Name _____

Mitochondrial DNA as a Molecular Clock

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We gratefully acknowledge David Micklos and the staff of the Dolan DNA Learning Center at Cold Spring Harbor Laboratory for their generous help. Some materials for this exercise were adapted, by permission, from the *Genomic Biology: Advanced Instructional Technology for High School and College Biology Faculty* laboratory manual, Cold Spring Harbor Laboratory, copyright 1999 and the *Genetic Origins* website at http://vector.cshl.org/geneticorigins.

Introduction

The DNA of every species on Earth is susceptible to change. Base pairs are lost. Base pairs are gained. One base pair can be substituted for another. We call these changes in DNA **mutations** or **single nucleotide polymorphisms (SNPs)** and they can arise by different mechanisms. By allowing organisms to adapt to environmental changes, mutation drives the steady and inevitable march of evolution.

Because a mutation within a gene can change the amino acid sequence of the encoded protein, many mutations spell disaster for that gene's function and potentially for the organism that bears it. Almost all genomes, however, from those of viruses to humans, carry segments of DNA that neither directly code for a protein nor are involved in the control of gene expression. A mutation occurring within such regions can usually be tolerated by the organism since it will most likely not impart any disadvantage to its survival nor impair its ability to reproduce.

Recently, forensic scientists, anthropologists, and evolutionary biologists have looked at mutations within the DNA of the mitochondrion to explore differences between peoples and populations. **Mitochondria** are found in all eukaryotic cells and are believed to have once been free living bacteria that were assimilated early in evolution. They provide the cell in which they reside with the genes needed for the synthesis of the energy-carrying molecule ATP. Each mitochondrion contains several copies of its own circular genome and each cell may contain hundreds of mitochondria.

Because of its abundance, mitochondrial DNA has become a target for those scientists who do not have a ready supply of blood, bodily fluids, or tissue to work with but who still need to examine differences between people at the molecular level. Whether it is the skeletal remains of a Neandertal or a trace amount of hair left at the scene of a crime, where intact genomic DNA might be hard to come by, mitochondrial DNA can often be readily recovered.

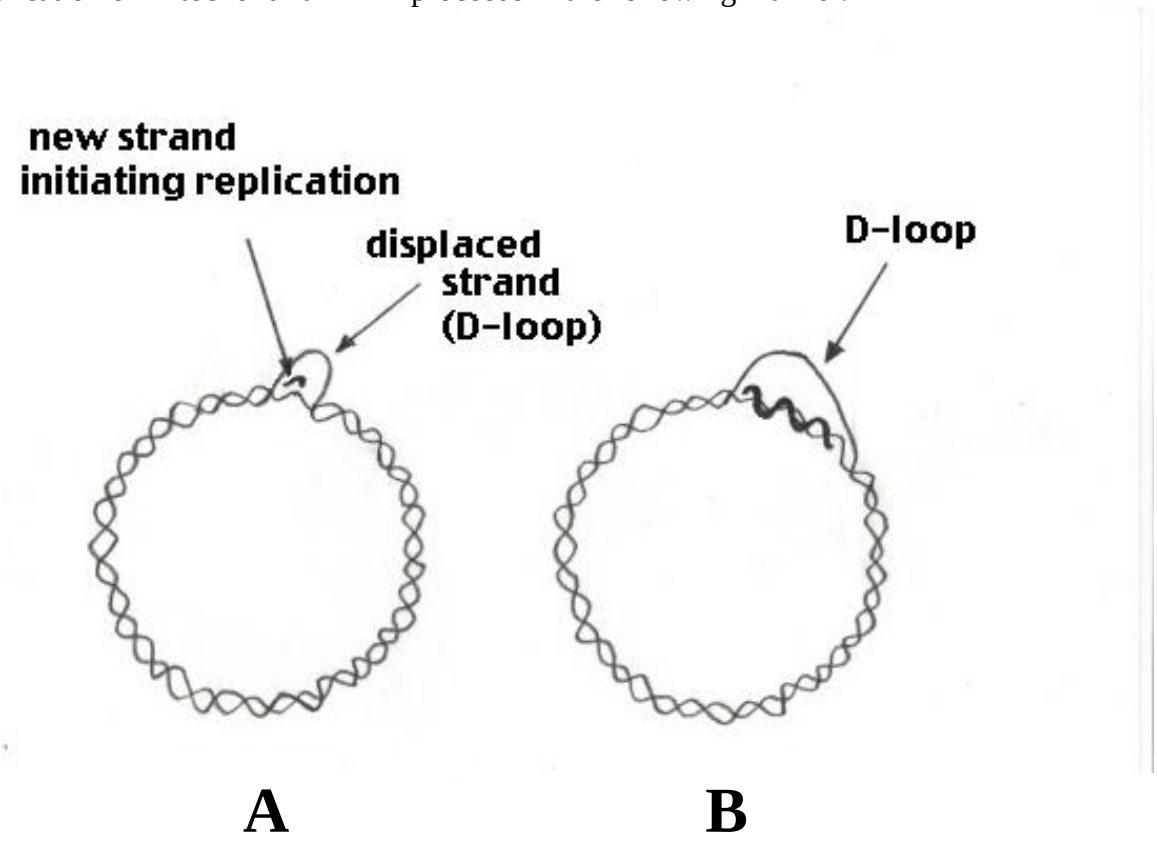
The mitochondrial genome is 16,569 bp in length and contains 37 genes. Within its structure, however, there is a 1200 base pair non-coding segment, called the **control region**, carrying the genetic signals needed for replication and transcription. Since much of this DNA segment is not vital to the survival of the mitochondrion or the host cell, it is free to accumulate mutations. By studying the number and variety of base changes within

this area, geneticists can determine the relatedness between individuals. Using the mutation rate within the mitochodrial control region as a "molecular clock," evolutionists can plot the course that hominid evolution has taken.

