

Development of NIST SRM 2394 Heteroplasmic Mitochondrial DNA Mutation Detection Standard

Human mitochondrial DNA (mtDNA) mutations are important for forensic identifications and mitochondrial disease diagnostics. If a mutation is present in every mtDNA molecule, detection is routine, but in the case of heteroplasmies, where the mutations occurs in only some of the mtDNA molecules, the low-frequency mutations are almost impossible to detect. Also, in pooled nuclear DNA samples used in population screening, single nucleotide polymorphisms (SNPs) may be present at very low levels and difficult to detect.

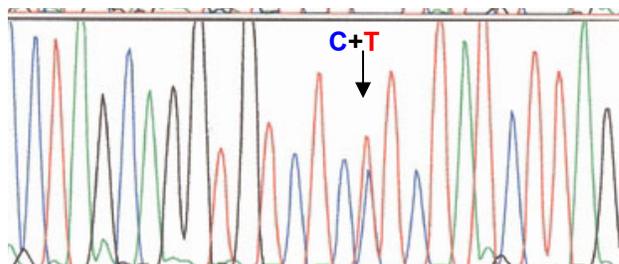
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NIST has developed heteroplasmic human mtDNA Standard Reference Material (SRM) 2394 to provide quality control to forensic, medical, and DNA scientists who wish to determine their sensitivity in detecting low-frequency mutations. SRM 2394 is composed of mixtures of two 285 base pair (bp) PCR products from two cell lines that differ at one nucleotide position. The CHRcell line designated polymorphic has a thymine (T) at nucleotide position 6371 and the “normal” cell line has a cytosine (C) at that site. SRM 2394 is composed of 10 tubes, one tube containing only the DNA with the polymorphism, one tube containing the normal DNA sequence, and 8 tubes containing different percentages of the polymorphic/normal mtDNA mixtures (in which the mass fraction polymorphic levels are 1%, 2.5%, 5%, 10%, 20%, 30%, 40% and 50%). Before the final SRM was prepared, twelve laboratories including NIST, participated in an Interlaboratory Evaluation (ILE) of a prototype of SRM 2394. This ILE was a blind study in which the investigators could use any mutation detection method of their choice. The methods in-

cluded automated DNA sequencing with three different chemistries and different sequencers; denaturing gradient gel electrophoresis (DGGE); a designer peptide nucleic acid (PNA) method; the Luminex 100 analysis; the LigAmp procedure; and denaturing high-performance liquid chromatography. Most of these procedures were unable to detect the heteroplasmy if present below 20% – an indication that, in many real life cases, low-frequency mutations remain undetected and that more sensitive mutation detection techniques are urgently needed.

SRM 2394 will soon be available and will serve as a positive control for the determination of low-frequency mutations and as an aid in the development of new analytical techniques.

Heteroplasmy detection by sequencing. Electropherogram of the 50:50 mixture (50% T:50% C) taken from the SRM 2394 kit. Although the heteroplasmy is readily apparent at this level, below 20% T the presence of the heteroplasmy cannot be distinguished from the baseline noise.



Publication

D.K. Hancock, L.A. Tully, and B.C. Levin, “A Standard Reference Material to determine the sensitivity of techniques for detecting low-frequency mutations, SNPs, and heteroplasmies in mitochondrial DNA,” *Genomics* (2005) 86, 446-461.