B189 A Reproducible Failure of One Base Pair Resolution in Casework Samples on the 3500xL Genetic Analyzer

Rachel Maragliano, Huntington, WV 25705; Jason M. Chute, MSFS, Marshall University Forensic Science Center, Huntington, WV 25701; Kelly Beatty, MSFS, Marshall University Forensic Science Center, Huntington, WV 25701*

Learning Overview: After attending this presentation, attendees will understand the forensic DNA workflow, the importance of the ability for instrumentation to accurately resolve DNA mixtures, and a recurring failure to resolve DNA mixtures with alleles one base pair apart.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by confirming a one base pair resolution failure and present some observations that can lead to fixing this issue.

Many items of evidence submitted for forensic DNA testing are mixtures of more than one individual. Therefore, it is critical that the instrumentation and chemistries used can correctly resolve all the alleles from all contributors. Recently, laboratories have been noticing that a mixture consisting of a homozygote as the major contributor and a heterozygote as the minor contributor at a locus where two of the alleles, the homozygote allele and one allele from the heterozygote within one base pair of each other are not properly resolving. At initial glance it appears to be a non-Gaussian peak caused by artifacts of the DNA process. However, standards require precision studies exhibiting one base pair resolution, so if it is not occurring that is cause for alarm. This study looked to replicate this resolution failure and to provide some insight as to why this issue is occurring.

The first study looked to see how well resolution occurred in the position of the minus A artifact. Samples were obtained from two females who at D12S391 are 18,18 (female A) and 17.3,20 (female B). Extracts were quantified, normalized, and mixed together at ratios of 1:1, 2:1, 4:1, 6:1, 8:1, 10:1, and 16:1, with female A as the major contributor. The mixtures were then amplified with a GlobalFiler™ PCR Amplification Kit at 1 ng and 3 ng, run on a 3500xL Genetic Analyzer, and analyzed with GeneMapper® *ID-X* v1.5.

The second study looked at resolution where the homozygote microvariant is the major contributor and a heterozygote is the minor contributor. This was to see the resolution on the trailing side of the homozygote peak. Samples were obtained from a female and a male who at TH01 are 9.3,9.3 A (female C) and 6,10 (male A). Extracts were quantified, normalized, mixed together at ratios of 1:1, 2:1, 4:1, 6:1, 8:1, 10:1, and 16:1. The mixtures were then re-quantified in triplicate to obtain actual mixture ratio values. The mixtures were then amplified in triplicate with a Globalfiler™ PCR Amplification Kit at 0.5 ng and 1.5 ng loads and run on a 3500xL Genetic Analyzer. The mixtures, apart from the 16:1, were also amplified at 0.5 ng and 1.5 ng loads with PowerPlex® Fusion 6C System and run on the same instrument, however, not in triplicate.

In the first study, the mixtures amplified with a 3 ng/µL load resolved better than those with a 1 ng/µL load. Six out of the seven mixtures with a 3 µL load resolved the 17.3 allele, while three of the six mixtures with a 1 ng/µL load resolved. Analysis of the peak height ratios show that the minus A artifact is contributing to the peak height, causing the peak to be resolved.

In the second study, only one of the 4:1 mixture replicates and the 1:1 and 2:1 mixtures amplified with GlobalFiler™ resolved. However, the 1:1, 2:1, 4:1, and 6:1 mixtures amplified with PowerPlex® Fusion 6C resolved the 10 allele at both amplification loads and the 8:1 at a 1.5ng load. This confirms that without the minus A artifact, alleles within one base pair of each other are not resolving correctly.

While there is no definite answer yet as to what is causing the resolution failure, it is to note that PowerPlex® Fusion 6C did resolve the mixtures better at lower mixture ratios than Globalfiler™. This could possibly be due to the dynamic range of the 3500xL increasing to greater than 10,000 RFU, while the analytical threshold did not increase proportionally. Future studies look to investigate this cause further, such as using a different polymer, running on a different capillary electrophoresis instrument, adjusting run voltage, and amplifying with an Investigator 24plex QS Kit.

DNA, Mixtures, Resolution