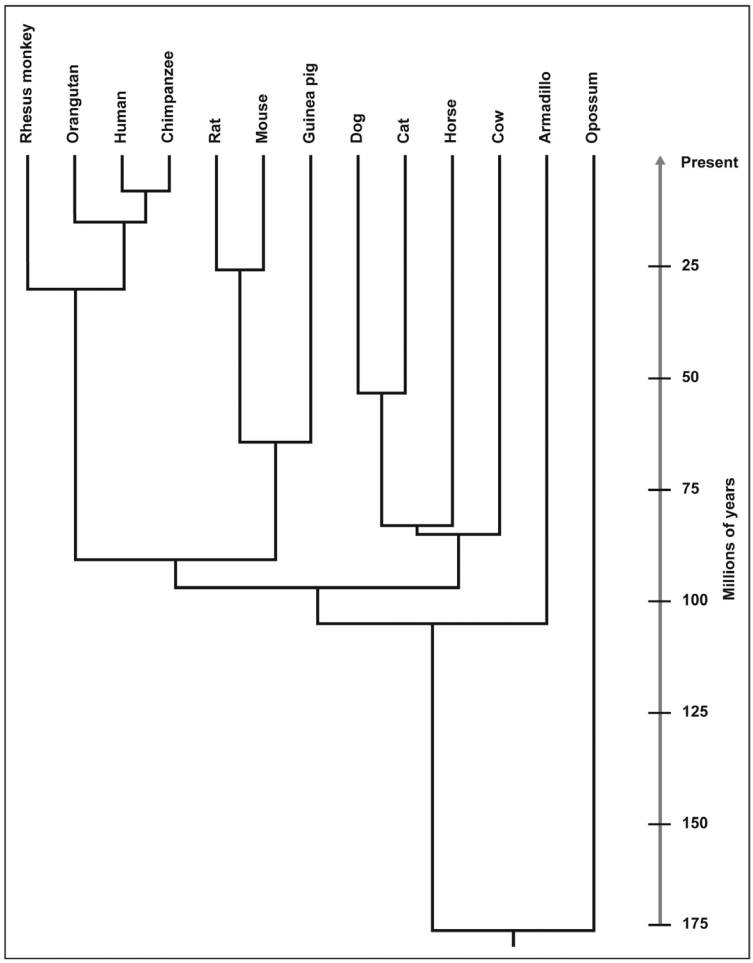
4. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.

Part 2: Guinea Pig, Mouse, and Rat

Table 3. Time Since Common Ancestry for Additional Species Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Guinea pig	Mouse	64
Guinea pig	Rat	64
Rat	Mouse	26

Figure 2. *Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.*



a. Use the data in Tables 1, 2, and 3 and Figure 2 to calculate the amount of time this evoprint represents.

b. Identify two regions with eight or more nucleotides in a row that have not changed over the amount of time calculated in the previous step. Write out the nucleotides for these regions.

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Irf6 Evoprints

Evoprint 1. *Comparison of* Irf6 *sequences from human and chimpanzee.*

Comparison: Human, Chimpanzee

```
GGTGTGTGCTtATAGTTCCAGCTACGTGGGAGGTTGAGGCGGGAGGATCGCTTGAGC
CTAGGAGGTCAGGGCTGCAGTGAGCTATGATCATGCCACTTGCACTCCAGCCTGGTT
GACAGAGCAAGACCATATCCCACAAAAAAAGAAAAAGAAAGAAAAACTTGCTGAGAG
CCCTCTATACCAATCACATAGGCTATACTGCGTGCCTGCTAACACCTGATAGTTCTG
TCCTGGTCTGAGTATCCATTCCCAGGCCCAAGCAaCTCTCTGCATAGCAAGAGCTCT
GAGTTATTGAGGAGGAAGAGTCAACTGCTGACCCTCGCTAGCTTTTGAAACAAACAC
AtCACTACAATCACTAATGAGAGTTTTAGCCAACCCCAGCTGGTATCCTTTGGGATG
TGAGTGAGTACCTTCAATGAGTGGGAAAGAATAAACGGCTTCAACCATTGCAGACAT
GCCCCCAAAAGAGGAATTACTATGCCTGCTGAGTTTGGGCACCCCCATCATAAGCAT
TCTCTCTGTTtCACCAGAGTTTTAGATCTAGTGTATTCCCCATGCCAAAAAAAAAAA
AAAAAAaaaaTCCAGAAAGGTCTGATGGTAGAAGAAGTCCTTTACCTTAAAAATGGT
ATTTTCCTCTTCTTGTTGAGGGCTATGCCGGGTGGCATGTTTCCAGGGAATCTGGAA
GCGTTTAGAGTCCCTGTGTAGCCAGATGAGCCCAGGGTAGAGGCCACTATCCACCTG
GGCCACCAGCCAGGGCTTTAGCCGGACTCTGCGGGGGTGGAGGGCCATGATCTGGGG
GGGTCAGAGGGAGAAATGGGAAGAGCAGAAGAATTAGGCCAGCCACTGGGAACCCTT
CCCAGCCACCtTTCCCATCTACTAGAGCTACAGTTTCACTAGATTACAGCAAAGAAA
CTAAATATGGGAATAAGCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTCCTTT
TTATTCTGATTCCAAAGCCATTTACACTTCATGCTCTAGCCAAATCAGACTTATCAG
CTAAGTAGATCACATTTCTTCTaTTTAAATCCTGAAGCAGAAAAATAAAAAGGTGAT
GTCATCCTGCTGGAACACAGTCCTTCCTGAAGCACCAAGGGCTTTGGAAGAGAAGGA
AAATTGCTCTTTTCAATCTCATCTCAGGAGAGGGAAGAAAAAAGTTATGGAAACAGC
AACAACTATAtAAGTGAGAAGgTGACACATGTTCTCTTTTTAAACAAGACAACTAAA
gGTGAATGGGAATATTTTAGAAATAAATGACACCACCATGATGAGGGAGAAGTAGAT
AAGGGGTAGATCCTGGACTCCAGAGTCCTCACTTACATGGAAGAGCAGGCAGTGTGT
CCaCAGGGATCTGAAGAGTCGGcTTGTCTTTCCCTTGACCGCTCAAAGATTCTGTAT
GGAGAAAAGGAGGGGTCTCAGCTGGCTATCCATACAACAATCCAGTGTCTGGGAGAA
GGCTTGGGAACACTGGCGACAAAGGCTGAGAACACGTCTCCACTACTGAGTCTGGTA
AAGCTCAGAAGCCCTAGAATAACCAAATACTAAAGTACTAAGAGTACTTAGAGTACT
AAAAGCAGTCATGAGACAAGCTTTGCATGGCTAGGACAGCCTCCTCCCTTGGGTCTG
TTTATCTCCCAgTGAAGGCTGGAGTGAACCAATTGGTTGCAGGAGCCA
```