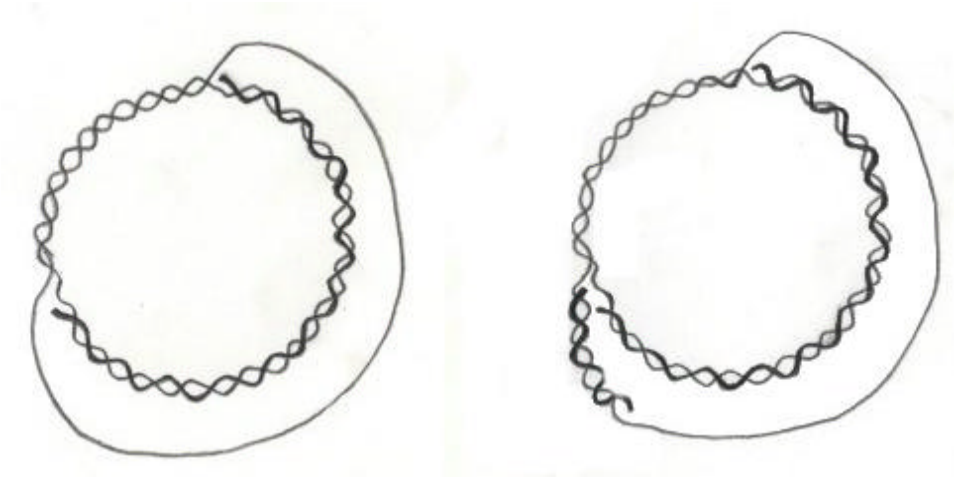
Mitochondrial DNA Replication

When thinking about the human genome and all the traits that make us what we are tucked away within those six billion base pairs, it is easy to forget that all other cells in our body contain another genome, that of the mitochondrion. In some ways, mitochondrial DNA resembles the small circular pieces of DNA called **plasmids** found in bacterial cells. Like a plasmid, mitochondrial DNA is circular with a genome a fraction of the size of that of its host cell. Also, like a plasmid, there are multiple copies within each cell. Some cells in the human body carry thousands of mitochodria, and therefore contain thousands of copies of the mitochondrial genome. Because our bodies are composed of trillions of cells, with hundreds or thousands of mitochondria present in each cell, our bodies may contain more than 5 quadrillion (5,000,000,000,000,000) copies of the mitochondrial genome. A great deal of DNA replication has taken place to reach the adult body's full complement of mitochondrial genomes!

Replication of mitochondrial DNA proceeds in the following manner.



Replication of mitochondrial DNA begins on only one strand within the non-coding "control" region. As this strand is replicated, the opposite strand of the original DNA duplex is displaced and forms a single-stranded loop (hence the name "**D-loop**" for Displacement loop in Figure A). If the mitochondrion is not committed to the replication of its genome, copying of the non-displaced strand stops close to the protein-encoding boundary (Figure B). The displaced strand is then broken down and the replication process begins again displacing a single strand in the process. These events are repeated again and again until a signal is received that commits the entire molecule to replication. The segment you will amplify by PCR is within the D-loop sequence.



C **D**

Once the signal is received to replicate the entire genome, replication continues around the circular molecule, increasing the size of the displaced strand (Figure C). When replication has proceeded approximately two-thirds of the way around the molecule, replication begins on the displaced strand (Figure D) until two new circular genomes are created.

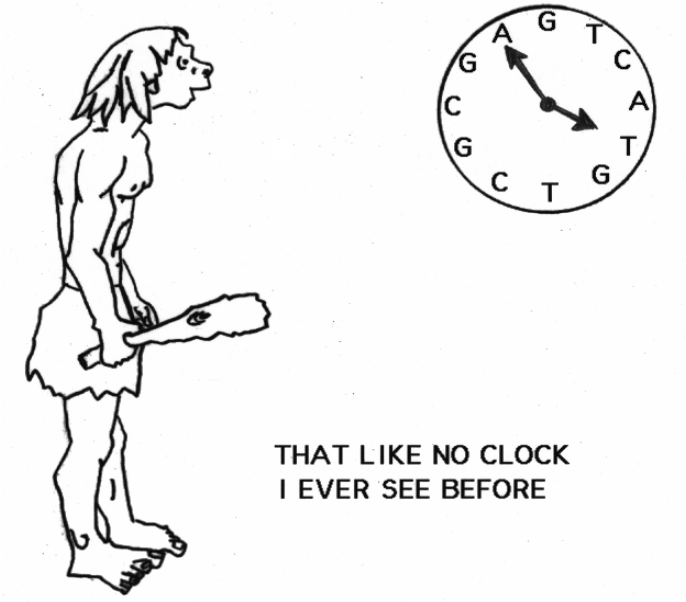
Setting the Molecular Clock

A **species** is defined as a group of organisms that are capable of interbreeding to produce viable, reproductive offspring. New species can arise when members of a population separate to form their own breeding group within a new environment that demands of its inhabitants a unique set of survival skills. As the separated group struggles to fill a new and different ecological niche, the genes that provide individuals an advantage in the competition to flourish and mate are selected for and passed on to the next generation. Those individuals carrying genes that do not provide a selective advantage may neither survive into adulthood nor mate. Their genes are lost to the population.

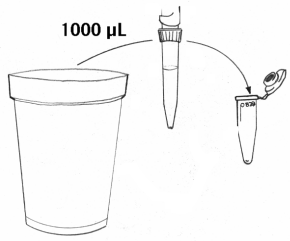
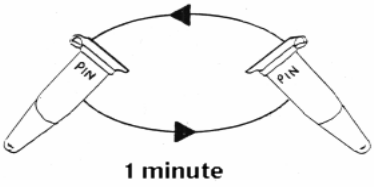
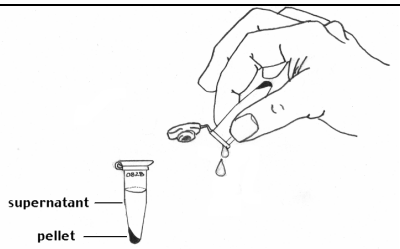
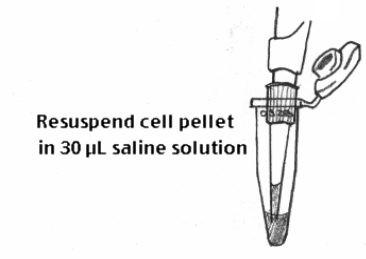
A population's ability to adapt to a new environment is driven by the process of natural selection. Mutation makes natural selection possible. Mutation alters genes, destroying or changing their function. Mutation molds the ability of members of a species to survive under a defined set of conditions. Eventually, during the process of adaptation and over many generations, enough mutations accumulate within the separated population group that its individuals are no longer capable of interbreeding with members of the original population. This marks the birth of a new species. The longer two species diverge from each other, the greater the number of mutational differences there will be between them.

