IMPROVEMENT OF THE NIST HUMAN MITOCHONDRIAL DNA STANDARD REFERENCE MATERIAL 2392 BY THE ADDITION OF DNA FROM THE HL60 CELL LINE

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Mitochondrial DNA (mtDNA) is used by the forensic community for human identification and by the medical community for diagnosis of a number of human diseases now known to be associated with specific mutations and deletions of mtDNA. An mtDNA Standard Reference Material (SRM 2392) was prepared by the National Institute of Standards and Technology to provide quality control to the scientific community when they amplify and sequence human mtDNA. This SRM includes two human DNA templates (CHR and 9947A) and all the information necessary to successfully conduct the PCR amplification process, cycle sequencing steps, gel separation, and data analysis to obtain the final DNA sequence. The information on the sequence of 58 unique primer sets that allow the sequencing of the entire mtDNA (16,569 bp) is also provided. Following an interlaboratory evaluation, Standard Reference Material 2392 was completed and released to the public in December of 1999 (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 forensic laboratories that are sequencing unknown samples are correct. On July 15, 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 Standard Reference Material or standard traceable to a NIST standard.” The FBI’s CODIS (Combined DNA Index System) program now includes mtDNA sequences (and nuclear DNA loci when possible) from unidentified remains, as well as from relatives of missing persons. In order for authorized laboratories to contribute or examine these indices, the FBI has deemed that certain quality standards must be met. In particular, a positive control from the human cell line HL60 must be run in conjunction with their unknown samples. HL60 was chosen by the FBI Laboratory as the positive control because of several features present in HL60 but not in the cell lines currently available in NIST 2392. Some of the advantageous features of HL60 are well-spaced polymorphisms throughout the HVI and HV2 areas of the mtDNA control region, and no insertions at the HV2 C-stretch area (position 303-310). The CHR DNA has a C-stretch in the HVI region caused by a T to C change at position 16189. Sequencing through this C-stretch is difficult and results in the need to perform additional sequencing reactions to resolve this region. The CHR template was chosen by NIST specifically for the C-stretch region since some laboratories wanted the opportunity to specifically address this difficult sequencing problem and to try to resolve it. The DNA from 9947A has only one polymorphism in the HVI region with respect to the Cambridge Reference Sequence and that polymorphism is a common site. For the work that the FBI is doing on human identification, several evenly spaced polymorphisms are more desirable to differentiate the positive control from the test sample. Thus, the FBI suggested to NIST that the addition of HL60 to SRM2392 would greatly increase its utility. This addition of HL650 to the NIST human mtDNA sequencing SRM 2392 is currently in progress and should provide more quality control when sequencing human mtDNA. Corroboration of the SRM results will provide assurance that any unknown DNA is also being amplified and sequenced correctly.