Laboratory Exercise # 10 — Evolution

Learning Goals:

To understand the significance of the Lucy fossil in human evolution

To compare chromosomes from humans, chimpanzees, orangutans and gorillas, and to construct a phylogenetic tree showing their relationships

To use bioinformatics tools to compare myoglobin or insulin amino acid sequences from different species, and to create a phylogenetic tree showing their relationships

A. Human Evolution video

View the video presentation "Becoming Human," at http://becominghuman.org. While you are watching the video, try to find the answers to the following questions. If you miss one, don't worry—the video is available online for your viewing at any time; you can go back on your own time to collect your missing answer. However, if you pay careful attention to the video during the lab, you won't have to re-view it later!

A1. How old is the Lucy fossil? Where was it found?

A2. What species was Lucy?

A3. Why is Lucy a significant anthropological "find"?

A4. How many of our (human) genes are the same in chimpanzees?

A5. What is bipedalism? What are its advantages?

A6. Was the expansion of the human race into Europe and Asia a migration or a dispersal? What is the difference between migration and dispersal?

A7. Summarize the two arguments presented as to why *Homo neanderthalensis* vanished from Europe after a 200,000 year run of success as a species.

A8. Compare and contrast cave art in Australia and Europe.

Since the video: The latest scoop on hominid ancestry!

Our oldest bipedal ancestor:

A new hominid ancestor, ***Australopithecus anamensis***, was discovered in Kenya by Maeve Leakey and Alan Walker in 1991. Radioisotope dating of the volcanic sediments in which these fossils were found indicates the fossils to be **4 million years old**. Fossils of *Australopithecus anamensis* show clear signs of **bipedalism**: the knee and ankle structures are those of a creature that walked upright, and the scientific community is in broad agreement about these features.

An even older possible hominid ancestor , *Sahelanthropus tchadensis*, was discovered in Chad by Michel Brunet and Alain Beayvilain in 2001. This fossil is dated to between 7 and 6 million years old. Scientists are still debating whether *Sahelanthropus tchadensis* could walk upright. Some scientists think that the foramen magnum, or “big hole” in the skull, is far enough forward for the creature to have walked upright, while others disagree. Unfortunately the remains of *Sahelanthropus tchadensis* do not include any bones other than a partial cranium, so scientists cannot yet examine the knee or ankle or foot structure to further test the hypothesis that *Sahelanthropus tchadensis* could have been bipedal.

Have a look at *Sahelanthropus tchadensis* yourself, and see what you think!

Photographs taken by Dr. Mary Morrison at the American Museum of Natural History

The great Neanderthal debate:

In the video, you heard about two hypotheses for the disappearance of *Homo neanderthalensis*: one, that *Homo sapiens* outcompeted them and they became extinct, and another, that they interbred with Homo sapiens. Since the video was produced, new samples of Neanderthal DNA have been isolated and sequenced. In the 7 May 2010 issue of the journal Science, the first data from these analyses were reported by Svante Paabo’s large international research group. And the great surprise was that there is substantial evidence supporting the idea that some Neanderthals interbred with some *Homo sapiens*!

Although the modern human genome and the Neanderthal genome are **99.84% identical**, Europeans and Asians share 1 to 4% of their nuclear DNA with Neanderthals, and Africans do not. This means that **Neanderthals probably interbred with Homo sapiens after Homo sapiens migrated out of Africa but before they spread out into Asia and Europe**.

B. Chromosomes and evolution - Read through the attached background for the Chromosome Connection exercise. Obtain an envelope with Chromosome Connection images, and go through the exercise, answering the Check Questions along the way.