Human

Chimpanzee

Evoprint 2. *Comparison of* Irf6 *sequences from human and dog.*

Comparison: Human, Dog

```
ggtgtgtgcttatagttccagctacgtgggaggttgaggcgggaggatcgcttgagc
ctaggaggtcagggctgcagtgagctatgatcatgccacttgcactccagcctggtt
gacagagcaagaccatatcccacaaaaaaagaaaaagAAAGAAAAACTTgctgAGAG
CCCTCTAtACCAATCACAtaggctatactgcgtGCCTGCTAACACctgaTAGTTCTG
TCCTGGTCTGAGtatcCATTCCCAGGCCCAAGCAaCTCTCTgCATAGCAAGAGCtCT
GAGTtAtTGAgGAGGAAGAGTCAaCTGCTGACCCtcGctAgCTTTTGaAACAAaCAC
ATCAcTACAATCACTaatgagAGTTTtAGcCAACccCAgCTGGtATCcTTTGGGATG
TGAGTGAGTACCTTCAAtgAgTggGAAAGAATAAACgGCtTCAAcCATTgCAGACAT
GCCCCcaaAAGAGgAATTACTATGCCTgctgAGTTTGGGCACCCCCaTCATAAgCAT
TCTctctgtttcaccagagTTTTaGATcTAGTGTATTCCcCaTGCCAAAAAAAAAAa
AAAAAAAAAATCCAGAAAGgTCTGATGGTaGAAGAAGTcCTTTACCTTaAAAATGGT
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GCGTTTaGAGTCCCTGTGTAGCCAGATGAGCCCAGGGTAgAGGCCACTATCCACCTG
GGCCACCAGCCAGGGCTTtAGCCGgACTCTGCGGGGGTGGAGGGCCATGATCTGGGG
GGGtcaGAGGGAGAaatGGGAAGAGCAGaAGAATTAGGCCAGCCaCTGGGAACccTT
CCCAGCCACcTTTCCCATcTacTAGAgCTACAGTTTCAcTAGATTACAGCAAAGAAA
CTAAATATGGGAATAAgCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTcCTTT
TtATTCTgATTCCAAAGCCATTTAcACTTCATGcTCTAGCCAAATCAGACTTATCAg
cTAAGTAGATCACATTTCTTCTATTTAAATCCTGAAGCAGAAAAATAAAAAggtGAT
GTCATCCtGCTGGAaCACAGTCCTTCCTGAAgcACCAAGGgCTTTGGAAGAGAAGga
AAATTGCTCTTTTCAATCTCAtctcaGGAGAGGGAagAAAAAaGTTATGGAAACAGC
AAcaactatataagtgagaaggtgacacatgtTCTCTTTTTAaACAAgacaaCtAAA
GGtGAATGGGAATATTTTAGAAatAAATGACACcAcCaTGaTGAGgGAGAAGTAGat
AAGGGGTaGaTCCTGGacTCCAgAGTCCTCACTTACAtgGAAgagCAGGCAGTGTgT
CCACaGGGATCtGaAGAGtCGGcttgtCTTTCCcTTGaCCgctCAAAGAtTCTGTAt
ggAGAAAAGGaGGGgTCTcAGcTGGcTATCCAtACAACAATCCAGTGtCTGGGAGAA
GgCTTGGGAACActGgcGACAAAGGCTGAGaacacgtctccactaCTGAGTCTGGtA
AAGcTCAGaAGCCCTAGAATAAcCAAATaCTAaagtactaagagtacttagagtact
aaaagcagtcatgagacaagctttgcatggctaggaCAGCCTCCTCCCtTGGGTCTG
TTTATCTCCCagtgaAGGCTggAGTGAACCaATTGGtTGCAGGAGCCa
```

Human

Dog

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Evoprint 3. *Comparison of* Irf6 *sequences from human and guinea pig.*

Comparison: Human, Guinea Pig

```
ggtgtgtgcttatagttccagctacgtgggaggttgaggcgggaggatcgcttgagc
ctaggaggtcagggctgcagtgagctatgatcatgccacttgcactccagcctggtt
gacagagcaagaccatatcccacaaaaaaagaaaaagaaagaaaaacttgctgagag
ccctctataccaatcacataggctatactgcgtgcctgctaacacctgatagttctg
tcctggtctgagtatccattcccaggcccaagcaactctctgcatagcaagagctct
gagttattgaggaggaagagtcaactgctgaccctcgctagcttttgaaacaaacac
atcactacaatcactaatgagagttttagccaaccccagctggtatcctttgggatg
tgagtgagtaccttcaatgagtgggaaagaataaacggcttcaaccattgcagacat
gccccccaaaagaggaattactatgcctgctgagtttgggcacccccatcataagcat
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aAAAAAAAAATcCAGAAaggtctgatGGTagaAGAAGTCCTTTACCTTAAAAATGGT
aTTTTCCTCtTCtTGtTGaGGGCTATGCCGGGTGGCATGTTTCCAGGGaATCTGGAA
GCGTTTAGAGTCcCtgTGTAGCCAGATGAGCCCAGGGTAGAGgCCaCTATCCACcTG
GGCCACCAGCCAGGGCTTtAGCCGGACTCTGCGGGGGTGGAGGGCCATGatCTGGGG
GggtCAGaGGGAGAAaTGGGaAGAGCAgaAGAATTAGGCCAGCCaCTGGGAACcctT
CCCAGCCaCCTTTCCCATctaCTAGAGCTACAGTTTCACTAGATTACAGCAAAGAAA
CTAAATATGgGAATAAgCtgTGCCAggTGGGAAAGAAAAGAGGTTAAAAGGTcCTTT
TtATTCTgATTCCAAAGCCATTTAcACTTCaTGCTCTAGCCAAATCAGACTTATCAg
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GTCATCCtgcTGGAAcacagtCCTTCCTGAAGCAcCAAgGGCTTTGGAAGAGAAGGa
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AAcaactatataagtgagaaggtgacacatgttctctttttaaacaagacaactaaa
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CCACAGggatctgaagagtcggcttgtctttcccttgaccgctcaaagattctgtat
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ggcttgggaacactggcgacaaaggctgagaacacgtctccactactgagtctggta
aagctcagaagccctagaataaccaaatactaaagtactaagagtacttagagtact
aaaagcagtcatgagacaagctttgcatggctaggacagcctcctcccttgggtctg
tttatctcccagtgaaggctggagtgaaccaattggttgcaggagcca
```

Human

Guinea Pig

Evoprint 4. *Comparison of* Irf6 *sequences from human and opossum.*

Comparison: Human, Opossum

```
ggtgTGTGCTTaTAGtTCCAGCTACgtGGGAGGtTGAGGCgGGAGGATCGCTTGAGC
CtAGGAGgTCaGGGCTGCaGTGaGCTATGATCATGCCACTtGcACTCCAGCCTGGtt
gacagagcaagaccatatcccacaaaaaaagaaaaagaaagaaaaacttgctgagag
ccctctataccaatcacataggctatactgcgtgcctgctaacacctgatagttctg
tcctggtctgagtatccattcccaggcccaagcaactctctgcatagcaagagctct
gagttattgaggaggaagagtcaactgctgaccctcgctagcttttgaaacaaacac
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GTCATCCTGCTGGAACACAGtCCTTCCTgAaGCACCaagggctttggaagagaagga
aaattgctcttttcaatctcatctcaggagagggaagaaaaaagttatggaaacagc
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ggtgaatgggaatattttagaaataaatgacaccaccatgatgagggagaagtagat
aaggggtagatcctggactccagagtcctcacttacatggaagagcaggcagtgtgt
ccacagggatctgaagagtcggcttgtctttcccttgaccgctcaaagattctgtat
ggagaaaaggaggggtctcagctggctatccatacaacaatccagtgtctgggagaa
ggcttgggaacactggcgacaaaggctgagaacacgtctccactactgagtctggta
aagctcagaagccctagaataaccaaatactaaagtactaagagtacttagagtact
aaaagcagtcatgagacaagctttgcatggctaggacagcctcctcccttgggtctg
tttatctcccagtgaaggctggagtgaaccaattggttgcaggagcca
```