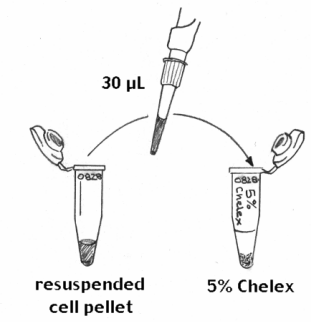
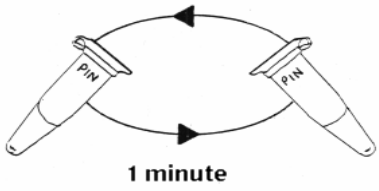
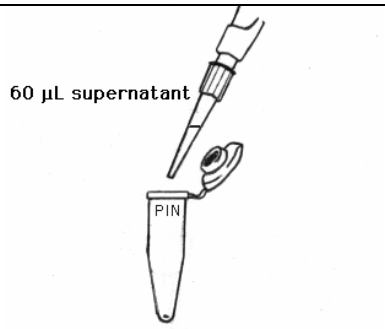
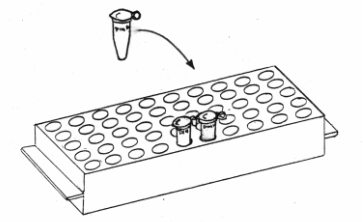
A clock measures the passage of time. Assuming that mutations occur at a constant rate, the accumulation of mutations in a DNA segment can be used as a "**molecular clock**" to measure the passage of time. In this case, the greater the number of mutations, the greater the amount of time passed. For example, if a new mutation appears in a defined region of DNA at a rate of one every 100,000 years, then after 500,000 years, 5 mutations will likely accumulate.

In this laboratory exercise, you will isolate mitochondrial DNA from cheek cells and amplify a 440 base pair segment of the control region by PCR. You will analyze the DNA sequence of the PCR product to reveal differences between you and the other students in your class. You can compare your sequence with those of the "Ice Man," "Lake Mungo Man," and other long-dead humans. How does your sequence compare to those of chimpanzee and Neandertal? Could you and other modern humans have arisen from Neandertals or did we evolve separately? If we evolved separately, at what point in time did modern humans and Neandertals diverge on the evolutionary tree? Could Neandertals have contributed to our gene pool? These are all questions you will investigate.

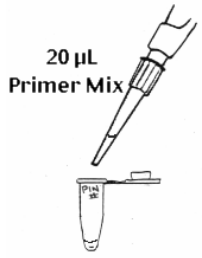
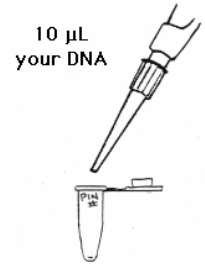
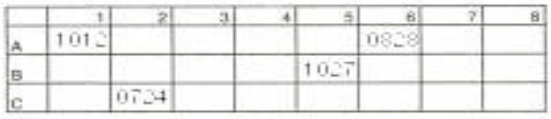
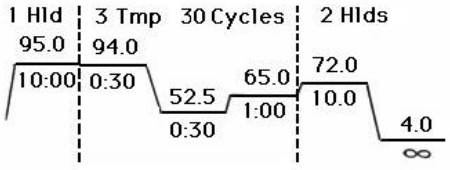


DNA Preparation Using a Saline Mouthwash

1. Swirl 10 mL of 0.9% saline in your mouth for 30 seconds.	
2. Expel saline into a cup and swirl to mix the cells.	
3. Transfer 1000 µL of the liquid into a 1.5 mL microfuge tube, labeled with your PIN.	
4. In a balanced centrifuge, spin sample for 1 minute.	
5. Observe your cell pellet at the bottom of the tube. Pour out the supernatant back into your cup, being careful not to lose your cell pellet. **Note:** It is okay if some supernatant is left in the tube.	
6. Resuspend your cell pellet in 30 µL of saline. Make sure the entire cell pellet is thoroughly mixed by vortexing or "racking" your tube. **Note**: To "rack" your sample, be sure the top of the tube is closed, hold tube firmly at the top, and pull it across a microfuge rack 2-3 times.	

7. Withdraw 30 µL of the cell suspension and add it to a 0.2 mL tube containing 200 µL of 5% Chelex, provided by your instructor.	
8. Place your 0.2 mL Chelex tube into a thermal cycler set for 99°C and heat for 10 minutes.	
9. Shake your tube well or briefly vortex it and then place it in a balanced centrifuge. Spin for 1 minute.	
10. Withdraw 60 µL of supernatant (no Chelex beads) and add to a new 1.5 mL tube, labeled with your PIN and "DNA." **Note**: This stored sample contains your DNA that you will add to your PCR reaction.	
11. Place your DNA tube in the class microfuge rack so that your teacher can refrigerate your isolated DNA until you are ready to prepare your PCR amplification.	

Polymerase Chain Reaction

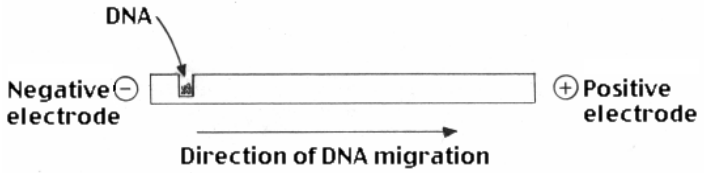
1. Label a 200 µL PCR tube with your 4 digit PIN.	
2. Set your micropipet to 20 µL and dispense **20 µL of Master Mix** into your PCR tube.	
3. Change your pipet tip and add **20 µL of Primer Mix** into your PCR tube.	
4. Using a clean tip, add **10 µL of your purified DNA** into your PCR tube. **Note**: Slowly pipet up and down several times to mix all the reagents in your reaction tube.	
5. Place your reaction into the thermal cycler and record the location of your tube on the grid provided by your teacher.	
6. The cycling protocol for amplification of this mitochondrial control region is: 95°C, 10 minutes; 94°C, 30 seconds; 52.5°C, 30 seconds; X 30 cycles 65°C, 1 minutes; 72°C, 10 minutes; 4°C, hold	

Controls

+Control: 20 µL Master Mix, 20 µL Primer Mix, 10 µL +Control DNA
-Control: 10 µL sterile water, 20 µL Master Mix, 20 µL Primer Mix

Agarose Gel Electrophoresis

To determine if you generated a PCR product that can be used for subsequent DNA sequencing, you will need to visualize the products of your amplification. This will be done using a process called **electrophoresis** in which electric current forces the migration of DNA fragments through a special gel material. Since DNA is negatively charged, it will migrate in an electric field towards the positive electrode (Figure 1). When electrophoresed through a gel, shorter fragments of DNA move at a faster rate than longer ones.

Figure 1. Side view of an agarose gel showing DNA loaded into a well and the direction of DNA fragment migration during electrophoresis.	

The gel material to be used for this experiment is called **agarose.** When agarose granules are placed in a buffer solution and heated to boiling, they dissolve and the solution becomes clear. A casting tray is set up with a comb to provide a mold for the gel. The agarose is allowed to cool slightly and is then poured into the casting tray. Within about 15 minutes, the agarose solidifies into an opaque gel having the look and feel of coconut Jell-O. The gel, in its casting tray, is placed in a buffer chamber connected to a power supply and buffer is poured into the chamber until the gel is completely submerged. The comb can then be pulled out to form the wells into which your PCR sample will be loaded.