THE CHROMOSOME CONNECTION

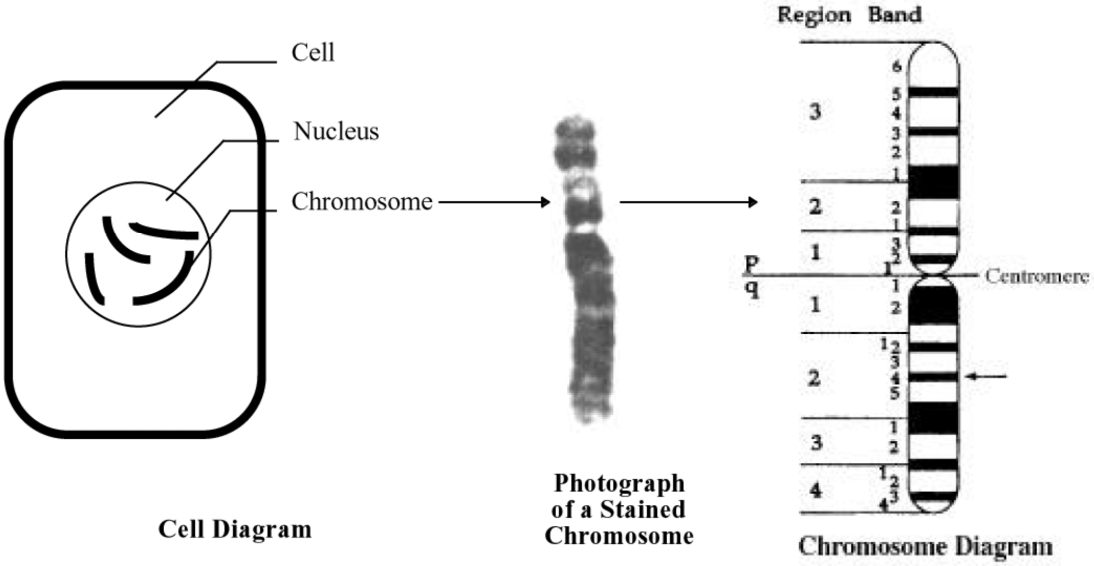
BACKGROUND INFORMATION

A. <u>THE BIG IDEA</u>: The degree of chromosome pattern similarity between two species indicates the degree of biological relationship.

B. <u>BACKGROUND</u>:

Chromosomes are microscopic strands found in the nuclei of the cells of most living things. As we learn in genetics, the genes (DNA code sequences) for the characteristics of an organism are located IN that organism's chromosomes. Similarities of the characteristics in the members of one species (humans for example) are due to the similarities in the information (DNA sequences) in their chromosomes.

To extend that logic, wouldn't similarities between members of **different species** also be due to the similarities in the information in their chromosomes? Comparing chromosomes is one of the several ways we can assess the evolutionary relationships between organisms of different species. Organisms get their chromosomes from their parents, and even further back in time, from their ancestors. According to evolution, we can predict that two species having a recent common ancestor should have chromosomes that are more similar than two species having a more distant common ancestor. In other words, **species that are closely related should have very similar chromosomes.**



Cell Diagram

Photograph of a Stained Chromosome

Chromosome Diagram

Chromosomes are usually prepared for viewing by squashing some of the organism's cells so their nuclei burst and release their contained chromosomes. These are then stained, and photographed through a microscope. Chromosomes are nearly impossible to see, much less distinguish, without adding a stain. A number of different stains can be used, but certain ones reveal characteristic banding patterns. When Giemsa stain is used, the **bands** produced are called G-bands. With Giemsa stain, regions where the chromosome proteins (histones) are **tightly packed** appear **dark**, while regions with less condensed proteins appear lighter.

Even with staining, photographs of the chromosomes still appear fuzzy, so scientists apply techniques to make the banding appear more distinct, observe many chromosomes, and develop **chromosome diagrams**, as shown above. NOTE: these bands are NOT genes; there may be hundreds of genes in a single band.

In addition, some studies use chromosomes taken from the metaphase stage (where chromosomes are seen as fairly short); other studies consider the chromosomes during their late prophase, when they are much longer, and show many more bands (appearing somewhat different from their metaphase versions). This enables a more detailed analysis. The chromosomes used in this activity came from a published study by Yunis, et al (1982) using the late prophase stage, showing about 1000 bands for the whole set.

C. SUMMARY:

Chromosome similarity implies biological relationship:

a. Numerous studies show that **chromosome similarity** is a good measure of **genetic relatedness**. The visible structure of chromosomes is an extremely complex pattern of bands and lines. The probability that two different chromosomes would independently have identical banding patterns by chance is essentially zero (Wallace, 1966).

b. Detailed studies provide clear evidence that any **identical chromosomes** in two different species **indicates common ancestry** just as surely as identical scratch patterns on two bullets indicate that both came from the same gun (Wallace, 1966).

