Genetic Origins: Mitochondrial Control Region Exercises (http://geneticorigins.org/mito/mitoframeset.htm)

## II. Using mt Sequences to Test Models of Human Evolution

1. Since their initial discovery in the Neander Valley of Germany in 1856, the heavy-set bones of Neandertal have fascinated scientists, as well as the general public. Neandertal was an archaic member of the genus Homo, which lived in Europe beginning about 300,000 years ago and became extinct about 30,000 years ago. Clearly, during part of its span on earth, Neandertal shared its European habitat with modern humans (Homo sapiens). There has long been controversy about whether or not Neandertal was the direct ancestor of modern humans. Alternatively, if Neandertal and Homo sapiens were separate, was there any significant exchange of genes between the two populations?

According to the multiregional model, modern humans developed concurrently from several different archaic populations living in different parts of the world. Under this model, Neandertal was the ancestor of modern Europeans, while Java man (Homo erectus) was the ancestor of modern Asians.

According to the displacement model, better know as "Out of Africa," Homo sapiens arose from a single founding population that emerged from Africa in the last 100,000-200,000 years. This group migrated successively to Europe and Asia, displacing archaic hominids.

In 1997, an international research team headed by Svante Paabo, extracted DNA from the humerus of the original Neandertal specimen, amplified the sample by PCR, and cloned the resulting products in E. coli. The cloned fragments were then used to reconstruct a 379-bp stretch of the mt control region. Now, you will use the DNA Sequence Server at the DNA Learning Center WWW site to recreate this study and answer the questions posed above. You will obtain mt control region sequences from several sources and then move them onto the analysis workspace of the DNA Sequence Server Page.

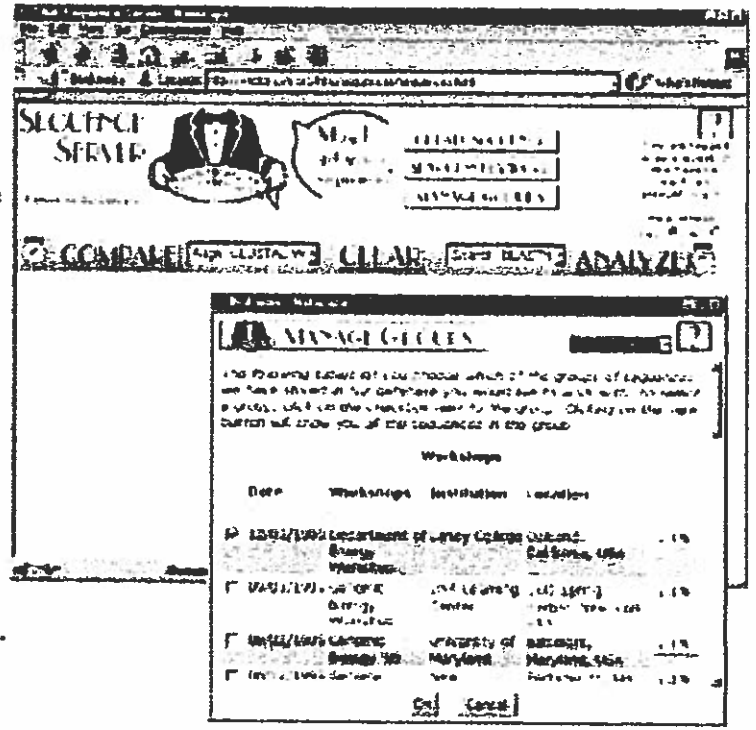
a. Determine the average number of nucleotide differences in the mt control region between Neandertal and 5 modern humans in the DNALC's own database.

1. Point your web browser to DNA Sequence Server. Log-into the Sequence Server either as a guest, or using a registered account. We suggest you print these instructions (using your browser's print function) so you can follow these instructions without switching between two windows.

2. Click MANAGE GROUPS to open a new window in which you can view mt control region sequences currently in the DNA Sequence Server database.

3. Use the pull-down menu in the upper right hand corner to select and view a list of student classes and/or several teacher workshops. Each class or workshop contains up to 30 individual DNA sequences.

4. Click in the check box to select classes or workshop sequences you want to make available for analysis (for example, "Genomic Biology Workshop"). (If you like, click VIEW to open a new window showing the sequences in



the selected group.)

5. In the "Manage Groups" window, select "Ancient DNA" from the pull-down menu in the upper right hand corner.

6. Click in the check boxes to select Modern Human DNA or Prehistoric Human DNA to access Mitochondrial DNA sequences from American Indians, African Americans, Europeans, Africans, Asians, Australian Aborigines, as well as Neandertal Man and Mungo Man.

7. Click OK. The window will close, and the selected group will now appear on the Sequence Server Workspace.

8. On the Sequence Server workspace, visually gauge the quality of several student or teacher sequences in the following way:

Use the pull-down menu displaying the sample names (e.g. Teacher 001) to select a student or teacher sequence. Then, click the adjacent "Open" box to display a new window showing the nucleotide sequences of the selected entry.