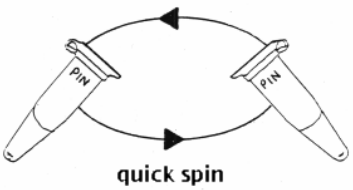
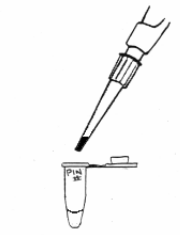
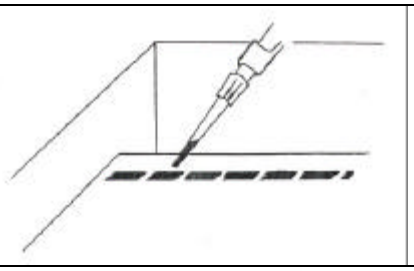
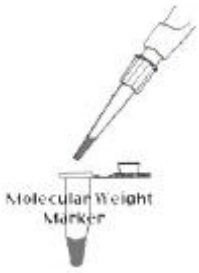
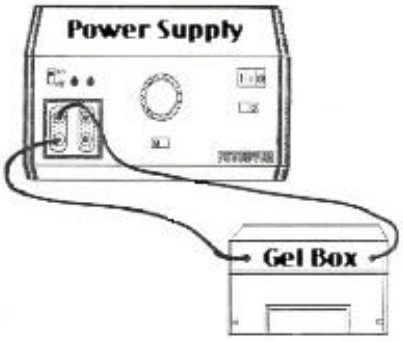
Loading dye is a colored, viscous liquid containing dyes (making it easy to see) and sucrose, ficoll, or glycerol (making it dense). You will add loading dye to your amplification reaction and then pipet an aliquot of the mixture into one of the wells of your agarose gel. When all wells have been loaded with sample, your instructor will switch on the power supply. The samples should be allowed to electrophorese until the blue loading dye is 1 to 2 cm from the bottom. The gel can then be stained with ethidium bromide and photographed.

You will need a 2% agarose gel for electrophoresis of your PCR products. If your agarose gel casting tray holds 50 mL, then you can calculate the amount of agarose you will need as follows:

$$(C_i)\,(M_i) = (C_f)\,(M_f)$$

C = concentration
M = Mass
i = initial
f = final

$(100\%)\,(M_i) = (2\%)\,(50\text{ g})$
$100\,(M_i) = 100\text{ g}$
$M_i = 1$ g agarose powder
50 g – 1 g = 49 g = 49 mL buffer

Electrophoresis of Amplified DNA	
1. Retrieve your PCR tube and spin it briefly to bring the liquid to the bottom of the reaction tube. **Make sure the centrifuge is balanced before you begin spinning your sample!**	
2. In a 0.5 mL new tube, labeled with your PIN, dispense **20 µL of PCR product and 2 mL of loading dye** (use a new tip). Slowly pipet the mixture up and down until the contents in the tube are uniformly colored.	
3. Carefully load **15 – 20 µl** of your reaction into a well in your gel. Avoid poking the pipette tip through the bottom of the gel or spilling sample over the sides of the well. Use a new tip for each sample.	
4. One student (or the instructor) should load **5 µl** of the 100 bp ladder (molecular weight marker) into one of the wells of each gel.	
5. When all samples are loaded, attach the electrodes from the gel box to the power supply. Have your teacher check your connections and then electrophorese your samples at **125 Volts** for **20 to 30 minutes**.	

Staining and Photographing Agarose Gels
Your teacher will stain your agarose gel and take a photograph for you so that you may analyze your amplification results. Gel staining is done as follows.

1. Place the agarose gel in a staining tray.
2. Pour enough ethidium bromide (0.5µg/ml) to cover the gel. Wait 15 minutes.

CAUTION: Ethidium bromide is a carcinogen. Always wear gloves and safety glasses when handling.

3. Pour the ethidium bromide solution back into its storage bottle. Pour enough water into the staining tray to cover the gel. Wait 5 minutes.
4. Pour the water out of the staining tray into a hazardous waste container and place the stained gel on a UV light box.

CAUTION: Ultraviolet light can damage your eyes and skin. Always wear protective clothing and UV safety glasses when using a UV light box.

5. Place the camera over the gel and take a photograph.

Figure 2. Ethidium bromide molecules stacked between DNA base pairs.

The PCR products run on your agarose gel are invisible to the naked eye. If you look at your gel in normal room light, you will not be able to see the amplified products of your reaction. In order to "see" them, we must stain the gel with a fluorescent dye called **ethidium bromide**. Molecules of ethidium bromide are flat and can nestle between adjacent base pairs of double stranded DNA (Figure 2). When this interaction occurs, they take on a more ordered and regular configuration causing them to fluoresce under ultraviolet light (UV). Exposing the gel to UV light after staining, allows you to see bright, pinkish-orange bands where there is DNA (Figure 3).

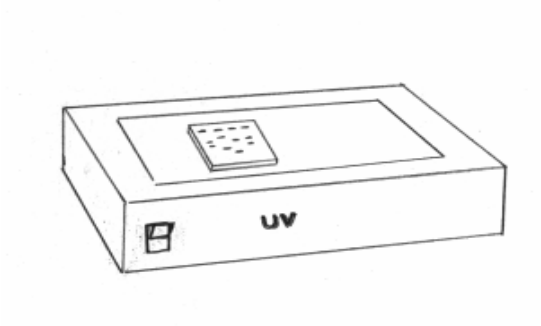


Figure 3. After staining an agarose gel with ethidium bromide, DNA bands are visible upon exposure to UV light.

Mitochondrial D-loop PCR Amplification Results

PCR amplification of the mitochondrial D-loop region using the primers for this protocol should produce a 440 bp product as shown in the figure below.

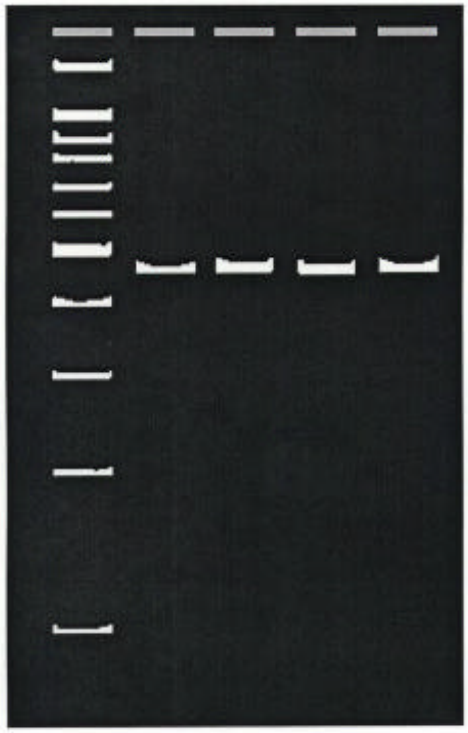


Figure 4. Representation of an agarose gel containing a 100 bp ladder (leftmost lane) and lanes showing 440 bp products from D-loop PCR amplification.