D. ACTIVITIES AND QUESTIONS

At this point, go to the **Activity Packet** provided and follow the directions found there. You or your team will also receive an **envelope** with 7 cutout pieces to use with the Activity Packet. At various points, answer the **Check Questions** to see if you have understood the concepts presented up to that point. Your teacher may ask that you number and record all answers on a separate sheet to hand in, and not mark on the packet.

When comparing chromosomes from different species, only one member of each pair of matching chromosomes is used (plus an X and a Y), since both members of each pair appear identical (except for the XY combination in males). In examining the chromosomes, the **length** of the chromosome, the location of the **centromere** (usually constricted, where spindle fibers attach) and the **banding patterns** are studied very closely. The similarities and differences are carefully noted.

Be sure to return all 7 pieces to the envelope when finished.

E. RESOURCES:

Alberts, Bruce, et al. 1989. *Molecular Biology of the Cell*. Garland Publishers. Chiasmata, p.848, fig. 15-11.
Flammer, Larry. 1983. “Karyotype Komparison.” Classroom activity.
Klein, Richard G. 1999. *The Human Career*. Univ. of Chicago Press. p. 71.
Kramer, Beth. 1995. “Comparison of Human and Chimpanzee Chromosomes”. Classroom activity.
Wallace, B. 1966. *Chromosomes, Giant Molecules, and Evolution.* New York: W.W. Norton & Co., Ch.2.
Yunis, J.J. and O. Prakash, 1982. "The origin of man: A chromosomal pictorial legacy". *Science*, 215, 1525-1529.

CHROMOSOME CONNECTION ACTIVITY PACKET

SELF-CHECK QUESTIONS: Based on your reading of the **Background Information**, check your understanding at this point by answering the following questions:

1. If two different species have extensive identical chromosome banding patterns, they...
   - A) must be related
   - B) are probably related
   - C) could be related, but not necessarily so
   - D) are probably not related
   - E) may or may not be related...no basis for judging this

2. If you found that two sets of chromosomes (one from species "X" and one from species "Y") showed a very close match (in the number of chromosomes and many identical banding patterns), it would be most reasonable to say that...
   - A) one species evolved from the other
   - B) both species evolved from a common ancestor relatively recently
   - C) both species may be distantly related
   - D) their chromosome similarities are only a striking coincidence

ACTIVITY

PART 1: Matching Bullet Marks

Imagine yourself as a scientist in training to become a CSI (Crime Scene Investigator). One of your tasks is to practice finding a match for bullets fired from the same gun. We know that bullets fired from the same gun have very similar markings caused by spiral grooves and imperfections inside the barrel of the gun.

Six guns were involved in a recent crime. Gun specialists fired each gun into a test chamber and retrieved the spent bullet from each gun. The bullet marks were photographed. You are shown photos of the scratch marks on those 6 bullets. You are then given a photo of the scratch marks on bullet (B) taken from that crime scene. Your job is to figure out which gun the crime scene bullet (B) was fired from.

1. Remove from your Chromosome Connection **Envelope** the strip of paper for Part 1, showing the bullet scratch pattern of bullet B, and the group of 6 other bullet scratch patterns (#1-6).
2. Place the B pattern next to each of the 6 patterns in turn. Which pattern matches the B pattern?

Return these two papers to the envelope when done.

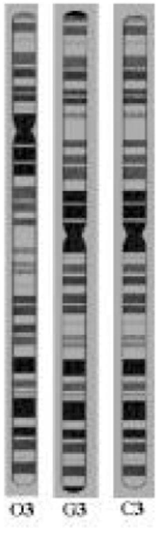
3. Since there is a perfect match, this tells you that these two bullets DID come from the SAME GUN...they both have a **common origin**.

PART 2: Identical Chromosomes?

Identical scratch patterns on bullets indicate they came from the same gun. The same principle applies when we look at chromosomes, the units of heredity found in each cell. **Identical chromosomes have a common origin. They are inherited from the same source: a common ancestor.**

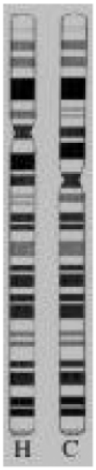
Chromosomes, when treated with a particular stain, reveal characteristic banding patterns, according to their molecular makeup. Where the chromosome proteins (histones) are tightly packed, they stain very darkly.