Human

Opossum

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Evoprint 5. *Comparison of* Irf6 *sequences from human, chimpanzee. orangutan, Rhesus monkey, dog, horse, cat, and cow*

Evoprint 6. *Comparison of* Irf6 *sequences from human, chimpanzee, orangutan, Rhesus monkey, dog, horse, cat, cow, rat, mouse, guinea pig, armadillo, and opossum.*

Image sources: PhotoDisc for human and rat; *Corel* for cow, chimpanzee, orangutan, rhesus monkey, guinea pig, dog, horse, opossum, and cat; *Vlad Lazarenko* for armadillo.

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Email from Viroformatics

From: Paul Monkeyflower, President of Viroformatics

To: Biology students

Subject: Information about influenza evolution

Greetings. Our company, Viroformatics, wants your help. Viroformatics does research on viruses in order to help improve human health. We have a strong presence in the community and want to encourage all students to pursue careers in biomedicine.

We would like to create a brochure for high school students that will help them understand how we use information about evolution to better understand influenza. Your teacher has agreed to let your class help us by creating an outline of the brochure. Because you are the same age as the target audience and you are knowledgeable about evolution and medicine, your perspectives will be very helpful.

Your task is to collect information about influenza and evolution that you think is important. To help you with this, we compiled some questions and sent them to your teacher. We also want to include in the brochure some descriptions of what high school students think about the topic. To help us do that, please write down your ideas about the questions we sent before you start gathering information. Once you have gathered information, compile bulleted lists in answer to the questions. We have our own writers who will make the final brochure.

We're giving you access to our Viral Genome Database, which should help you with your task. Certain parts of the database will give you a glimpse of some of the ways scientists study influenza.

We look forward to working with you.

Sincerely,

Paul Monkeyflower
President of Viroformatics

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Notes about Influenza and Evolution

Name: _____

Questions from Viroformatics

Write your answers to the following questions before the lesson and after the lesson. You may want to create a new page in your notebook for each question. This will help you with your brochure outline and with keeping track of your understandings as you proceed through the lesson. Use a bulleted-list format for your answers.

1. **What is influenza, or the "flu"?**
   What I think before the lesson:

   What I think after:

2. **How do scientists use data to explore how influenza genes evolve?**
   What I think before the lesson:

   What I think after:

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3. **Not all individual influenza viruses are identical. What causes viruses to differ from one another?**
   What I think before the lesson:

   What I think after:

4. **How does natural selection help explain the evolution of influenza? (Keep in mind the major principles of natural selection.)**
   What I think before the lesson:

   What I think after:

5. **How does evolution help explain why researchers need to make a new vaccine for influenza every few years?**
   What I think before the lesson:

   What I think after:

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Introduction to Influenza

Influenza basics

Influenza, also called the flu, is an illness caused by a virus. The influenza virus infects lung cells and causes respiratory problems. The flu can be mild, severe, or even deadly, and it has a large impact on human health. For example, on average each year,

- 5 to 20 percent of Americans suffer from the flu,
- complications from the flu result in hospital stays for over 200,000 Americans, and
- flu-related effects cause the death of tens of thousands of Americans and about 500,000 people worldwide.

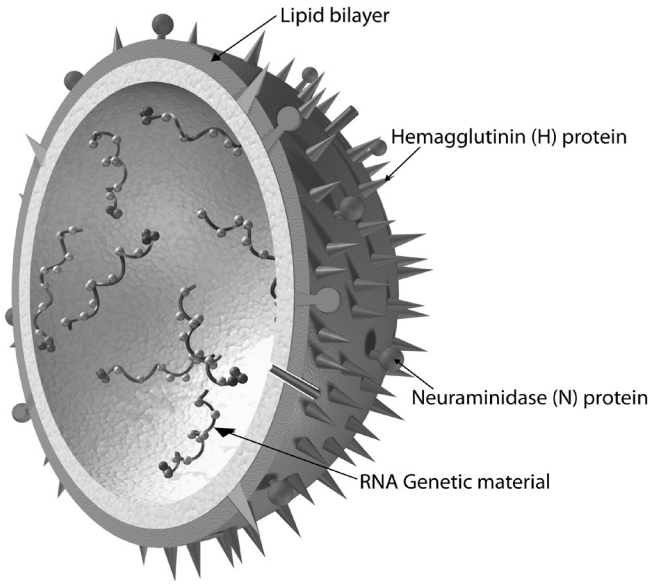
Symptoms of a flu infection include a high fever, extreme tiredness, muscle aches, a dry cough, a sore throat, and a stuffy nose. In people who are otherwise healthy, symptoms from the flu are usually gone after four to seven days, but they can last longer.

Occasionally, new strains of influenza emerge that cause global pandemics. A pandemic is a large-scale outbreak of an infectious disease that spreads throughout the world. In the "Spanish flu" pandemic of 1918–1919, hundreds of millions of people were infected and tens of millions of people died.

Structure of the virus

The influenza virus is simply genetic material surrounded by a membrane. Inserted like spikes in the membrane are two main proteins, hemagglutinin (H) and neuraminidase (N) (Figure 1). Influenza has eight segments of RNA that contain 11 genes. Importantly, the genetic material is RNA, not DNA. RNA is more prone to mutations than DNA.

Figure 1. *Influenza virus.*



Source: National Institute of Allergy and Infectious Diseases.

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Types of influenza viruses

Three main types of influenza viruses exist: A, B, and C. The forms of specific proteins found in influenza determine the type. Types A and B cause the seasonal flu that sweeps across the globe every year. Type A causes some of the flu outbreaks that result in a larger number of deaths than normal. Type C causes only a mild illness and does not cause epidemics.

Each type of the virus can be further broken down into specific groups:

- Type A: Subtypes are based on forms of hemagglutinin (abbreviated H or HA; 16 forms, named H1–H16) and neuraminidase (abbreviated N or NA; 9 forms, named N1–N9). H3N2 and H1N1 are examples of subtypes. Within each subtype, different strains exist.
- Type B: There are no subtypes, but different strains exist.
- Type C: There are no subtypes, but different strains exist.

Influenza A (H1N1 and H3N2) and influenza B strains are included in each year's seasonal flu vaccine. The specific strains in the vaccine change over time, however.

Though influenza infects many humans and pigs each year, it's considered a bird disease. This is because many more subtypes and strains of influenza are found in birds than in other animals.

How do you get the flu?

The common way to get the flu is to inhale a virus from another infected person. Infected people release viruses when they cough and sneeze. You can also get the flu by touching your mouth or nose after touching something with flu viruses on it. You can start infecting other people one day before you show symptoms and continue to infect people five to seven days after you get sick. As you can imagine, this makes it very difficult to control the spread of the flu.