Tape your gel photo in the space below.

Cycle Sequencing Reactions

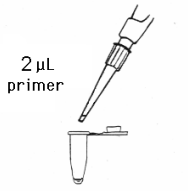
1. Label a 0.2 mL tube with your 4 digit PIN.

2. Set your p20 micropipet to 8 µL and add **8 µL of sterile water** to your 0.2 mL tube.

3. Change your tip and dispense **8 µL of Big Dye Terminator RR Mix*** into your 0.2 mL tube.

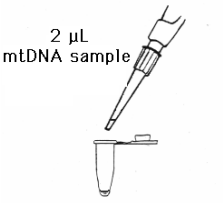
*"RR"stands for **R**eady **R**eaction. A Ready Reaction Mix contains the dNTPs, ddNTPs, buffer, enzyme, and magnesium chloride.

4. With a new tip, add **2 µL of primer** into your 0.2 mL tube.



5. Using a clean tip, add **2 µL of your mtDNA PCR** sample into your 0.2 mL tube. Slowly pipet up and down several times to mix.

Note: You may need to centrifuge the tubes for 5-10 seconds if the reaction components are along the walls of the tube.

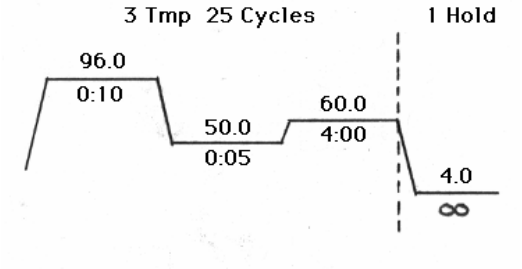


6. Place your reaction into the thermal cycler and record the location of your tube on the grid provided by your teacher.

	1	2	3	4	5	6	7	8
A	1012					0828		
B					1027			
C		0734						

7. Follow the cycling parameters below for cycle sequencing of this mitochondrial control region.
   25 cycles of:
   96°C, 10 seconds
   50°C, 5 seconds
   60°C, 4 minutes

   4°C hold



+ Control: 6 µL sterile water, 8 µL Ready Reaction Mix, 4 µL –21 M13 Control Primer, 2 µL pGEM – 3Zf(+) Control DNA Template

Spin Column Purification and Loading the Automatic Sequencer
1. Rehydrate the Centri-Sep column in 750 µl sterile water. Remove any air bubbles from the column by tapping on the column until the bubbles float up. Allow them to sit for at least 30 minutes.
2. If any bubbles appeared during rehydration, tap them out. Remove upper cap and then lower cap and place the column in the wash tube. Make sure that the column is dripping. If it is not, seal the top of the tube with Parafilm and press your finger on the Parafilm to start the flow.
3. Place the wash tube with spin column in the centrifuge and spin for 2 minutes at 4,000 rpm.
4. Dump the wash tube and place the column into a 1.5 mL microfuge tube, labeled with your initials.
5. Apply your cycle sequencing reaction to the top center of the column matrix. Do not allow the micropipet tip to touch the matrix.
6. Spin the column and microfuge tube as a unit for 2 minutes at 4,000 rpm with the column in the same orientation as in the first spin.
7. After centrifugation, throw away the column and vacuum-dry the sample.
8. Add 5 µL of loading buffer to resuspend the cycle sequencing sample. Pipet up and down repeatedly along the bottom sides of the tube to ensure complete resuspension of the reaction products.
9. After a quick 5 second spin, transfer the sample into a 200 µL PCR tube.
10. Heat samples for 2 minutes at 95°C in the thermal cycler and then immediately place your sample in ice.
11. Load 1.5 µL into the appropriate well on the sequencing gel, following the indications on the Sample Sheet (on the computer screen).

Using the Sequence Server at the CSHL DNA Learning Center

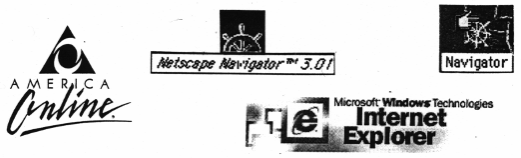
Objectives:
You should be able to view your class data in the Cold Spring Harbor Laboratory Sequence Server database.
You should be able to perform pair-wise sequence alignments between diverse modern humans.
You should be able to perform pair-wise sequence alignments between diverse modern humans and Neandertals.
You should be able to set the "molecular clock" based on the number of sequence differences between modern humans.
You should be able to use a "molecular clock" to estimate when Neanderthals and modern humans diverged.

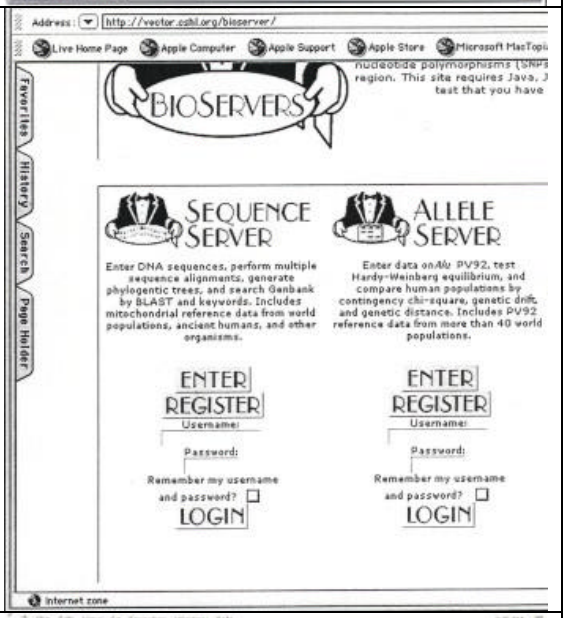
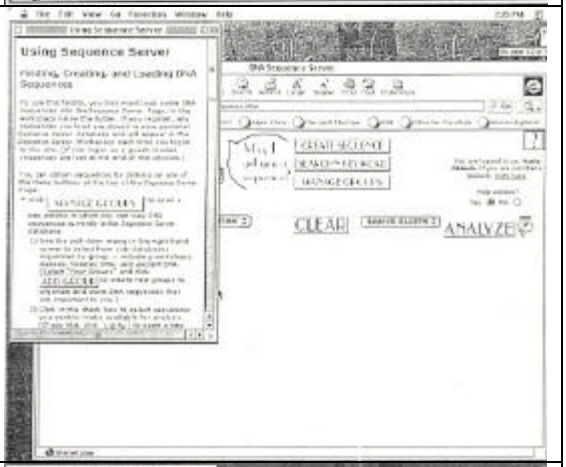
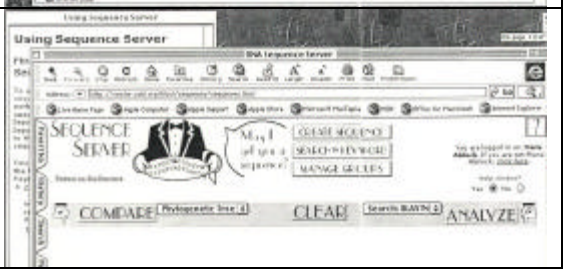
The DNA Learning Center at Cold Spring Harbor Laboratory has developed a number of **bioinformatics** tools for student use. Bioinformatics tools are computer programs used to help scientists make sense of biological data and solve biological problems. You will be using the Sequence Server for three different activities to help you learn more about the origins of our species.

