EVALUTION OF NEXT-GENERATION SEQUENCING TECHNOLOGIES FOR PCR-BASED STR GENOTYPING

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Next-generation sequencing (NGS) technologies have recently emerged as potentially valuable tools for forensic sciences. Laboratory methods and analysis software are being developed to accelerate growth and test the utility of NGS platforms. In this study, we evaluated three commercially available benchtop NGS instruments, Illumina MiSeq, Roche GS Jr, and IonTorrent PGM, for the ability to perform STR genotyping using a front-end PCR enrichment step similar to existing capillary electrophoresis (CE)-based methods. Cell line, buccal swab, FTA-blood, and saliva derived DNA for single reference and mixture samples were assessed on each platform for an STR 18-plex including the CODIS loci. Custom software was designed to interpret NGS data and to measure concordance with CE profiles from matched samples. A scoring matrix comprised of over 60 criteria was implemented to rate the three systems for operation and overall performance. The results demonstrated critical attributes of each instrument in regards to performing human identification with NGS. First, high-quality read length was a critical requirement to obtain NGS data on full length STR alleles. Sequencing errors and low quality data were the main drivers for failing to consistently and accurately generate STR genotypes. Instruments with low error rate and longer read lengths provided the optimal data sets. Second, the data yield (bandwidth) proved to be a factor for the ability to multiplex and provide sufficient data for mixed samples. Lastly, the forensics lab readiness level had a major influence on the scoring of the instruments for ease-of-use, reproducibility, sample contamination, and validation potential. In conclusion, this study demonstrates the pros and cons of the currently available NGS technology as related to human identification using STR-based approaches and provides recommendations for further development and implementation of this technology in the forensics laboratory.