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Influenza Sequences

In this exercise, you will analyze genetic sequences from three different influenza viruses that scientists have collected from people around the world over a span of 10 years. The sequences come from the gene that codes for hemagglutinin. This protein is found in the membrane of an influenza virus.

Procedure

1. Cut out the three sequences on page 2 so you can move them easily.

2. Select two of the sequences. Tape one of the sequences to a blank piece of paper. Place the second sequence beneath the first sequence. Slide the bottom sequence back and forth until many of the nucleotides line up with the upper sequence. When you are satisfied with its placement, tape the lower sequence into place.

3. Repeat Step 2 with the third sequence. The third sequence should align with the second sequence.

4. Scientists today use sophisticated computer programs to help line up sequences. This is called an "alignment." You just performed a sequence alignment.

5. Underline the nucleotides that line up. Then draw boxes around the nucleotides that differ among the three samples. How many nucleotides differ among the three samples?

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Sample 1: Virus from a patient in Finland in 2003

Sample 2: Virus from a patient in Auckland, New Zealand, in 1997

Sample 3: Virus from a patient in Madrid, Spain, in 1993

1. CACACTGGAGTTTAACAATGAAAGCTTCAATAT

2. CCTGGAGTTTACCAATGAAAGCTTCAATATTGG

3. AGGCACCCTGGAGTTTACCAATGAAGACTTCAA

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Aligned Influenza Sequences

Sample 1: Virus from a patient in Finland in 2003.

Sample 2: Virus from a patient in Auckland, New Zealand, in 1997.

Sample 3: Virus from a patient in Madrid, Spain, in 1993.

```
1.   CACACTGGAGTTTAACAATGAAAGCTTCAATAT
2.      CCTGGAGTTTACCAATGAAAGCTTCAATATTGG
3.    AGGCACCCTGGAGTTTACCAATGAAGACTTCAA
```

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Exploring a Genetic Database

Name: _____

In this portion of the activity, you will work with a partner to explore the Viral Genome Database at Viroformatics. For security reasons, you will only have access to certain portions of it. To access the database, go to the Evolution and Medicine Web site: http://science.education.nih.gov/supplements/evolution/student. Click on "Lesson 4: Using Evolution to Understand Influenza" to get to the home page for Viroformatics. Once there, go through the following steps to learn how scientists at Viroformatics use evolution in their studies of influenza.

Procedure

1. Your first goal is to get a sense of the types and amount of data stored in the database. Click on "Viral Genome Database," then "Full Virus Database." Once there, explore the number of sequences available for influenza in humans for the hemagglutinin gene. Record the number available for types A, B, and C.

   (You can explore the data for specific sequences by clicking on the accession number. By convention, the genetic sequences are recorded as DNA. Influenza viruses store their genetic information as RNA.)

2. Why do you think the number of sequences for each type is different?

3. Researchers at Viroformatics are studying how influenza viruses have changed over time. They obtained the genetic sequence for hemagglutinin from 11 viruses isolated from people around the world at different times. Scientists store influenza samples from the past in freezers. This way, future scientists have access to the influenza "fossil record." To access the 11 sequences, return to the "Viral Genome Database" page. Then click on the "Influenza-Over-Time Project." Note the years and the countries in which the samples were collected. (Again, you can explore specific sequences by clicking on the accession number.)

4. Click on "Align Sequences" to see a portion of the alignment for the hemagglutinin gene in all 11 viruses.

5. Calculate the number of changes per nucleotide in this 100-nucleotide sequence by using the following formula:

$$\frac{\text{number of nucleotides that have at least one change}}{\text{toal number of nucleotides}}$$

6. The viruses in this study were collected over a span of 35 years. Calculate the number of changes per nucleotide per year by using the following formula:

$$\frac{\text{answer from Step 5}}{\text{total number of years}} = \text{number of changes per nucleotide per year}$$

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7. In Lesson 3, you investigated a portion of the sequence of a gene called *Irf6* that is involved in the development of the head and face. You compared the sequence of this gene in many different species. Thirty nucleotides from the *Irf6* sequence are shown below:

TGGGCCACcAGCCAGGGCTTtAGcCGgACT

The lowercase (gray) letters show nucleotides that differ among some of the species. The uppercase (black) letters did not change. The amount of time represented in this comparison is 1,009 million years (or 1.009 billion years). Use the same formulas you used in Steps 5 and 6 to calculate the expected number of changes per nucleotide per year in this sequence.

8. Compare the rate of change per nucleotide per year for the hemagglutinin gene in influenza with the rate for the *Irf6* gene. Do this by dividing the rate for the hemagglutinin region by the rate for the *Irf6* gene. The number you calculate will show how many times faster one region changes than the other.

9. To see a diagram that summarizes the relationships among the viruses, click on "Build a Tree." Does this diagram show evidence that the influenza virus is changing over time?

10. Explore how the number of changes in the genetic sequence relates to time. Do this by selecting two sequences and then clicking on the "Compare" button. Hint: A useful comparison is to record the number of changes in all the samples compared with the most recent sample (FIN_2003). Use the data you collect to answer the following questions.

    a. How do the number of changes to the sequence relate to time?

    b. What do you think this means?

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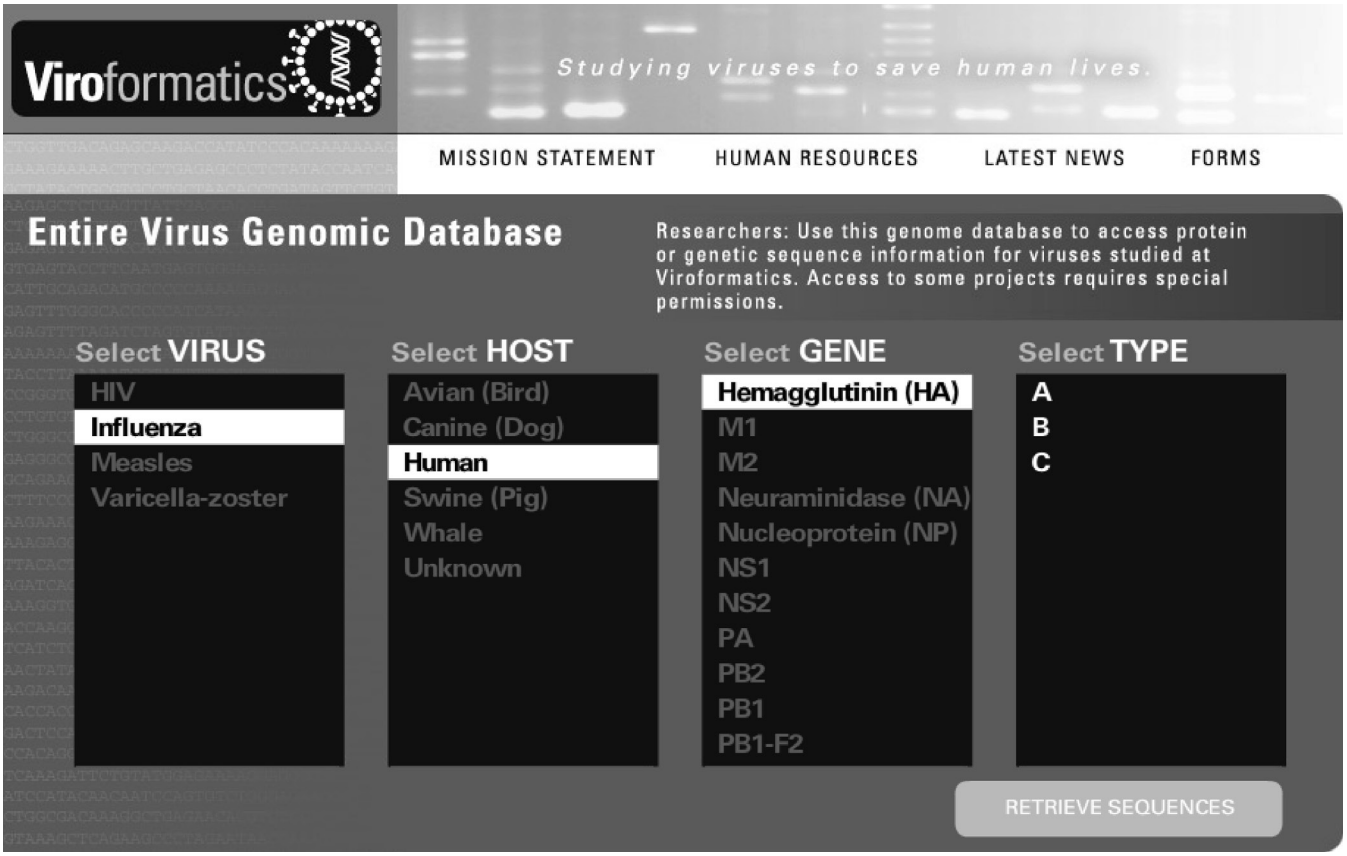
Viroformatics Virus Database