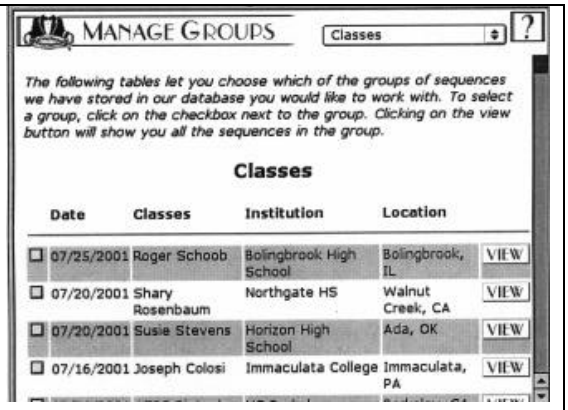
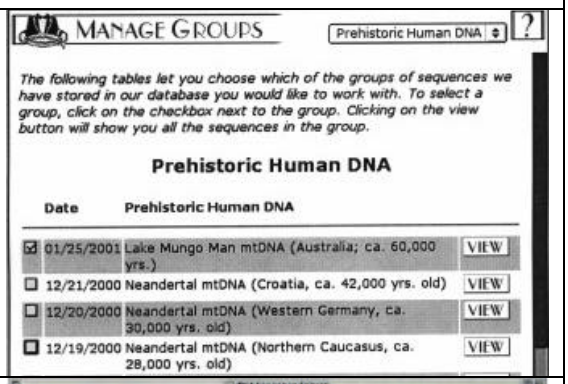
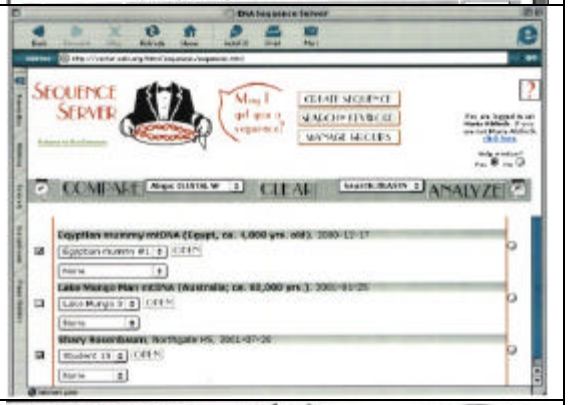
In the following exercise, you will compare DNA sequence between individuals from several different population groups. You will first compare sequences between modern humans. This information will be used to set a "molecular clock" (described on page 19). You will then compare modern humans to Neandertals to see if Neandertals might have contributed to our gene pool. The molecular clock you derive will be used to determine when modern humans and Neandertals diverged. In your final comparison, you will align modern human sequences to that of a chimpanzee to derive a new molecular clock. The molecular clocks will be used to estimate when modern humans first appeared.

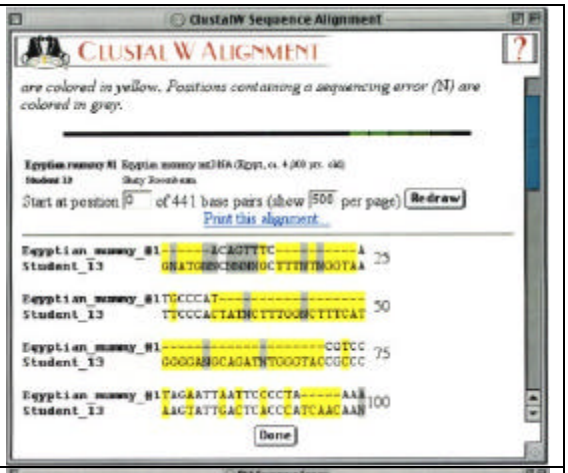
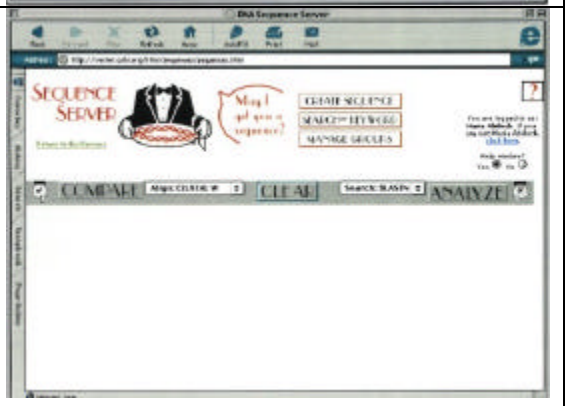
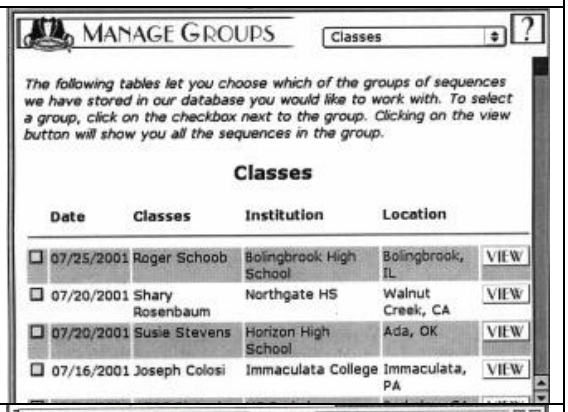
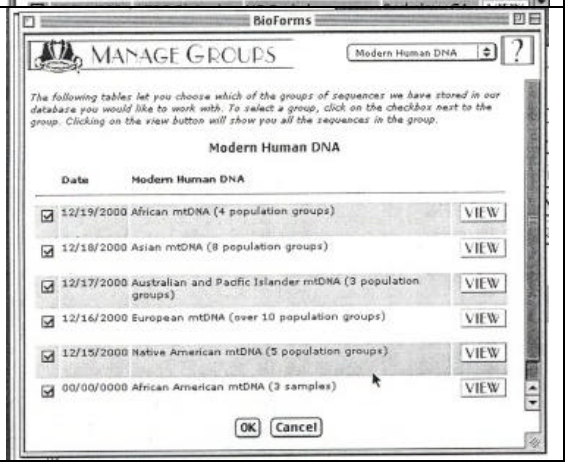
Using the Sequence Server to Align Mitochondrial DNA Sequences

