DETECTION OF LOW-FREQUENCY HETEROPLASMIC MITOCHONDRIAL DNA SINGLE NUCLEOTIDE POLYMORPHISMS

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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 identifying humans, accurately 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 been examining heteroplasmy from the following different perspectives: A. NIST heteroplasmic mtDNA Standard Reference Material (SRM 2394) is now available. This SRM enables investigators to determine the sensitivity of any technique to detect low-frequency heteroplasmic mutations and hopefully will provide a tool to help investigators to perfect even more sensitive mutation detection techniques. In this way, low-frequency mitochondrial heteroplasmies may become detectable, and mitochondrial heteroplasmic diseases, once detectable, may be treatable, and perhaps even preventable prior to the appearance of any symptoms. B. We have examined a control region heteroplasmy in thirteen maternally-related family members over three generations. The goal was to determine the stability of the inheritance of that heteroplasmy within and across generations as well as examine the heteroplasmic variation among tissues of single individuals. C. We have designed and are using a peptide nucleic acid (PNA) for detecting the specific low-frequency heteroplasmy associated with the disease MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like Episodes). These experiments were designed to develop a methodology to detect specific single nucleotide mutations that are present in very low to undetectable levels in a population of wild-type mtDNA molecules. The goal is to help improve public health since early detection of these heteroplasmic mutations could lead to advances in the treatment of the disease and perhaps even its prevention.

1. K.Sekiguchi, K.Kasai and B.C.Levin, Intergenerational Transmission of a Human Mitochondrial DNA Heteroplasmy among Thirteen Maternally-Related Individuals and Differences between and within Tissues in Two Family Members. Mitochondrion 2:400-413 (2003).
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).