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STUDY OF A C/T HETEROPLASMY AT NUCLEOTIDE 12071 IN MITOCHONDRIAL DNA (mtDNA) OF THE HL-60 CELL LINE USING A PLEXOR® qPCR SYSTEM

Michael Adam[1], Joseph Reiner[2], Rani Kishore[2], Kristian Helmerson[2], and Barbara Levin[3], Ashley Knipe[1] and Koren Holland Deckman[1]

[1]Department of Chemistry, Gettysburg College, Gettysburg, PA 17325

[2]Laser Cooling and Trapping Group, Physics Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD

[3]DNA Biotechnology Group, Chemical Science and Technology Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD.

Single nucleotide polymorphisms (SNPs) and heteroplasmies are present in mtDNA in a wide variety of cells and can cause problems in forensic identifications. For example, a heteroplasmy found in single hair shaft from the crime scene but not in the suspect's sample could lead to ambiguity in the forensic identification. In this study, we sought to optimize a genotyping method - Plexor qPCR - using a C/T heteroplasmy specific to the mtDNA in the human leukocyte (HL-60) cell line. An improved genotyping method would help in successfully characterizing other ambiguous heteroplasmies. Traditional amplification and sequencing of extracted total DNA shows an approximately equal ratio of C to T in HL-60 by examining the electropherogram peak intensities. Control samples only contain T at 12071. The Plexor qPCR system was then used to amplify the same region around nucleotide 12071 to quantify the C/T ratio. The ratio as demonstrated by ABI BigDye v.1.1 chemistry was compared to the ratio generated by allele-specific qPCR and Promega Plexor chemistry. The optimized method was then applied to single cells and single mitochondria, both isolated via the optical tweezers methodology. By learning more about heteroplasmic distribution at the single cell and single mitochondria level, the forensic community can begin to define the limitations of heteroplasmic mixtures in forensic samples.