On the right are the detailed diagrams of chromosomes from three different animals which share many characteristics with humans. In fact, even these chromosomes are very similar to the human chromosome #3. You will find in your Chromosome Connection **Envelope** a strip of paper with the diagram of **human chromosome #3**. Place it next to each of the three chromosomes on the right. Is there a perfect match? If so, which one is it?



That's it!!! These two chromosomes **match perfectly**, band for band. Just as with the perfectly matching bullet scratch marks, this is taken as clear evidence that they must have had a **common origin**. Even though these chromosomes come from two different species, they both had to have a common ancestor. The perfectly matching chromosome is from a chimpanzee, so this tells us that humans and chimps must have descended from a fairly recent **common ancestor** that was neither human nor chimp.

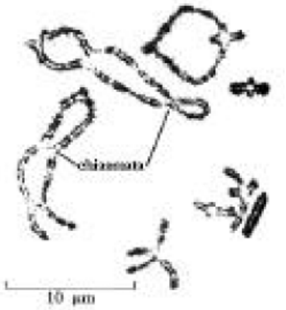
PART 3: Alike, but Different: Inversions



Sometimes similar chromosomes don't match perfectly. This is because even if humans and chimps once shared a common ancestor, they have both changed since that time, and it's reasonable to expect that their chromosomes have changed also. But even apparent differences can be more superficial than real.

On the right are two very similar human and chimp chromosomes: identical in both upper and lower regions, but apparently different near their centromere constrictions. In the Chromosome Connection **Envelope**, find the paper strip for part 3, showing just that centromere region. First, align that region so it matches the orientation in the **human** chromosome (H). Then, simply turn that segment upside down (rotate it 180°), and place it next to the **chimpanzee** chromosome (C) so that their centromere constrictions are at the same level. Identical, aren't they! You've just produced a pericentric (around the centromere) **INVERSION!**

Analysis suggests that this is exactly how the chimp chromosome was formed from the chimp/human ancestor. In fact, according to the authors of that study (Yunis and Prakash, 1982), the human chromosome #4 is considered as the ancestral type "because it's the only one from which the others [chimp, gorilla, and orangutan] can be derived by a single, but differing, pericentric inversion ..." That ancestral chromosome #4 has continued unchanged in humans. Scientists typically infer the simplest explanation from all the evidence, unless there are strong reasons to do otherwise.



The tell-tale signs of inversion have been observed in many organisms. It is very common and normal, and typically occurs during meiosis (typical in sex cell production). During this process, chromosomes are seen in peculiar "crossing" configurations called "chiasmata", as shown here. Chromatids twist about each other, sometimes forming loops. These segments may break and reattach, reversed (inverted).

Many of the chromosomes in apes and humans are essentially identical except for their inversions. We know from numerous examples that inversions can occur (in humans and other animals) without affecting their fertility and normal development. Pericentric inversions are fairly common. See if you can find examples of inversions in other chromosomes shown in this lesson.

PART 4: Alike, but Different: Fission or Fusion?

In addition to inversions, chromosomes undergo other changes which can account for some of the differences seen in ape and human chromosomes. Sometimes, a chromosome will break apart, producing two shorter chromosomes from one longer one. This is called "**fission**". Other times, two short chromosomes will stick together, end to end, forming a single long chromosome where there were two before. This is called "**fusion**"

Humans have 23 pairs of chromosomes, chimps and the other apes have 24. Could this have happened as a result of an early ancestor chromosome splitting apart to produce the two smaller chromosomes (fission) we find in apes? Or, was there a common ancestor in which two smaller chromosomes linked together to make one, but only in the human line of descent? Let's see if we can find out. When we study the chromosomes carefully, we find that the long human chromosome #2 (shown at right) has banding patterns which look very similar to two shorter chromosomes found in apes (which we'll call "2p" and 2q").

In your Chromosome Connection **Envelope**, find the two "Part 4" strips, one marked "**#2p**" and the other marked "**#2q**". These are chimpanzee chromosomes. Be sure the blank paper at both ends of each chromosome are cut off. Now place them end to end, and align them next to the #2 human chromosome on the right. If they don't match, invert one or the other chromosomes, and/or exchange the "attachment" ends, until you get the combination which matches the human #2 chromosome. How would you describe that match comparison?
A) identical; B) very similar; C) somewhat similar; D) totally different.