Figure 1. *Viroformatics Virus Genomic Database home page.*



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Influenza Hemagglutinin Sequence

Figure 1. *Viroformatics data for influenza A virus H3N2 hemagglutinin.*

Viroformatics

Studying viruses to save human lives.

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Data for Accession Number: 2546935698

# of bases = 987
RNA sequence, recorded as DNA

Virus: Influenza A Virus H3N2
Gene / Protein: Hemagglutinin (HA)

Amino acid sequence

```
QKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYAS
LRSLVASSGTLEFNNESFNWTGVTQNGTSSACKRRSNNSFFSRLNWLTHLKFKYPALNVTMPNNEKFDKLYIWGVHHPGTDNDQIFLYAQASGRITVSTKRSQQTVI
PNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKMRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLKLATGMR
NVPEKQTR
```

Nucleotides

```
  1 caaaaacttc ccggaaatga caacagcacg gcaacgctgt gccttgggca ccatgcagta
 61 ccaaacggaa caatagtgaa aacaatcacg aatgaccaaa ttgaagttac taatgctact
121 gagctggttc agagttcctc aacaggtgaa atatgcgaca gtcctcatca gatccttgat
181 ggagaaaact gcacactaat agatgctcta ttgggagacc ctcagtgtga tggcttccaa
241 aataagaaat gggacctttt tgttgaacgc agcaaagcct acagcaactg ttacccttat
301 gatgtgccgg attatgcctc ccttaggtca ctagttgcct catccggcac actggagttt
361 aacaatgaaa gcttcaattg gactggagtc actcaaaatg gaacaagctc tgcttgcaaa
421 aggagatcta ataacagttt ctttagtaga ttgaattggt tgacccactt aaaattcaaa
481 tacccagcat tgaacgtgac tatgccaaac aatgaaaaat ttgacaaatt gtacatttgg
541 ggggttcacc acccgggtac ggacaatgac caaatcttcc tgtatgctca agcatcagga
601 agaatcacag tctctaccaa aagaagccaa caaactgtaa tcccgaatat cggatctaga
661 cccagagtaa ggaatatccc cagcagaata agcatctatt ggacaatagt aaaaccggga
721 gacatacttt tgattaacag cacagggaat ctaattgctc ctaggggtta cttcaaaatg
781 cgaagtggga aaagctcaat aatgagatca gatgcaccca ttggcaaatg caattctgaa
841 tgcatcactc caaatggaag cattcccaat gacaaaccat ttcaaaatgt aaacaggatc
901 acatatgggg cctgtcccag atatgttaag caaaacactc tgaaattggc aacagggatg
961 cgaaatgtac cagagaaaca aactaga
```

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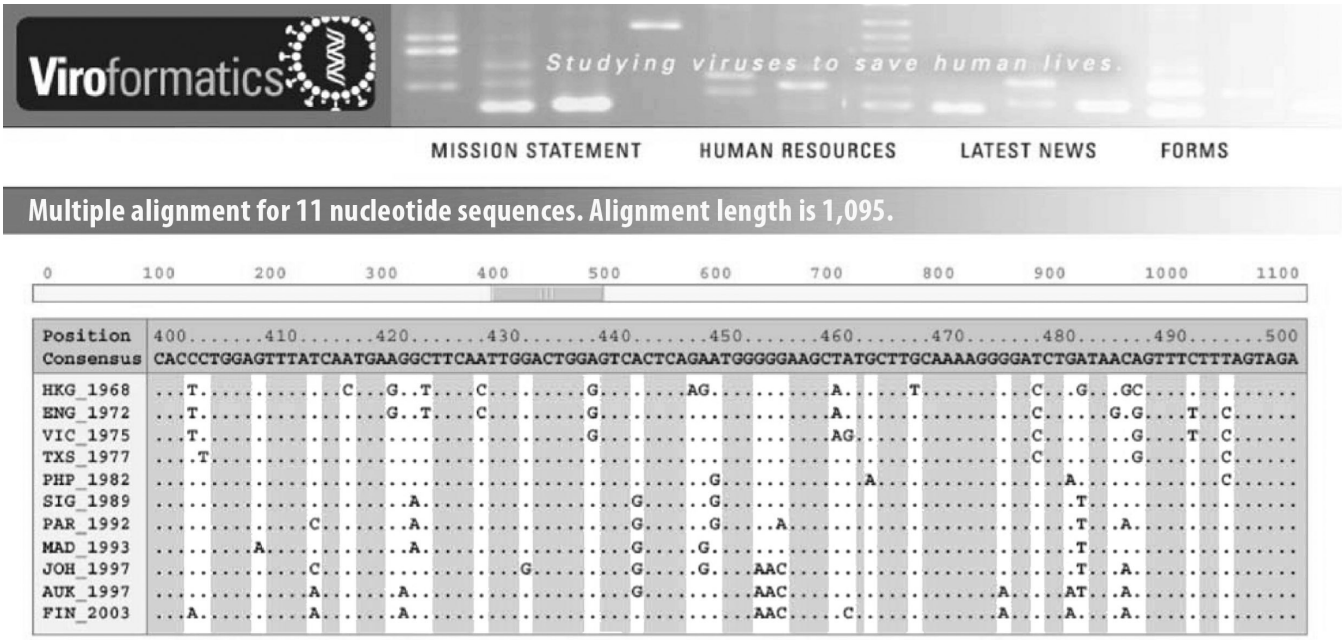
Influenza-Over-Time Alignment

Figure 1. *Viroformatics multiple alignment for 11 nucleotide sequences.*



The diagram above shows a portion of the multiple sequence alignment for the gene that codes for the hemagglutinin protein in 11 influenza viruses. The full gene has about 1,000 nucleotides. Samples are identified in the left column by the country or city of origin and the year the virus was isolated from a patient. In this alignment, a period (.) means that the nucleotide is the same as the most common nucleotide in the sample. Nucleotides that remain the same in all samples have a darker gray background. Nucleotides that vary across the samples have a lighter background. Use the alignment to answer the questions on page 2.