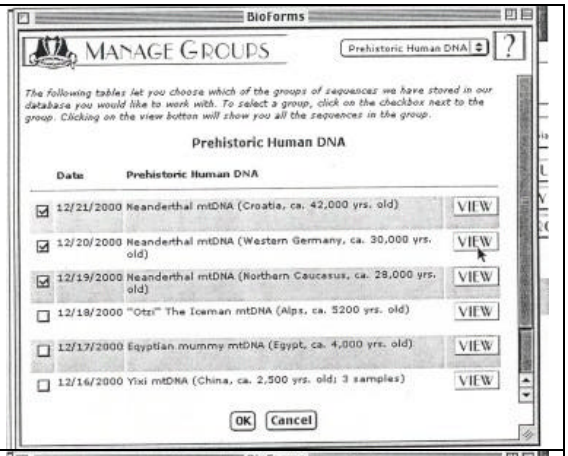
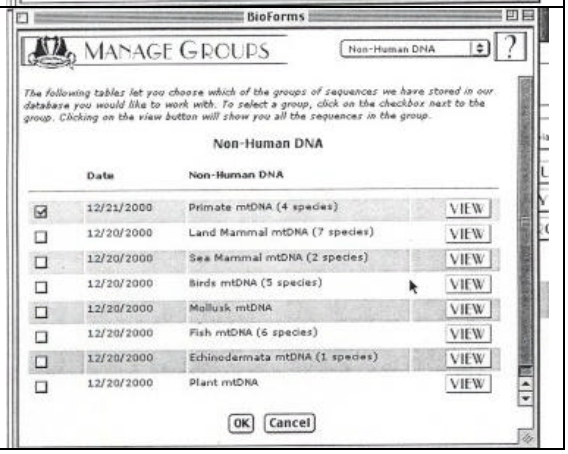
Your class data has been entered into the Sequence Server database at the Dolan DNA Learning Center at the Cold Spring Harbor Laboratory. Use the following steps to access and utilize that data.

Mitochondrial DNA Sequence Comparisons	
1. Click on the icon for your Internet Service Provider to gain access to the internet. (This might be America Online, Netscape Navigator, Microsoft Internet Explorer, etc.)	
2. In the internet address box, type in the following URL: http://vector.cshl.org and press the Enter (or Return) key on the keyboard. The DNA Learning Center main page will be brought up.	

3. In the DNA Learning Center main page, click on the "Bioservers" image towards the bottom right of the page.	
4. Click on the "REGISTER" button if you have not previously registered with Bioservers. Fill out the required information and then hit "SUBMIT." If you are already registered, enter your username and password, then press "LOGIN."	
5. The "Using Sequence Server" instruction window will appear on top of the Sequence Server Workspace. The instructions contained in this box can be used if you need more information about using this site. Click on the Sequence Server Workspace to bring it forward on the desktop.	
6. Click on the "MANAGE GROUPS" box. This is where you can identify groups of data to add to your workspace.	

7. In the upper right hand corner of the Manage Groups window is a scroll menu. If it isn't already showing, select "Classes." This will bring up a list of classes from across the country that have sequence data stored in the Sequence Server database. Use the scroll bars on the right side of the window, if needed, to locate your class. When you have located your class, click the box to its left to select it. **Note:** The entire window must load before attempting to change the selection.	
8. Select "Prehistoric Human DNA" from the Manage Groups window. Click the box to the left of one or more of the following: "Lake Mungo Man," "Otzi, the Iceman," "Egyptian Mummy," "Yixi mtDNA," or "Cahokian mtDNA." Press "OK" when finished.	
9. Your class data and several prehistoric humans' data should now be added to your workspace. Select your sequence by using the scroll menu below your class name. Deselect all the check boxes on the left except for your sample and one prehistoric human of your choice.	
10. Next to the "COMPARE" button below the Sequence Server icon, use the arrows to scroll to "Align:CLUSTAL W" then click the "COMPARE" button.	

11. Your sequence and the prehistoric human sequence you chose should align where bases are complementary. You may notice some yellow highlight regions, dashes and gray highlight regions with "N's." What do you think these indicate?	
12. When you have examined the alignment to your satisfaction, press the "CLEAR" button to clear your workspace. Next, you will work with various diverse modern humans, Neandertals, chimpanzee, and your classmates' data to investigate your genetic origins. The following steps will guide you through the procedure to add members of these groups to your workspace.	
13. In the Manage Groups window, use the scroll menu to locate your class. Select it by checking its box.	
14. Using the scroll menu again in the upper right corner of the Manage Groups window, locate and select "Modern Human DNA." Check all boxes in this Modern Human DNA window.	

15. Locate and select the “Prehistoric Human DNA” category from the Manage Groups window. Place check marks in the boxes to the left of the Neanderthal mtDNA sequences.	
16. Select the “Non-Human DNA” category from the Manage Groups window. Click the box to the left of “Primate mtDNA (4 species).” Click on the “OK” button at the bottom of the window. This will place all selected DNA sequences onto the Sequence Server Workspace.	

Sequence Comparisons

You will perform a series of sequence alignments that will allow you to estimate a mutation rate and to calculate the timing of crucial events in human evolution.
Use the following guidelines for each comparison.

- Identify a region spanning 200 bases where there is good alignment between the two sequences you are comparing. This region should contain few, if any, "N's."
- Excluding N's (in gray) and dashes that may occur at the beginning or end of the alignment, count how many yellow-highlighted base positions are found in the alignment. If you find a run of three or more dashes in a row, count such a run as a single nucleotide difference.
- If you find a sequence that does not align for 200 bases, use a different sequence.

Follow the steps below to fill in the spaces in the chart that follows.