Awesome! Detailed studies have shown that human chromosome #2 was most likely the result of the **fusion** of the two smaller chromosomes found in the common ancestor of chimps and humans (and still found in chimps). According to Yunis and Prakash (1982), the "... ancestral chromosome 2p [was probably] similar to that of orangutan and gorilla, with a pericentric inversion accounting for the chimpanzee 2p. The ancestral 2q, on the other hand, resembled that of gorilla and chimpanzee, and [the] human chromosome #2 can be explained [most simply] by fusion of a chimpanzee-like 2p and the ancestral 2q." This, in fact, is one of several indications that humans and chimps are more closely related to each other than either is to gorillas, and that orangutans are even more distantly related.

PART 5: How do the Other Apes Compare?

Not only do humans and chimps have very similar chromosomes, but (as you may have suspected by now) their chromosomes are also very similar to those of gorillas and orangutans.

From the Chromosome Connection **Envelope**, remove the paper showing 7 selected sets of chromosomes, with 4 chromosomes in each set. Each set consists of the corresponding chromosome from each of four species. In some cases, you may find that one chromosome in a set is significantly different from the other three. In other cases, all four are very similar, with none significantly different.

As you compare the chromosomes in each set, you can **ignore the dark-staining tip-ends** found on some chromosomes...they are composed of "heterochromatin", and are not considered as important differences. For each item below, if none of the sets show the specified difference, say "NONE of these"

1. Look for those sets in which the **fourth (O)** chromosome is significantly **different** from the other three. You might want to discuss your selections with a partner. **Record** the numbers of those sets.

2. Now look for those sets set in which the **third (G)** chromosome is clearly **different** from the other three. **Record** the number (or numbers) selected.

3. Finally, look for those sets in which the **1st** or **2nd chromosome (H or C)** is significantly **different** from the others. **Record** the number (or numbers) selected.

When you have answered 1-3 above, check with your teacher for the numbers most would choose. As a matter of fact, detailed analysis of **all** the chromosomes from these four species reveals that the H and C chromosomes are most alike (13 virtually identical), the G chromosomes are next most like H (9 virtually identical to H), and the O chromosomes are least like the others (8 virtually identical to H).

The **H** chromosomes are human, the **C** chromosomes are chimpanzee, the **G** chromosomes are gorilla, and the **O** chromosomes are orangutan.

CHECK QUESTIONS

3. How would you now compare the chromosomes (generally) of these four organisms (H, C, G & O)?
   A) identical
   B) very similar
   C) somewhat similar
   D) mostly different
   E) totally different

4. Based on the above observation (and the background information), what would be the most logical assumption about the relationship between these four organisms?
   A) they are members of the same species
   B) they all evolved from a common ancestor relatively recently
   C) they are most likely distantly related
   D) no basis for an assumed relationship...could be related or totally unrelated

NEW INFORMATION: Species C, G, and O are all in different genera which, based on anatomy, have all been traditionally classified in the **same taxonomic family:** the **pongids** (great apes), while humans have been placed in the **hominid** family**.** However, when we compare the full sets of chromosomes for all 4 species, we find that 1) all four species are very similar, and 2) the chimp set is more like the human set than either is like the other apes.

CHECK QUESTION

5. Given this information, should apes and humans all be placed in the same classification family? A) yes; B) no

PART 6: Chromosome Analysis and Degree of Similarity

Let's see what the scientists say. All the differences in the chromosomes from these four animals were recorded, counted and analyzed in great detail by the scientists doing this study, and from these data, it was possible to create a diagram, showing the likely sequence of steps in the evolution of these species relative to each other, and how closely related they are. In the diagram below (based on a similar one by Yunis and Prashak, 1982), the branching points and lengths of each branch are based on the types and relative numbers of chromosome differences between the four species.

