THE COMPLETE mtDNA GENOME SEQUENCE OF HUMAN CELL LINE HL-60 AND ITS INCLUSION IN THE NIST HUMAN MITOCHONDRIAL DNA STANDARD REFERENCE MATERIAL SRM 2392

Barbara C. Levin, Koren A. Holland, Diane K. Hancock, and Kristy L. Richie
National Institute of Standards and Technology, Gaithersburg, Maryland

In 1999, the National Institute of Standards and Technology (NIST) announced the availability of Standard Reference Material (SRM 2392) to provide quality control for the amplification and sequencing of human mitochondrial DNA (mtDNA) or any DNA. This SRM was designed to provide assurance that the human identifications being done by the forensic community and the diagnosis of mtDNA diseases being conducted by the medical community were correct. A third area of potential use is the detection of single nucleotide polymorphisms (SNPs) and is currently a major effort of geneticists wishing to determine the locations and significance of these variations in the human genome. SRM 2392 includes two human DNA templates (CHR and 9947A) and all the information necessary to obtain the final DNA sequence. Fifty-eight unique primer sets for PCR and sequencing the entire mtDNA without gaps are described. An interlaboratory evaluation of SRM 2392 was completed and a paper has been published (Levin *et al*., 1999. Genomics 55:135-146).

The Federal Bureau of Investigation (FBI) needs DNA SRMs to provide the quality control and assurance that the results from sequencing unknown samples are correct. In 1998, the FBI Director signed Standard 9.5 that stated “The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST SRM or standard traceable to a NIST standard.” The FBI asked NIST to add the DNA from cell line HL-60 to SRM 2392 to increase its utility and provide more quality control to the forensic community. Since the CHR and 9947A DNA are from apparently normal individuals and HL-60 is from an acute promyelocytic leukemia patient, it was of interest to determine if there were unique mutations in this leukemia cell line.

NIST has completed all of the amplifications and sequencing necessary to determine and verify the correct sequence of the 16569 bp that comprise the entire human mtDNA of HL-60. The FBI Academy, the Armed Forces DNA Identification Laboratory (AFDIL), and the Georgia Bureau of Investigation (GBI) participated in an interlaboratory evaluation of the HL-60 sequence. All the laboratories, including NIST, obtained identical results. HL-60 has 12 differences from the two normal templates in SRM 2392; six of these (one of which is heteroplasmic) result in amino acid changes, four are silent changes, two are in transfer RNAs (alanine and serine). Four of the mutations that produce amino acid changes are associated with Leber’s Hereditary Optic Neuropathy (LHON) and are considered intermediate or secondary mutations (Wallace *et al*., 1997. Emery and Rimoin’s Principles and Practice of Medical Genetics, 3rd Ed. Churchill Livingstone, NY, pp. 277–332.)
(MITOMAP http://www.gen.emory.edu/mitomap.html). These intermediate or secondary mutations may increase the probability of having LHON or may be linked to one of the primary mutations associated with LHON. We expect this new SRM 2392A to be available by January 2003. Corroboration of the SRM results will provide additional assurance that any unknown DNA is also being amplified and sequenced correctly. Funding was provided by the National Institute of Justice through the Office of Law Enforcement Standards at NIST.