Every sequence will entail nucleotides (A,T,C,G) interspersed with Ns, indicating that the nucleotides could not be determined at these positions. In "good" sequences, where experimental conditions were near optimal, the sequence will have very few, if any, Ns.

In non-optimal cases, a large number of Ns will be interspersed throughout the sequence.

When possible, use good sequences (those without internal Ns) in your subsequent comparisons.

9. Now, compare the Neandertal sequence to a modern sequence using the ClustalW algorithm available in the Sequence Server Workspace. Click in the check boxes to the left of the Neandertal sequence and one student or teacher sequence. Above the list of groups, make sure the pull-down menu next to the "Compare" button reads "Align: CLUSTAL W." If not, use the menu to select CLUSTAL W. Then click on "Compare" to send your sequence to the CLUSTAL W tool at the European Bioinformatics Institute in Hinxton, UK, or to a local server at Cold Spring Harbor Laboratory (the computer will determine which one is available at the time), and align the checked sequences. Your results will be returned in a new window.

10. Count the number of differences between the Neandertal sequence and the student/teacher sequence:

A yellow box indicates positions with a nucleotide difference. A gray box indicates positions with an "N," where a nucleotide could not be determined.

Read the sequence and count the number of yellow highlighted nucleotide differences. Do not count any Ns.

Also count dashes (-), which indicate a deletion – a nucleotide that is absent at that position in the sequence.

Record the number of differences.

11. If the readable student or teacher sequence does not contain as many bases as the Neandertal sequence, use the sequence coordinate numbers to estimate the length of the comparable region. If the student or teacher "read" was shorter than the Neandertal sequence, "normalize" the differences in this way:

Normalized Difference between two sequences = (Number of observed differences) divided by (number of nucleotides in the stretch of sequences with overlap)

12. Repeat Steps 9-11 to do four more comparisons of the Neandertal sequence to a different teacher or student sequence. For each pairwise comparison, record the raw number of differences and the Normalized value.

b. Now, determine the average number of differences between any two modern humans. Repeat Steps a9-11 to compare five pairs of student or teacher sequences. For each pairwise comparison, record the number of differences.

c. Next, repeat Steps a9-11 to compare a modern human sequence to a primate sequence. When viewing the results of these analyses, you may find alignments that contain one or more short runs of dashes. In these instances, count the entire run of dashes as a single nucleotide difference.

d. Next, repeat Steps a9-11 to determine the average number of differences between selected pairs of Diverse Modern Humans. Because we want to test the theory that Neandertal was a direct ancestor to modern Europeans, be sure to include the European group in your comparisons.

e. Now repeat Steps a9-11 to compare the Neandertal sequence to the Diverse Modern Human sequences you used in Step d.

f. Next compare a "good" human sequence (one that contains no or only very few Ns) to all three Neandertal sequences simultaneously. By looking closely you will find that sequence differences fall into one of three categories:
1) the human sequence differs from the Neandertal sequence, at a spot where all three Neandertal sequences are identical;
2) the human sequence differs from one or two Neandertal sequences but shares homology with at least one Neandertal sequence;
3) the human sequence differs from all three Neandertal sequences which among themselves show variation as well.
Report the number of occurences for each of these three categories 1)-3).

g. Now compare the mtDNA sequences of two modern humans (e.g. Germany #1 and Japan #1) to all three Neandertal sequences simultaneously. Count the occurences for each of these three categories:
1) German and Japanese sequence are identical, but differ from one or more Neandertal sequences;
2) German sequence differs from Neandertal sequences, which resemble the Japanese sequence;
3) Japanese sequence differs from Neandertal sequences, which resemble the German sequence.
What conclusions can you draw from your observation, especially in regard to the assumption that Neandertals may have been the direct ancestors of Europeans?

2. The number of differences in mt sequence provides a measure of the genetic distance between populations -- that is the amount of time that has elapsed diverged from a common ancestor. Before one can use mt mutations as a "molecular clock," one must set the clock by some reference. The reference for hominid evolution is the estimated divergence between humans and chimpanzees 4 million years ago.

a. Assuming that mt mutations occur at a constant rate, use the human-chimp divergence estimate and the average number of chimp-human sequence differences to calculate the average time span between mt mutations. Hint: the unit of your answer will be years/mutation.

b. Now, use this value to calculate a divergence time for all modern humans.

c. Scientists have used mt and chromosomal DNA mutations to calculate a divergence time for modern humans of about 150,000 years. Why is this number 3-4 less than your calculation?

d. Now, calculate the divergence time for Neandertal-modern humans, using the modern human divergence estimate of 150,000 to set your mt clock.

e. What does this tell you about the relationship between Neandertal and modern humans?

In order to check your conclusions, visit again the Theory chapter and the 'Neandertal Mystery' in the Media/Animation chapters.