H97 Comparing Resolution of Mixtures by DNA Sequencing Using the Illumina® MiSeq® FGx System

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Learning Overview: The goal of this presentation is to show that the MiSeq® FGx system is concordant with current Capillary Electrophoresis (CE) -based analysis and provides additional sequencing data.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing a possible alternative to CE-based Short Tandem Repeat (STR) analysis that can provide additional sequencing information to mixture samples.

The use of STRs for genotyping forensic case samples has long been an effective tool for human identification. However, deconvolution of forensic STR mixture samples can be difficult and being able to obtain additional information to aid in this process will be important. Allele overlap and stutter during PCR can cause drop-out of the minor contributor's alleles and result in incorrect allele calling. The GlobalFiler® Polymerase Chain Reaction (PCR) Amplification kit targets 21 autosomal STRs, amelogenin, and 2 Y-indels. In comparison, the ForenSeq™ Signature Prep Kit on the MiSeq® FGx targets an additional 6 autosomal STRs, 24 Y-chromosomal Short Tandem Repeats (Y-STRs), 7 X-chromosomal Short Tandem Repeats (X-STRs), and 94 Single Nucleotide Polymorphisms (SNPs), and it also provides the DNA sequence of those targets. The additional loci as well as separation of alleles by sequence should provide much more information for resolving mixture samples. The contributor ratio accuracy and MiSeq® FGx performance is analyzed here and compared to current CE-based methods. The DNA sequencing process used here requires three PCR amplification steps overall. Additionally, there are many wash steps and transfer steps involved in the purification and normalization of the libraries prior to sequencing. Together, the number of steps may increase profile variability.

A side-by-side assessment of the ForenSeq™ Signature Prep Kit with the MiSeq® FGx system and the GlobalFiler® PCR Amplification Kit using equivalent samples containing two-person DNA mixtures at three different mixture ratios is presented. The ratio of two contributors was calculated at three mixture ratios (1:1, 1:4, and 1:9) to use as a means of comparison. Each mixture was analyzed in quadruplicate twice on two separate amplifications. The mean contributor ratios calculated on the MiSeq® FGx were 1.799, 7.595, and 13.524 for the 1:1, 1:4, and 1:9 mixtures, respectively. This was not significantly different from the CE mean contributor ratios of 1.818, 7.722, and 14.827, respectively. There was a total of 68 minor contributor alleles (9.09% of the total possible alleles) that were lost on the MiSeq® FGx™ and only 20 on the CE (3.38% of the total possible alleles). Most of those alleles lost on the MiSeq® FGx (61 or 8.15%) and CE (18 or 3.03%) were from the 1:9 mixtures. This difference may seem large, but there were an additional five minor contributor alleles, per replicate, that could be identified by sequence. A high preferential amplification of the D22S1045 was seen, which resulted in the loss of that locus for contributor ratio calculations.

The SNP loci were also analyzed here to determine if they present a more accurate representation of mixture ratio at lower concentrations, as they are shorter in length and more readily amplified. The contributor ratios for the SNP loci were calculated to be 1.787, 8.726, and 21.077 for 1:1, 1:4, and 1:9, respectively. Only three SNP alleles dropped out, one at 1:4 and two at 1:9; however, the contributor ratio values were significantly higher than STRs calculated on either the CE or sequencing platform. Overall, the data showed that the MiSeq® FGx was concordant with the CE, which should facilitate the introduction of sequence data as an additional tool for use in forensic DNA testing.

Next Generation Sequencing, Mixture Interpretation, Contributor Ratio

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