Teacher Prep & Follow-up

STUDENT LAB BRIEFING

To get the most out of the laboratory experience, we strongly suggest that you prepare your class prior to coming to the DNA Learning Center. Make sure your students have a basic understanding of the structure and function of DNA. Notions of heredity and will also be useful although not essentials. They should read the enclosed **Carolina Tips** article, "Polymerase Chain Reaction." After reading this article, students should be able to discuss:

- The mechanism and applications of polymerase chain reaction.
- Use of electrophoresis and agarose to separate DNA fragments.

You may also wish to have students read over the lab theory and protocol accessible online at: http://www.geneticorigins.org/geneticorigins/mito/mitoframeset.htm.

AT THE DNA LEARNING CENTER

Before starting the experiment, the instructor will briefly review the purpose of the laboratory and the techniques involved. Students will be introduced to the lab equipment. The lab protocol will be discussed step by step. Students will have 45 minutes during the amplification step to eat their lunch/snack if they wish to. Teachers must supervise students in the lunchroom, or students may choose to eat in the school bus if available.

RESULTS AND DISCUSSION

In addition to the questions in the lab protocol available on line, you can discuss the ethical ramifications of DNA fingerprinting and genetic screening, the Human Genome Project, and genetic engineering. There are often articles in journals on teaching bioethics, for example "Genetic Engineering—A Lesson on Bioethics for the Classroom," by Kerri Armstrong and Kurt Weber (*The American Biology Teacher*, May 1991, Volume 53(5)). There is also a very good section on societal issues in *A Sourcebook of Biotechnology Activities* from the National Association of Biology Teachers and the North Carolina Biotechnology Center.

Answers to student questions are in bold.

2. How would you interpret a lane in which you observe primer dimer, but no 440-bp band?

   The presence of primer dimer confirms that the reaction contained all components necessary for amplification, but that there was insufficient template to amplify the 440-bp target sequence.

3. The mt control region mutates at approximately ten times the rate of nuclear DNA. Propose a biological reason for the high mutation rate of mt DNA.

   The mitochondrial genome is housed within the cell's energy producing factory, where it is exposed to reactive by-products of oxidative phosphorylation. Notably, oxygen free radicals are potent mutagens. The number of reactive by-products, in turn, increases as enzymes involved in energy production accumulate mutations that make them function less efficiently. It is hypothesized that this decline in mitochondrial efficiency is a major contributor to aging. Also, the mt DNA polymerase lacks the high-level of mutation repair of nuclear polymerase.

4. The high mutability of the mt genome means that it evolves more quickly than the nuclear genome. This makes the mt control region a laboratory for the study of DNA evolution. However, can you think of any drawbacks to this high mutation rate?

   The mutation rate is so high that some nucleotides have mutated several times over evolutionary history. This makes it difficult to determine the actual mutation rate and to ascertain the ancestral (original) state of a DNA sequence. These make it difficult to accurately calibrate the "mutation clock."

5. There are numerous insertions of mt DNA into nuclear chromosomes. Notably, scientists recently discovered a 540-bp fragment of the mt control region that inserted into chromosome 11 approximately 350,000 years ago. Would you expect any difference in the mutation rates of the control region sequence in the mt genome versus the chromosome 11 insertion? What implication does this have in the study of human evolution?

   Once removed from the context of the mitochondrion, the mt insertion is subject to the lower mutation rate of nuclear DNA. The "mutation clock" effectively stops, preserving the insertion as a "molecular fossil" from that moment in DNA history. This means that one can study human evolution within oneself by comparing the sequence of an ancient DNA sequence (nuclear mt insertion) with the modern sequence (mt control region).