## B45 Isoalleles Revealed by Massively Parallel Sequencing (MPS) Provide Increased Resolution and Discrimination in Forensic Casework

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After attending this presentation, attendees will understand what isoalleles are and how they can be used in forensic casework.

This presentation will impact the forensic science community by demonstrating the power MPS holds for increased discrimination in forensic casework.

Short Tandem Repeat (STR) typing by Capillary Electrophoresis (CE) is the standard for DNA processing in forensic laboratories; however, this method is limited in the amount of information that it yields for analysis by only providing length-based information from STRs. In contrast, STR typing by MPS yields both size- and sequence-based information. This ability makes it possible to detect isoalleles (alleles that are homozygous by length but heterozygous by sequence). Because these are sequence-based differences, they are not observable in CE-based STR assays. Not only can sequence variation between individuals at the same loci be revealed, but this kind of diversity is often observed at one or more alleles in a DNA profile.

Battelle and the Ohio Bureau of Criminal Investigation (BCI) have collaborated to evaluate a customized MPS workflow utilizing the Promega® PowerSeq™ Auto/Y prototype amplification kit and the Illumina® TruSeq® DNA PCR-Free library preparation kits for sequencing on the Illumina® MiSeq® instrument. Sixteen buccal swabs (extracted and quantified) and 49 database samples (directly amped) were processed with this workflow and analyzed for the presence of isoalleles. In this study, 17 of 65 samples displayed at least one isoallele. The most common locus displaying isoalleles was D3S1358, a locus with a compound repeat, where it was observed in five individuals. Isoalleles were also observed in four samples for locus D21S11, which is a complex locus. The sample with the highest number of isoalleles contained three at different loci within the profile.

Due to the relatively common appearance of this characteristic during this study, isoalleles present a unique potential for resolving contributors of mixed samples. Several mixtures at different ratios were prepared and sequenced. Analysis of these mixtures revealed that isoalleles are able to yield valuable information when attempting deconvolution of MPS mixture data. In one mixed sample, there were three loci where the contributors to the mixture showed isoalleles. There was an additional locus where the sequence data allowed the allele type of the minor contributor to be differentiated from the stutter of the major contributor's allele. These preliminary studies suggest MPS will be a valuable tool for analyzing mixed samples in forensic casework.

As one part of a larger validation study, genetic transmission was studied to ensure that the markers included in the PowerSeq™ Auto/Y prototype amplification kit are consistent with the expected Mendelian inheritance pattern. The pattern of transmission from mother and father to offspring allowed an evaluation of the inheritance pattern as well as the stability of genetic transmission events. Isoalleles were observed in this study and were traceable from parents to their offspring. In one family, a male child had a heterozygous genotype at locus D1S1656 with isoalleles of two different sequence variants that were traced back to the mother and father. In another family, the father exhibited isoalleles at D2S441 (10a and 10b). Upon sequencing the amplified products in the offspring, it was observed that the daughter had inherited the 10a allele while the son inherited the 10b allele.

The studies performed as part of this evaluation highlight the prevalence of isoalleles and their high value to forensic casework. Whether in mixture cases, paternity cases, missing persons cases, or familial searches, MPS can provide valuable information that extends beyond those of traditional CE-based methods.

**MPS, Isoalleles, Casework**



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