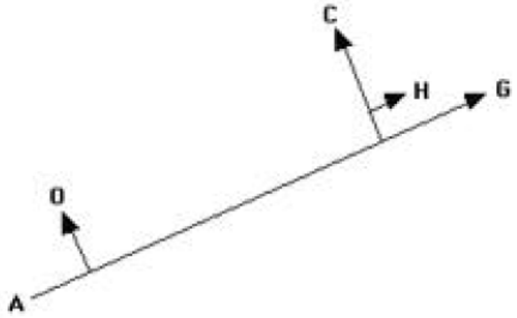
A = an extinct primate, common ancestor to great apes and humans

O = orangutan

H = humans

C = chimpanzees

G = gorilla



CONCLUSIONS

Traditionally, based mostly on anatomy, all **humans** and all **apes** have been placed in the same superfamily: the **hominoids**. Humans (all modern and fossil humans) have been placed in their own **family** (the **hominids**), while the great apes (chimps, gorillas, and orangutans) have been grouped in their own **family** (the **pongids**).

However, extensive "...biomolecular studies demonstrate unequivocally that the living African pongids - Gorilla (*Gorilla*) and the chimpanzee (*Pan*) - are actually more closely related to people than either is to the living Asian pongid, the orangutan (*Pongo*)." (Klein 1999). Reflecting the growing evidence (in fact **M**ultiple **I**ndependent **L**ines of **E**vidence), it has been proposed that **all apes and humans** be placed into the **same family** (the hominids), and that **humans even be placed in the same subfamily as the great apes** (orangutans, gorillas, and chimpanzees):. The **Chromosome Connection** provides evidence which clearly supports that thinking.

Multiple Independent Lines of Evidence...

This pattern of similarity in ape and human chromosomes closely matches the patterns and inferred biological relationships based on anatomical and molecular comparisons. It is also consistent with their geography (orangutans in Southeast Asia; gorillas, chimps, and earliest hominids in Africa). Here we have an excellent example of one important criterion for how scientists select the "best" explanation: **M**ultiple **I**ndependent **L**ines of **E**vidence, all pointing to the same conclusion. With all these "MILEs", we have a very strong case that humans and apes have a close kinship, relatively recent common ancestors. Apes truly ARE our COUSINS!

WHEN FINISHED WITH THIS ACTIVITY, BE SURE TO RETURN ALL 7 ITEMS TO THE CHROMOSOME CONNECTION ENVELOPE

REFERENCES: See the list of REFERENCES in the BACKGROUND pages of this lesson.

C. Phylogenetic relationships via Comparative protein sequence analysis

Myoglobin is an oxygen-carrying molecule found in the muscles of essentially all animals, even those as distantly related as humans and fish. When two populations become reproductively isolated and form separate species, most of their genes are probably nearly identical. However, over time, different mutations will occur in these two species, and because they are reproductively isolated, the genes will become more and more different from each other over time.

- _______________ mutations do not change the amino acid sequence and are usually **not** deleterious and accumulate relatively rapidly.

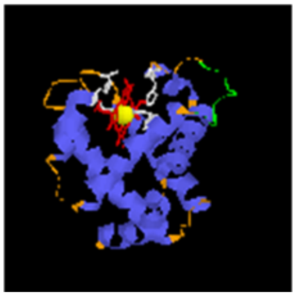
- _______________ mutations change one amino acid in a protein and may or may not be deleterious. A change top an amino acid with similar chemical properties (e.g. substituting one hydrophobic residue for another hydrophobic residue) often will not significantly affect the protein's folded structure or its function.

  When the substitution is deleterious, it is usually lost due to natural selection. (What might be the phenotype associated with a poorly functioning myoglobin?

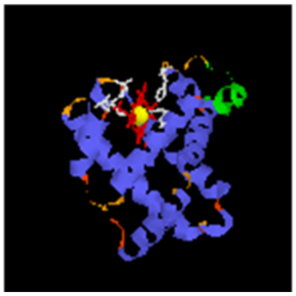
- _______________ and ______________ mutations are almost always deleterious and often lost by natural selection.

- Which do you think changes fastest over evolutionary time, the DNA sequence, the amino acid sequence, or the protein's folding pattern?

The structures of human myoglobin and yellow fin tuna myoglobin are shown below. You can see that they look very much alike, their function is the same, yet they have different amino acid sequences. In this activity, we will compare the myoglobin sequences from a variety of different animals to identify mutations that have occurred in the common ancestor of different groups and how these patterns of mutations can be used to construct a phylogenetic tree to illustrate the relationships among the organisms.



Yellow-fin Tuna



Human

C1. Retrieve Myoglobin amino acid sequences from database.