HKG = Hong Kong; ENG = England; VIC = Victoria, Australia; TXS = Texas, USA; PHP = Philippines; SIG = Singapore; PAR = Paris, France; MAD = Madrid, Spain; JOH = Johannesburg, South Africa; AUK = Auckland, New Zealand; FIN = Finland

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Questions

1. Calculate the number of changes per nucleotide in this 100-nucleotide sequence by using the following formula:

$$\frac{\textit{number of nucleotides that have at least one change}}{\textit{total number of nucleotides}}$$

2. The viruses in this study were collected over a span of 35 years. Calculate the number of changes per nucleotide per year by using the following formula:

$$\frac{\textit{answer from Step 1}}{\textit{total number of years}}$$

3. In Lesson 3, you investigated a portion of the sequence of a gene called *Irf6* that is involved in the development of the head and face. You compared the sequence of this gene in many different species. Thirty nucleotides from the *Irf6* sequence are shown below:

   TGGGCCACcAGCCAGGGCTTtAGcCGgACT

   The lowercase (gray) letters show nucleotides that differ among some of the species. The uppercase (black) letters did not change. The amount of time represented in this comparison is 1,009 million years (or 1.009 billion years). Use the same formulas you used in Steps 1 and 2 to calculate the expected number of changes per nucleotide per year in this sequence.

4. Compare the rate of change per nucleotide per year for the hemagglutinin gene in influenza with the rate for the *Irf6* gene. Do this by dividing the rate for the hemagglutinin region by the rate for the *Irf6* gene. The number you calculate will show how many times faster one region changes than the other.

5. The number of changes in the hemagglutinin gene for six samples compared with the sample from Finland in 2003 is as follows:
   - Hong Kong, 1968 = 140 changes
   - Victoria, Australia, 1975 = 128 changes
   - Philippines, 1982 = 95 changes
   - Singapore, 1989 = 74 changes
   - Madrid, Spain, 1993 = 71 changes
   - Auckland, New Zealand, 1997 = 34 changes

   a. How do the number of changes to the sequence relate to time?

   b. What do you think this means?

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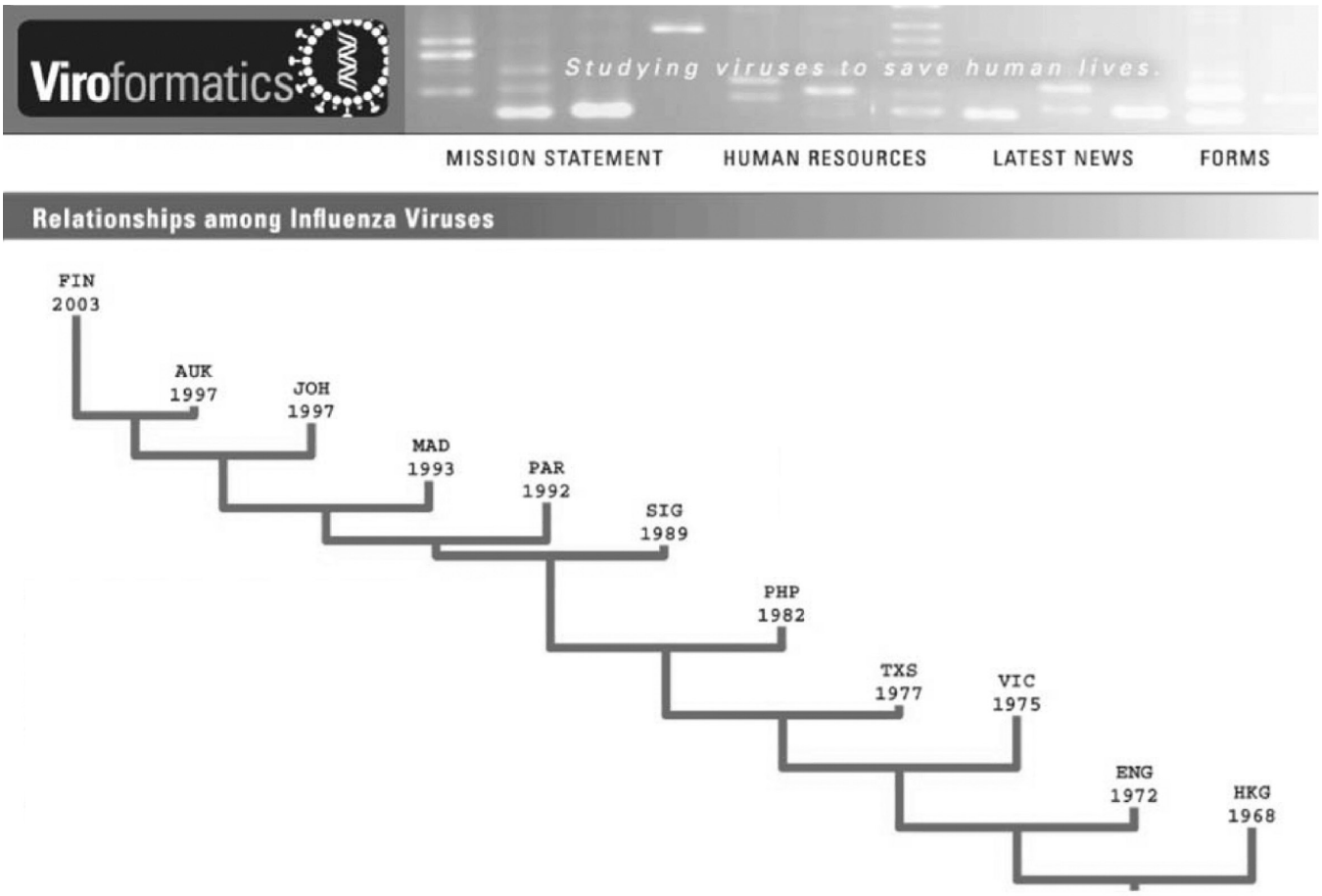
Relationships among Influenza Viruses

Figure 1. *Viroformatics diagram of relationships among the 11 flu viruses sampled over 35 years.*



This diagram shows the relationships among the influenza viruses sampled over 35 years. The length of the lines indicates the number of changes among the sequences. Each sample is named for the place and the year in which the virus was isolated from a patient.

FIN = Finland; AUK = Auckland, New Zealand; JOH = Johannesburg, South Africa; MAD = Madrid, Spain; PAR = Paris, France; SIG = Singapore; PHP = Philippines; TXS = Texas, USA; VIC = Victoria, Australia; ENG = England; HKG = Hong Kong.

