COMPARISON OF MITOCHONDRIAL DNA (MTDNA) CONTROL REGION NEXT-GENERATION SEQUENCE DATA OBTAINED USING THE ROCHE GS JUNIOR® AND ILLUMINA® MISEQ PLATFORM

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Next-generation sequencing (NGS) technologies are currently being evaluated in our laboratory for usefulness in forensic science due to their ability to reliably detect low-level variants in mixtures of mitochondrial DNA. We are currently evaluating two popular benchtop NGS instruments, the Roche® GS Junior and the Illumina® MiSeq on their ability to quantify minor variants in a mixed sample, sample preparation strategies, ease of use and cost effectiveness. Libraries prepared from mtDNA control region amplicons were multiplexed and deep-sequenced using both platforms. A mixture study was designed in which quantified extracts were combined in defined ratios and deep-sequenced to determine the minor variant limit of detection of each instrument. Additionally, a tissue comparison study using extracts from blood, buccal, and hair shaft samples was designed to determine if sequence differences exist between sample types, and if differences somehow relate to NGS chemistry used. Platform dependent data comparisons have allowed us to determine degree of multiplexing achievable by each instrument to reach a defined depth of coverage and minor variant limit of detection given a defined depth of coverage. We were also able to corroborate unexpected sequence variants from a highly conserved nuclear insertion of mitochondrial DNA (NumtS) initially detected by our laboratory in HV1b mitochondrial datasets using the Roche® GS Junior next-generation sequencer. Finally, we have quantified tissue dependent variants. Our results provided a useful context for those laboratories wishing to implement and validate NGS protocols using these burgeoning technologies. ⌘