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ANALYSIS OF HETEROPLASMIC DISTRIBUTION IN A SINGLE MITOCHONDRION BY THE OPTICAL TWEEZERS METHODOLOGY AND QUANTITATIVE PCR

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Mitochondrial DNA (mtDNA) heteroplasmies are well documented at the multi-cell level; however, PCR and sequencing (the gold standard for the determination of the presence of heteroplasmy) can only distinguish heteroplasmy if it is present at least in 20% of the sample. Also the presence of heteroplasmy may differ depending on the tissue examined; for example, it may be present in blood, but not in hair samples, leading to the conclusion that the hair at the crime scene is not from the suspect whose blood was tested. Therefore, the presence and degree of heteroplasmy is important in the forensic community. It is also important in the medical community as heteroplasmies have been linked to mitochondrial-based diseases. We examined the questions of whether heteroplasmy is present in a single cell and if so, is it present in a single mitochondrion. We developed an effective methodology to isolate single human leukocyte cells (HL-60) and single mitochondrion from those cells and to examine the single cells and single mitochondrion for a specific heteroplasmy. The cells from an HL-60 culture were labeled with Mitotracker Green FM, and individually separated using optical tweezers (5 watt fiber IPG Photo Optical laser) and an Axiovert 100 M fluorescent microscope. We found that the individual cells were heteroplasmic (single cell PCR and sequencing showed a 50/50 C/T heteroplasmy at nucleotide position 12071). Individually-dyed mitochondrion from the single cell were separated by this optical tweezer method and tested in the same manner. Our results suggest that the single mitochondrion also contains the heteroplasmy indicating the presence of multiple genomes in each mitochondion. We are now developing a method utilizing quantitative PCR to try to quantify the mtDNA genome copy number per mitochondrion. Preliminary results with this method will be discussed.