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Influenza and the Immune System

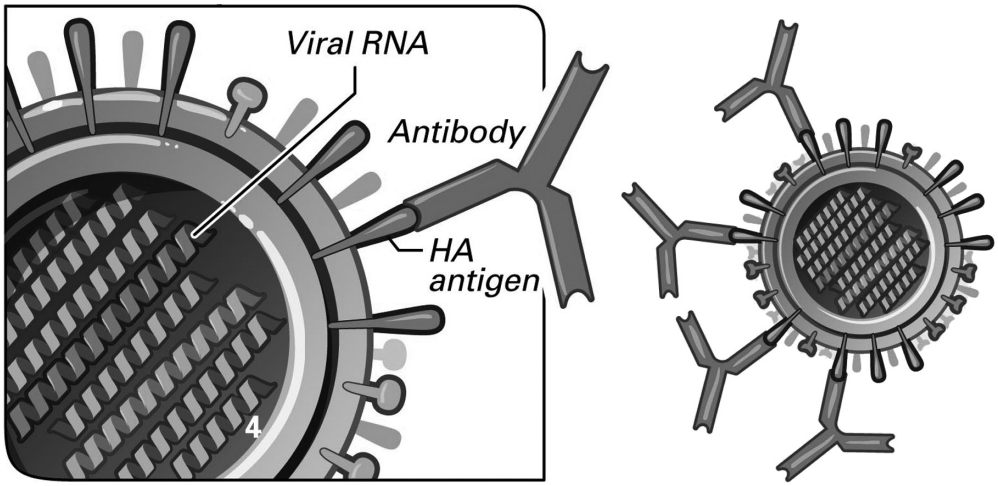
Name: _____

How your body fights influenza

Influenza is a respiratory illness caused by a virus that infects the cells lining the lungs, nose, and throat. To infect your cells, the virus has to enter them. To do that, the hemagglutinin protein on the virus binds to a receptor on the cell. The binding of the virus causes the cell to undergo endocytosis. Once inside, the virus starts making more viruses. Viruses use the "machinery" of the host cell to make new copies of their genetic material and to make proteins. These parts are assembled into a large number of new viruses.

One way your body fights influenza infections is by developing antibodies to the hemagglutinin on the virus. When antibodies attach to hemagglutinin, they keep the virus from attaching to healthy cells. This keeps the virus from infecting these cells.

Figure 1. *Schematic of antibodies binding to the surface of the influenza virus.*



Source: National Institute of Allergy and Infectious Diseases.

Influenza, the immune system, and natural selection

Scientists have explored changes in the hemagglutinin gene in many influenza viruses that circulated around the world. Some mutations resulted in changes in amino acids that make up hemagglutinin. These changes affected how the protein bound to cell receptors. Because these changes altered the phenotype, they affected a virus's ability to infect a cell.

Other mutations in the hemagglutinin gene did not cause a change in any amino acids that make up hemagglutinin. These mutations did not affect a virus's ability to infect cells. As a result, these mutations are mostly "invisible" to the process of natural selection.

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Scientists test for natural selection by comparing the number of mutations that caused amino acid replacements with the number that did not. For hemagglutinin in influenza viruses, scientists found that the number of mutations that cause amino acid replacements is higher than expected. This helped scientists conclude that the influenza virus seems to be evolving by natural selection by avoiding detection by the immune system. Natural selection is part of why influenza virus populations change over time.

Questions

1. Assume there is a change in the gene for hemagglutinin in the influenza virus. Describe how this change could alter the ability of an animal's antibodies to bind to the virus.

2. Imagine an influenza virus that has a mutation that changes the shape of its hemagglutinin protein. Because of this change, antibodies no longer bind to the virus. A second virus does not have the mutation, and antibodies can bind to its hemagglutinin protein. Which virus would leave more descendants? Describe why you answered as you did.

3. How does learning about the immune system help explain the rapid rate of change in the hemagglutinin sequences you observed?

4. When you get an influenza vaccine, you develop antibodies to the hemagglutinin protein for the strains of virus included in the vaccine. Use what you learned in this portion of the activity to revise and improve the answer you gave to Question 5 on the *Notes about Influenza and Evolution* (Master 4.2) handout.

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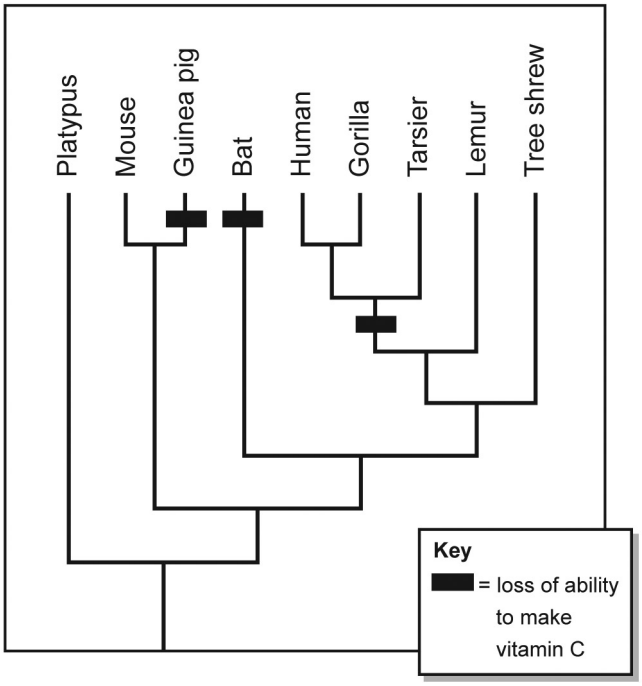
Editing an Article about Vitamin C and Evolution

Recently, the emergency room at the local hospital had a case that challenged the doctors. A three-year-old child was brought in by his parents because the child was pale, had unusual spots on his skin, and was weak; in addition, walking was very painful for the child. After interviewing the parents to learn more about the child's medical history and running some tests, the doctors determined that the child had scurvy. Scurvy is caused by a lack of vitamin C. The child's diet was unusual in that it did not include enough vitamin C. Most people only know of scurvy from history books. In the 1700s, sailors at sea for long periods of time developed scurvy and could be cured by eating citrus fruits. What made this child's diagnosis tough for the doctors was that scurvy is uncommon now. Most people get plenty of vitamin C from the foods in their diets. An understanding of evolution may help us understand how humans can develop scurvy.

Humans must take in vitamin C in their food—our bodies can't make it. This may be surprising to some people because plants and many other animals do synthesize vitamin C. However, humans are not the only mammals that can't make vitamin C—other primates, guinea pigs, and many bats also must get vitamin C from their diets. One question scientists want to investigate is how knowledge of evolution can help us understand when and how some species lost the ability to make vitamin C. One tool that scientists use is an evolutionary tree. Figure 1 shows a tree of the relationships among several types of mammals. Scientists know this tree is correct because it has already been published in a scientific journal. If primates, guinea pigs, and some bats all can't make vitamin C, then they must be each other's closest relatives. Reading the tree from left to right shows that the loss of the ability to

Figure 1. *This evolutionary tree diagram shows the relationships among a number of species. The loss of the ability to make vitamin C is shown with bars.*



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make vitamin C first occurred in guinea pigs. The trait was then passed to bats, and then to primates. Reading the tree from the bottom to the top leads to a different conclusion. Read this way, the tree shows that the ability to synthesize vitamin C was lost three separate times in mammals. Because the evolutionary tree provides this evidence, it's logical to conclude that humans are susceptible to scurvy because we inherited the lack of an ability to make vitamin C from a common ancestor with gorillas and tarsiers. Bats and guinea pigs lost this ability separately.

