MITOANALYZER – A NEW NIST HUMAN MITOCHONDRIAL DNA INTERACTIVE WEB SITE

Barbara C. Levin and Michael S. Lee
National Institute of Standards and Technology, Gaithersburg, MD

Every human cell has from a few dozen to several thousand mitochondria, each of which contain mitochondrial DNA (mtDNA). The sequence of the entire human mtDNA (16,569 base pairs) was determined and published by Anderson *et al*. in 1981 and is referred to as the Cambridge Reference Sequence (CRS). Most current mtDNA investigators use the same numbering system as the CRS and report their results as differences from the CRS. A mtDNA Standard Reference Material (SRM 2392) is now available to provide quality control when sequencing the entire human mtDNA for forensic identification, medical diagnosis or mutation detection (Levin *et al*., 1999). The DNA used in SRM 2392 came from apparently normal cell culture lines or individuals. Therefore, it was surprising that multiple changes in the coding region were found when the sequence was compared to the CRS. We examined each of these base pair (bp) changes, insertions or deletions to determine if the change would result in a silent change or an amino acid (aa) change in the ensuing proteins. It was extremely difficult and time consuming to manually determine the effect of each bp change, insertion or deletion in the total 12,569 bps. One needs to determine the codons that start and end the proteins and determine whether the first, second or third bp of the codon had changed and then using the human mtDNA code, which differs slightly form the universal code, determine if an aa had been affected. To complete this examination of all the bp changes, it was necessary to know that some tRNAs and NADH dehydrogenase 6 are transcribed on the heavy stand (antisense strand) rather than the light strand.

Therefore to facilitate this difficult manual procedure in the future, we developed an interactive web site called MitoAnalyzer to enable investigators to determine the effects of any single bp polymorphism or mutation in human mtDNA. The investigator enters the number and whether the change is an insertion, deletion or substitution and the identity (A, C, G, T) of the substitution. The program compares that to the CRS and determines if the single bp change is a transition or transversion, whether it occurs in the noncoding (HV1 or HV2) or the coding region, and if it is in the first, second or third bp of the codon. MitoAnalyzer also provides information on whether the change affects a ribosomal RNA, a transfer RNA, or a messenger RNA coding for a protein, whether it causes an aa change, the nature of that change, the position of the aa change in the protein (e.g., aa # 25 in a protein containing 200 amino acids), and the new aa sequence of the changed protein as well as the original Cambridge Reference Sequence. Since a number of human diseases are know to be associated with specific mutations and deletions of mtDNA, mutation associated with published mitochondrial diseases are noted. Thus, this program facilitates rapid analysis and evaluation of single nucleotide polymorphisms (SNPs) and mutations found in human mtDNA.

This web site can be accessed at: http://www.sctl.nist.gov/biotech/strbase/mitoanalyzer/html

Anderson, S. *et al*. (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457-465.

Levin, B.C. *et al*. (1999) A human mitochondrial DNA Standard Reference Material for quality control in forensic identification, medical diagnosis, and mutation detection. *Genomics* 55:135-146.