B186 The Application of Short Tandem Repeat (STR) Sequence Variation for the Selection of Novel STR Markers to Enhance DNA Mixture Deconvolution: What Do We Know and Where Are We Headed?

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After attending this presentation, attendees will better understand Massively Parallel Sequencing (MPS) of forensically related STRs, the value of capturing entire amplicon sequence information (primer-to-primer sequence data), and the potential impact increased allelic data will have on applications in forensic DNA human identification.

This presentation will impact the forensic science community by providing information on data that supports implementation of MPS and STRs into forensic laboratories, discussing best-practice analysis methods, and exploring the next steps in novel forensic STR determination for maximal DNA mixture deconvolution efforts.

DNA mixture deconvolution of crime scene samples remains one of the biggest challenges faced by forensic laboratories today. When DNA mixtures are encountered in a sample, the analyst would like to identify individual components of the mixture and characterize each component of the sample using mixture interpretation guidelines and statistical calculations. Once the different components of the mixture have been resolved, the analyst then is able to make comparisons of the mixture components to reference samples. Currently, the basis of forensic interpretations relies on the amplification of STR markers using the Polymerase Chain Reaction (PCR), allele sizes determined using Capillary Electrophoresis (CE), and the results used to query reference samples or existing profiles stored in the Federal Bureau of Investigation (FBI) Combined DNA Index System. DNA mixtures often complicate STR genotyping of samples, and complex mixtures of three or more contributors are being encountered routinely in forensic casework.

MPS is a novel yet robust technology for capturing large amounts of DNA data from minimal input DNA, which is useful for processing forensic evidence that is frequently limited in quantity and quality. Using MPS, multiplexing a large number of STR markers simultaneously is possible and will provide greater genetic information for STR genotyping of multiple contributors in mixture DNA samples. MPS also provides the opportunity to identify intra-allelic sequence variation within STRs not possible using conventional CE protocols. Although this technology offers promise, current panels that capture the core STR loci may not contain sequence variants that have sufficient variability to individualize components in a mixture sample. Additionally, the ability to capture flanking region sequence variation to exploit the full power of forensically relevant STR loci is needed. The application of such data will increase knowledge and understanding of each locus, increase the power of discrimination of a locus, and potentially aid in mixture deconvolution efforts. The underlying genetic variation needs to be described through studies on various population groups.

An in-depth examination of sequence variation in 58 STRs was performed on 780 individuals using the MiSeq® FGx™ Forensic Genomics System, STRait Razor, and in-house Excel® workbooks. A total of 747 autosomal, 228 X chromosome, and 324 Y chromosome STR alleles were identified by sequence compared to 357 autosomal, 107 X chromosome, and 188 Y STR alleles that were identified by length. Within the observed sequence variation, more

than 500 novel alleles were identified and described. These population data illustrate the genetic variation that exists in the commonly used STR markers and suggest that a great deal of genetic diversity has yet to be uncovered in STR markers.

Continued efforts are focused on identifying substantially polymorphic STR loci for the creation of a novel STR panel. The characterization of novel STR markers containing high-resolution intra-allelic sequence variants will allow the forensic scientist to overcome certain challenges of interpretation of some complex mixture samples, increase the number of resolved profiles being compared to reference and suspect profiles, and expand the DNA database by increasing the number of forensic samples uploaded. The benefit from this revolutionary application will be an increase in the number of investigative leads and the overall resolution of more crimes.

Massively Parallel Sequencing, STR Sequence Variation, DNA Mixture Deconvolution