MORE AND MORE MARKERS: USE OF THE PRECISION ID GlobalFiler MIXTURE ID PANEL TO ANALYZE CHALLENGED AND MIXED SAMPLES

J.D. Churchill[1], J.L. King[1], and B. Budowle[1,2]
[1] Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center
[2] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University

Massively parallel sequencing (MPS) enables forensic scientists to gather more information from the same amount of template than traditional capillary electrophoresis (CE) based methodologies. With MPS, different types of markers can be multiplexed and sequenced simultaneously. These larger and mixed-marker multiplexes can be useful in making identifications, performing and enhancing database searches, and employing alternate approaches for developing investigative leads. The Applied Biosystems™ Precision ID GlobalFiler™ Mixture ID panel is comprised of 30 STRs, 2 indels, 36 microhaplotypes, and 43 identity SNPs. The Ion Chef™ and the Ion S5™ Systems provide a workflow for typing this large multiplex that is conducive for use in forensic casework. In this study, samples were sequenced using the Precision ID GlobalFiler™ Mixture ID Panel on the Ion S5™ and compared with markers typed with the GlobalFiler kit on the Applied Biosystems 3500 XL Genetic Analyzer. Data were evaluated for accuracy, quality, and reliability. Challenged and mixed samples were analyzed to determine the panel's success with forensically relevant samples.

Ten samples were typed on the Ion S5™ System and 3500 XL Genetic Analyzer. Full profiles were obtained for each sample and genotypes were concordant between MPS and CE methodologies for common markers. Metrics such as strand balance, allele coverage ratios, and noise also indicated that quality and reliable data had been produced. A dilution series with input DNA ranging from 1 ng to 125 pg demonstrated the panel's sensitivity of detection. Challenged samples, including touch samples, hair samples, bones, blood stains, semen stains, and saliva, were typed successfully. Finally, mixtures with DNA ratios ranging from 1:1 to 1:640 (minor contributor:major contributor) and mixtures with up to six contributors were analyzed. This mixture analysis allowed for a comparison of resolution limits of MPS and CE methodologies and illustrated the benefits additional markers and intra-allelic sequence variants provide in analyzing mixtures. The results generated in this study support the utility of large, mixed-marker panels in forensic analysis, and the data quality produced in this study support the Precision ID GlobalFiler™ Mixture ID panel and Ion S5™ System as a viable MPS methodology for forensic genetic analysis.