Inter-Laboratory Internal Validation Study of the ForenSeq DNA Signature Prep Kit

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The ForenSeq DNA Signature Prep kit allows typing of 27 autosomal, 24 Y, and 7 X-STRs, and 94 identity, 56 ancestry, and 22 phenotypic SNPs in a single reaction. The following studies were performed on both DNA primer mix A and B: (1) concordance, (2) reproducibility and repeatability, (3) sensitivity and stochastic, (4) case-type samples, (5) mixtures, (6) contamination assessment. Studies were performed at AFDIL, DC Department of Forensic Sciences, and UNTCHI. Over 1600 samples were tested across 51 sequencing runs on 5 MiSeq FGx instruments according to manufacturer's recommended protocols for processing samples ranging from 8 to 96 simultaneously.

The concordance study compared FGx System allele data with data from positive control DNA, NIST SRM samples, and known reference CE profiles. For reproducibility and repeatability studies, the locus call rate, genotype accuracy, and genotype precision were 0.999, 0.997, 0.998, respectively, with data loss generally isolated to the DYS392 and D22S1045 loci. Sensitivity and stochastic studies demonstrated the dynamic range, ideal target range, and limit of detection/quantitation using samples at 0.008ng - 4ng input DNA. Full profiles were observed down to 31pg in some replicates; however, allele dropout was observed in some replicates throughout the series particularly with the previously mentioned loci. Despite data loss at these loci, >90% of STR alleles were observed at 62pg and 40% of STR alleles were observed at 8pg. For the case-type samples study, results from sample types typically encountered in casework, such as blood, saliva, semen, epithelial cells, sexual assault sperm and non-sperm fractions, touch samples, hair, bone, teeth, cigarette butts, chewing gum, etc., were compared with previous CE data to determine performance with potentially challenging samples. Concordance with CE data was >99% in high quality samples with stochastic effects evident in low quality/quantity samples. The mixture study evaluated the capability of the system to detect and resolve mixtures using NIST SRM component D and known male and female reference DNAs combined as 2 and 3 person mixtures at several ratios. Unique minor alleles were detected down to 1:499 (6.8%) with >50% of alleles detected at 1:19. The contamination assessment evaluated all blanks and known profiles for detection of exogenous DNA. The data support that the kit yields reliable results and will be submitted for NDIS approval.