A TALE OF TWO PLATFORMS: AN EVALUATION OF THE ROCHE GS JUNIOR AND ILLUMINA® MISEQ NEXT-GENERATION SEQUENCING INSTRUMENTS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

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Next-generation sequencing (NGS) refers to a suite of technologies that enable cost-effective, rapid generation of large amounts of detailed sequence information from clonal populations of individual template molecules. These methods are proving to be particularly well-suited for mitochondrial DNA analysis, and may provide forensic DNA analysts with a powerful tool that enables deconvolution of mtDNA mixtures. Recently, Illumina® has been working with members of the community to establish a human mtDNA forensic genomics consortium (IFGC) for concerted evaluation of NGS methods for potential use in mtDNA casework and databasing. In June, a set of samples was prepared consisting of quantified buccal extracts from two donors, as well as a series of mixtures of the buccal extracts at defined ratios (5, 2, 1 and 0.5%). This sample set has been distributed to participating IFGC laboratories for sequencing on multiple NGS platforms including the Ion PGM™, Roche GS Junior, and Illumina® MiSeq, to enable a cross-laboratory comparison of sequencing methods using identical samples. In our laboratory, the samples were sequenced on both the Roche GS Junior, and Illumina® MiSeq NGS platforms. Libraries from hypervariable regions 1 and 2 (HV and HV2) were sequenced on the Roche GS Junior using an amplicon library preparation approach where PCR primers were designed to include required adaptors and multiplexing indices. For sequencing on the Illumina® MiSeq, libraries were prepared using Nextera® XT in which two large amplicons covering the whole mtGenome as well as HV1 and HV2 amplicons were randomly fragmented, and adapters and indices incorporated enzymatically. The resulting data was analyzed using SoftGenetics® Next*GENe*® software and variant calls were compared. The Illumina® MiSeq resulted in significantly higher coverage across all positions sequenced, giving rise to higher certainty with low-level variant calls. Further, the Miseq allowed for detection of minor variants in all mixtures where the majority of minor variants were undetected in the 0.5% mixture with the Roche GS Junior. Finally, data from the MiSeq showed lower background noise overall, especially in homopolymeric regions when compared to data from the GS Junior. The Illumina® MiSeq offers a streamlined enzymatic library preparation approach, higher-throughput and variant detection sensitivity and basecalling than the Roche GS Junior. As a result, we feel that the MiSeq is better suited for forensic mtDNA analysis in both casework and databasing laboratories.