A131 Technical Challenges of Developing Large Multiplex STR Assays for CE Platforms

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After attending this presentation, attendees will gain a better understanding of the complexity of developing robust assays for human identification.

The presentation will impact the forensic science community by providing a better understanding of how multiplex STR assays are developed for human identification.

Multiplex STR genotyping assays using fluorescent detection and capillary electrophoresis represent the most popular method of human identification due to the highly polymorphic nature of STRs, and their small fragment size (~100-400 bp). These multiplex STR assays have a high discriminatory capacity. For example, when a complete DNA profile is obtained using the 13 CODIS loci, the probability of a chance match with a randomly chosen individual is usually less than 1 in 1 X 10^{12}. These assays have been used as supportive evidence not only to convict the guilty but also to exonerate the innocent. Given the potential consequences of STR evidence, it is important to have robust and reliable assays.

However, obtaining successful co-amplification of all STR loci in a multiplex reaction with good peak height balance between loci, specific amplification, minimal stutter, maximal non-template-dependent +A addition, and no non-specific products which might interfere with proper interpretation of a sample's DNA profile is very challenging. Furthermore, with every new primer set added to the reaction, the complexity of the PCR increases and exponentially increases the possible primer-primer and non-specific interactions. The development of an efficient multiplex PCR reaction requires careful planning and numerous tests and efforts in the area of primer design, balancing reaction components, and optimizing thermal cycling conditions. In addition, the contributions to data quality of oligonucleotide synthesis and purification, elimination of dye artifacts, and electrophoresis conditions need to be assessed.

The forensic community is concerned with many aspects of the assay such as: stutter products, non-template addition, micro variants, null alleles, accuracy, and species specificity since these can pose challenges to accurate data interpretation. They also have unique needs to handle mixtures, degraded DNA samples, PCR inhibition, and contamination since forensic casework samples do not come from a pristine, controlled environment.

Recently, the Combined DNA Index System (CODIS) Core Loci working Group in the United States published a paper stating that the number of current CODIS loci are insufficient to accommodate goals of wider data sharing internationally (greater risk of adventitious matches) and that there is a need for additional information to improve success of obtaining a profile with challenging samples, such as missing person cases. Thus, it was recommended expanding the required minimal core loci for the database from 13 autosomal STR loci to 19, plus another sex identification marker using a Y-chromosome STR locus and three optional STR loci. This recommendation increases the potential number of markers in one multiplex assay to twenty-three.

In this presentation, an overview of the challenges typically encountered when developing highly multiplexed STR assays for human identification purposes to meet the needs of the forensic community will be presented. Examples of the problems encountered and how they were resolved will be covered. Some of the examples will cover primer design, PCR artifacts, species specificity, electrophoresis, dye artifacts, and intralocus balance.

STR, CE, PCR

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