1. **Modern Human vs. Modern Human**
   a. Select any two modern humans from the groups on your workspace. Fill in the identifying information in the table.
   b. Compare these two individuals by ClustalW alignment. Count the number of mismatches, or SNPs, and record this number in the table.
   c. Repeat steps "a" and "b" using different modern humans.
   d. Now compare two students in the class and fill in all the appropriate information in the table.
   e. Calculate the average number of SNPs for this group and record in the table.
2. **Modern Human vs. Neandertal**
   a. Select any African modern human and any Neandertal to compare by ClustalW alignment. Fill in the identifying information in the table.
   b. Compare these two individuals and record the number of SNPs in the table.
   c. Repeat steps "a" and "b" with any Asian modern human and any Neandertal.
   d. Repeat steps "a" and "b" again using any European modern human and any Neandertal.
   e. Now compare your (or another student's) sequence with any Neandertal and record all appropriate information in the table.
   f. Calculate the average number of SNPs for this group and record in the table.
3. **Neandertal vs. Neandertal**
   a. Select Neandertal #1 and Neandertal #2 to compare by ClustalW alignment. Record the number of SNPs in the table.
   b. Do the same with the other two combinations of Neandertals.
   c. Calculate the average number of SNPs for this group and record in the table.
4. **Modern Human vs. Chimpanzee**
   a. Select any modern human to compare with Chimp #2. Fill in the identifying information in the table.
   b. Compare these two sequences by ClustalW alignment. Count the number of SNPs and record this number in the table.
   c. Repeat steps "a" and "b" using different modern humans.
   d. Now compare your (or another student's) sequence with Chimp #2 and record the number of SNPs along with the appropriate identifying information.
   e. Calculate the average number of SNPs for this group.

Student Data

Sequence Server Clustal W Alignments: SNPs			
Modern Human vs. Modern Human	**Number of SNPs**	**Your Average**	**Class Average**
_____ vs. _____			
_____ vs. _____			
_____ vs. _____			
Student _____ vs. Student _____			
Modern Human vs. Neandertal			
African _____ vs. Neandertal # _____			
Asian _____ vs. Neandertal # _____			
European _____ vs. Neandertal # _____			
Student _____ vs. Neandertal #____			
Neandertal vs. Neandertal			
Neandertal #1 vs. Neandertal #2			
Neandertal #1 vs. Neandertal #3			
Neandertal #2 vs. Neandertal #3			
Modern Human vs. Chimpanzee			
_____vs. Chimp #2			
_____vs. Chimp #2			
_____vs. Chimp #2			
Student _____ vs. Chimp #2			

1. Calculating a Molecular Clock

Archaeologists use a number of different techniques to estimate the age of fossils. These include radiocarbon dating, measuring changes in carbonates and tooth enamel brought about by exposure to radiation over time, and determining the age of the geological strata in which the fossil was found. By dating human fossils discovered in Africa, scientists estimate that modern humans first appeared approximately 150,000 years ago. Using this value and the class average number of differences for "Modern Humans vs. Modern Humans," derive a **molecular clock**, or mutation rate, in years/mutation. Use the formula below.

$$\frac{150{,}000 \ \textit{years}}{\_\_\_ \ \textit{mutations}} = \_\_\_\_\_ \textit{years / mutation}$$

2. Did Modern Humans Evolve from Neanderthals?

Neandertal fossils have been discovered in Europe and the Middle East. Dating the fossils by radiocarbon decay suggests that Neandertals inhabited the European continent as recently as 28,000 years ago. Estimates of when Neandertal first appeared in Europe are far less precise but many scientists believe it may have been as long as 300,000 years ago. Although they are frequently depicted as stocky and brutish individuals, Neandertals cared for their sick and injured, fashioned stone tools, used fire, lived and hunted in social units, and ritually buried their dead.

As far as we know, Neandertals did not inhabit regions far outside the European continent. If modern Europeans descended from Neandertals, you would expect that Neandertals would be more closely related to modern European populations than to any other modern human population in the world. Based on your "Modern Human vs. Neandertal" data, does it appear as though Europeans or any other modern world population descended from the Neandertals? Explain.

3. Human - Neandertal Divergence

How many years ago did the common ancestor of modern humans and Neandertals live? In the equation below, use the average number of differences (mutations) you found between modern humans and Neandertals and your calculated mutation rate (page 20) to estimate this number.

$$\_\_\_\ mutations \times \frac{\_\_\_\_\_\ years}{mutation} = \_\_\_\_\_ years$$

4. Did Neandertals Contribute to the Modern Human mtDNA Gene Pool?

A **gene pool** is the collection of all genes in a population. Members of a single gene pool would be expected to have fewer differences between them than would be expected between members of different gene pools. Did Neandertals have a separate gene pool from that of modern humans? Could Neandertals have contributed their mitochondrial DNA to the gene pool of modern humans? Use the comparisons below (4a through 4e) to answer this question.

a. Average difference between Neandertals = ____

b. Average difference between modern humans and Neandertal = ____

c. Average difference between modern humans = ____

d. The *closest* modern human/Neandertal alignment discovered by your class showed ____ differences.

e. The two most divergent modern humans discovered by your class showed _____ differences.

f. Do you think the Neandertals used in this study are members of a single gene pool (assume that modern humans are of a single gene pool)? Explain.

g. Do you think Neandertals contributed their mitochondrial DNA to the modern human mtDNA gene pool? What other data would you want to answer this question?

5. A Molecular Clock Based on Chimpanzee/Hominid Divergence

Based on the fossil record, scientists believe that chimpanzees and modern humans may have diverged 5,000,000 years ago.

a. Would the molecular clock be different if you used the time since chimpanzees and modern humans evolved to determine the mutation rate? Calculate a new mutation rate using the formula below and the 5 million year divergence estimate.

$$\frac{5{,}000{,}000 \ \textit{years}}{\_\_\_ \ \textit{mutations}} = \_\_\_\_\_ \ \textit{years / mutation}$$

b. Is this value different than the one you calculated based on "Modern Human vs. Modern Human" differences? Explain.

c. Using the mutation rate you calculated in 5a, when did "Mitochondrial Eve," the mitochondrial ancestor of all modern humans, live? Use the formula below for this calculation.

$$\_\_\_\ mutations \times \frac{\_\_\_\_\_\ years}{mutation} = \_\_\_\_\_\ years$$

How does this estimate compare with the value you used to calculate a molecular clock in Problem 1 (pg 20)?

d. Using the same molecular clock (calculated in 5a, pg 22), when did Neandertals and modern humans diverge and how does this estimate compare with the value you calculated in Problem 3?

$$\_\_\_\ mutations \times \frac{\_\_\_\_\_\ years}{mutation} = \_\_\_\_\_\ years$$

e. How many mutations would you need between chimpanzee and modern humans to give the mutation rate you calculated in Problem 1 (pg 20)? Use the equation below for your calculation.

$$\frac{5{,}000{,}000 \ \textit{years}}{x \ \textit{mutations}} = \_\_\_\_\_ \ \textit{years / mutation}$$

$x =$ _____ *mutations*

How does this number compare with the average number of SNPs your class found for the "Modern Human vs. Chimpanzee" comparisons and how can you account for any discrepancy?

f. Which mutation rate might be more accurate, that derived from the modern human/modern human comparisons or that derived from the chimpanzee/modern human comparisons? Explain.

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