1. Use a web browser to go to uniprot.org. Search for "myoglobin"

2. If you only get the default 25 sequences, change the number of results to show to 100 (upper right).

3. Place checkmarks to select 12-15 sequences – including many pairs and triples (3 birds, 2 reptiles, 2 rodents, 2 aquatic mammals, 3 primates…). Be sure to click the human (Homo sapiens) sequence. Click the download button at the top of the page.

4. Select the "FASTA (canonical)" option, then click "Go". When the lis of sequences appears, select and copy the text.

5. Open the Notepad program, and paste the sequences into the document. Save the file to your network space. First change the file typee from text to "all files", then save with the filename myoglobin.fasta.

6. To facilitate identification of the owner of printouts later and interpretation of the trees, **we will change the headers for each sequence**. Choose Format/Wordwrap.

7. Add a hard return (press enter) before each header (starts with a ">"), and change the header information to just the common name by referring back to the uniprot page. (insert space for the common name as selected). Use YOUR name for the Homo sapiens sequences, and use underscores "_" instead of blanks. **Do not delete the ">" sign at the start of the header lines.** The file should appear as below. Re-Save the file when finished editing.

```
>cow
MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKH
LKTEAEMKASEDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKI
PVKYLEFISDAIIHVLHAKHPSDFGADAQAAMSKALELFRNDMAAQYKVLG
FHG

>Charles_Darwin
MGLSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKH
LKSEDEMKASEDLKKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKI
PVKYLEFISECIIQVLQSKHPGDFGADAQGAMNKALELFRKDMASNYKEL
GFQG

>horse
MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKH
LKTEAEMKASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKI
PIKYLEFISDAIIHVLHSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGF
QG

>Loggerhead_sea_turtle
MGLSDDEWNHVLGIWAKVEPDLSAHGQEVIIRLFQLHPETQERFAKFKNL
TTIDALKSSEEVKKHGTTVLTALGRILKQKNNHEQELKPLAESHATKHKIPV
KYLEFICEIIVKVIAEKHPSDFGADSQAAMKKALELFRNDMASKYKEFGFQ
G

>mouse
MGLSDGEWQLVLNVWGKVEADLAGHGQEVLIGLFKTHPETLDKFDKFKN
LKSEEDMKGSEDLKKHGCTVLTALGTILKKKGQHAAEIQPLAQSHATKHKI
PVKYLEFISEIIIEVLKKRHSGDFGADAQGAMSKALELFRNDIAAKYKELGF
QG
```

C2. Align Myoglobin Sequences with MEGA5, construct Phylogenetic tree.

1. Open the program MEGA6 (under course files, Biology). Click the align button, then edit/build alignment, then retrieve sequences from a file, and open the Myoglobin.fasta file.

2. On the alignment menu, choose align by clustalW,
   in the dialog box, choose click OK to select all,
   in the ClustalW Parameters dialog box, keep all of the default options and click OK.

3. On the data menu, choose export alignment into MEGA format,
   Save the file with the default filename, then

click OK on the next dialog box—leave the Input filename of data field blank. DO NOT close the alignment explorer window.

4. On the main MEGA window, click the phylogeny button, choose construct neighbor joining tree, choose the myoglobin.meg file.

5. When the tree appears, on the file menu, choose print in a sheet, then OK.

6. Return to the alignment explorer window.
   Click and drag the sequences from their labels to arrange them in the same order as they are on the tree. Set it up so that the whole sequence is visible on the screen at one time.

7. Click anywhere on the screen to ensure that you don't have one row selected/highlighted blue.

8. Press the print screen key on the keyboard to capture the screen image into the clipboard.

9. Open Microsoft PowerPoint, and paste the clipboard image onto slide.

10. On the Picture tools tab, click crop, and crop away most of the screen grab, except for the sequence alignment. Adjust the size to fill the slide, using View/Zoom to see the sizing grab bars, and crop grab bars.
    Print the slide (in color preferred)

11. Identify specific mutations that occurred during the evolution of specific lineages and is present only in descendants of that lineage. Circle the mutation on the sequence alignment and assign a number (1-5).

12. Place the same number on the phylogenetic tree where the mutation would have occurred.

13. Identify 5 mutations that occurred at 5 different places on the tree and turn in these pages in as your quiz #12.