AVAILABILITY OF A HETEROPLASMIC MITOCHONDRIAL DNA STANDARD REFERENCE MATERIAL (SRM 2394) FOR QUALITY CONTROL IN THE DETECTION OF LOW FREQUENCY HETEROPLASMIES

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Human mitochondrial DNA (mtDNA) is a useful tool in forensic studies and disease diagnostics. However, heteroplasmy (the existence of two DNA nucleotides at the same site) can be problematic; for example, when trying to match samples from a crime scene and a suspect, the presence of an unknown heteroplasmy producing one base pair (bp) difference can cause ambiguous or false negative results. One bp difference should not be sufficient for an exclusion since it has been shown that different hairs from the same individual can have widely different proportions of the base pairs contributing to a heteroplasmy (1). MtDNA single nucleotide polymorphisms (SNPs), heteroplasmies, insertions, and deletions have also been implicated in many human diseases primarily involving the neuromuscular system, but deafness, diabetes, epilepsy, progressive dementia, hypoventilation, cardiac insufficiency, renal dysfunction, and sudden onset blindness have also been correlated with mtDNA heteroplasmic mutations. Recent results found that about 1/8000 individuals either have or are at risk of developing a mitochondrial disease (2). Therefore, the ability to detect a low-frequency heteroplasmic mutation in the presence of a majority of wild-type mtDNA is extremely important for correctly diagnosing diseases, predicting the risk of developing mitochondrial diseases and for providing pertinent genetic counseling to families at risk. If the mutation is present in every mtDNA molecule, detection is routine; however, low frequency mutations scattered throughout the DNA, are almost impossible to detect. Therefore, NIST has developed heteroplasmic human mtDNA SRM 2394 to provide quality control to forensic, medical, and toxicological scientists who wish to determine their detection limits when examining low frequency mutations or heteroplasmic sites in mtDNA. SRM 2394 is composed of 10 tubes containing different percentages of a polymorphic/wild-type mtDNA mixture (i.e., 1, 2.5, 5, 10, 20, 30, 40, and 50% as well as one tube that is 100% polymorphic and one tube that is 100% wild-type). These mixtures have been constructed from PCR products from two different cell culture lines that differed by one base pair in the 285 bp amplified region. An interlaboratory evaluation in which 12 laboratories determined their mutation detection sensitivity has been completed. NIST also examined various techniques [e.g., PCR and sequencing, denaturant gradient gel electrophoresis (DGGE), peptide-nucleic acid (PNA) complementary to the wild-type, and Luminex 100] to try to determine which was the most sensitive. The Luminex system was the only technique able to detect the heteroplasmy at the 1% level (the lowest concentration tested). Investigators will be able to use SRM 2394 to determine the resolution of their mutation detection techniques and, hopefully, to perfect even more sensitive methods.

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2. P.F.Chinnery, M.A.Johnson, T.M.Wardell, R.Singh-Kler, C.Hayes, D.T.Brown, R.W. Taylor, L.A. Bindoff, D.M.Turnbull, The Epidemiology of Pathogenic Mitochondrial DNA Mutations. Ann Neurol. 48:188-93 (2000).