Scientists are also comparing gene sequences from a variety of different organisms to learn more about vitamin C and evolution. Humans, other primates, some bats, and guinea pigs can't make vitamin C on their own because they lack an enzyme. The name of this enzyme is GULO, which stands for L-gulonolactone oxidase. As with other enzymes, a specific gene codes for the GULO enzyme. Scientists have found this gene in many organisms that can make vitamin C—for example, mice, dogs, cows, pigs, and horses. Figure 2 compares short segments of the gene sequences for a mouse and a cow. These animals make their own vitamin C, so individuals do not survive or reproduce well if they have mutations to the GULO gene that result in a nonfunctional protein. Eventually, these changes are weeded out by natural selection. Because of natural selection, the sequences for GULO in mice and cows have not changed much over time. Figure 3 compares the gene from a mouse and the gene from a human. Scientists found many differences between these two sequences. Some changes in the human gene stop the protein from even being made. Humans do not need the gene anymore because humans usually get vitamin C in their diets. This causes mutations to the GULO gene to occur. However, if people stop eating enough vitamin C, evolution will quickly make the gene functional again.

Figure 2. *DNA Sequences from a mouse and a cow for a segment of a gene that codes for a protein that helps animals make vitamin C.*

```
CTTCTGGCTGCTGTTCAA Mouse
CTTCTGGCTCCTGTTCAA Cow
```

Figure 3. *DNA sequences from a mouse and a human for a segment of a gene that codes for a protein that helps animals make vitamin C.*

```
CTTCTGGCTGCTGTTCAA Mouse
TTTCTGACTCCTGTTTGC Human
```

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Editing an Article about Vitamin C and Evolution, Answer Key

Misconceptions or misinterpretations in the second and third paragraphs are shown in **bold.**

Paragraph 2

Humans must take in vitamin C in their food—our bodies can't make it. This may be surprising to some people because plants and many other animals do synthesize vitamin C. However, humans are not the only mammals that can't make vitamin C—other primates, guinea pigs, and many bats also must have vitamin C in their diets. One question scientists want to investigate is how knowledge of evolution can help us understand when and how some species lost the ability to make vitamin C. One tool that scientists use is an evolutionary tree. Figure 1 shows a tree that depicts the relationships among several types of mammals. **Scientists know this tree is correct because it has already been published in a scientific journal.**

Evolutionary trees are claims based on evidence. Like all claims in science, they are subject to change with new evidence.

If primates, guinea pigs, and some bats all can't make vitamin C, they must be each other's closest relatives.

Estimates of relationships are based on multiple lines of evidence, not just one.

Reading the tree from left to right shows that the loss of the ability to make vitamin C first occurred in guinea pigs. The trait was then passed to bats, and then to primates.

This evolutionary tree should not be read from left to right. The pattern of branching underneath the names should be used to determine patterns of evolution.

Reading the tree from the bottom to the top leads to a different conclusion. Read this way, the tree shows that the ability to synthesize vitamin C was lost three separate times in mammals. Because the evolutionary tree provides this evidence, it is logical to conclude that humans are susceptible to scurvy because we inherited the lack of an ability to make vitamin C from a common ancestor with gorillas and tarsiers. Bats and guinea pigs lost this ability separately.

Paragraph 3

Scientists are also comparing gene sequences from a variety of different organisms to learn more about vitamin C and evolution. Humans, other primates, some bats, and guinea pigs can't make vitamin C on their own because they lack an enzyme. The name of this enzyme is GULO, which stands for L-gulonolactone oxidase. As with other enzymes, a specific gene codes for the GULO enzyme. Scientists have found this gene in many organisms that can make vitamin C—for example,

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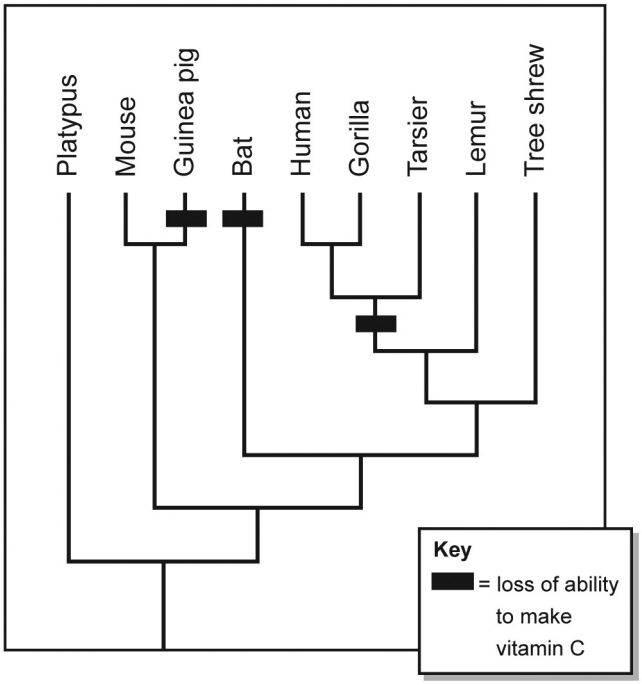
mice, dogs, cows, pigs, and horses. Figure 2 shows a comparison of a short segment of the gene sequences for a mouse and a cow. These animals make their own vitamin C, so individuals do not survive or reproduce well if they have mutations to the GULO gene that result in a nonfunctional protein. Eventually, these changes are weeded out by natural selection. Because of natural selection, the sequences for GULO in mice and cows have not changed much over time. Figure 3 shows a comparison of the gene from a mouse and the gene from a human. Scientists found many differences between these two sequences. Some changes in the human gene stop the protein from even being made. **Humans do not need the gene anymore because humans usually get vitamin C in their diets. This causes mutations to the GULO gene to occur.**

Mutations do not occur because an organism "needs" them.

Also, after students talk with other students who read Paragraph 2, they should realize that the loss of the ability to make vitamin C occurred in an ancestor to humans, gorillas, and tarsiers. The change did not occur only in the human lineage. This second error may be difficult for students identify.

However, if people stop eating enough vitamin C, evolution will quickly make the gene functional again.

Figure 1. *This evolutionary tree diagram shows the relationships among a number of species. The loss of the ability to make vitamin C is shown with bars on the diagram.*



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This is another argument based on need. Lineages of organisms do not change simply because they need to change.

Figure 2. *DNA Sequences from a mouse and a cow for a segment of a gene that codes for a protein that helps animals make vitamin C.*

```
CTTCTGGCTGCTGTTCAA Mouse
CTTCTGGCTCCTGTTCAA Cow
```

Figure 3. *DNA sequences from a mouse and a human for a segment of a gene that codes for a protein that helps animals make vitamin C.*

```
CTTCTGGCTGCTGTTCAA Mouse
TTTCTGACTCCTGTTTGC Human
```

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