While current techniques and technologies provide for reliable reporting of mitochondrial DNA (mtDNA) haplotypes, the methods in place today do not effectively identify heteroplasmic variants (especially low-level variants), and even when heteroplasmy is observed at high levels, the information is typically not used during the forensic investigation. The ability to identify, report, and leverage the discrimination potential of heteroplasmy will significantly enhance the value of using mtDNA analysis in forensic casework. A next generation DNA sequencing (NGS) approach will allow the community to achieve this goal. Our initial work illustrated that the increased resolution provided by NGS produces a higher rate of detectable heteroplasmy across the mtDNA genome. The objective of our current research (NIJ 2014-DN-BX-K022) is to measure the rate of observing mtDNA heteroplasmy on a per sample and per nucleotide basis, using mtDNA isolated from buccal cells collected from 550 unrelated individuals of European descent. This poster will present our progress on the current project for which we have sequenced a portion of the 550 samples with paired-end reads on an Illumina MiSeq using a Nextera XT library preparation on amplified products targeting the control region (CR) of the mtgenome. Secondary analysis, using the NextGENe software platform, evaluates haplotype, haplogroup, and heteroplasmy information for self proclaimed European males and females ranging from 18 to 81 years of age. The NGS approach is clearly capable of detecting heteroplasmy, including low-levels of heteroplasmy, but a standardized approach to data analysis, along with software designed to evaluate NGS mtDNA data, is needed. Establishing the rates of heteroplasmy for the CR of the mtDNA genome will allow forensic laboratories to report out heteroplasmy on a routine basis, significantly enhancing the discrimination potential of mtDNA testing. This will have a major positive impact on laboratories currently performing mtDNA analysis, and could be the impetus for other laboratories to bring mtDNA typing on line if the discrimination potential were improved, and the results of the testing become more valuable to a forensic case.