B149 Electrospray-Ionization Mass Spectrometry for Exploitation of Sequence Variation in Human Short Tandem Repeats

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The goal of this presentation is to demonstrate the use of Electrospray-Ionization Mass Spectrometry (ESI-MS) to exploit sequence variation in human short tandem repeat loci (STRs). This information provides another dimension of discrimination between STR alleles sharing the same number of repeat units. Preliminary studies have successfully demonstrated correct allele assignments of common CODIS STR loci in multiplexed PCR reactions using DNA extracted from buccal and blood samples. A substantial number of variant alleles were observed that contain polymorphisms within alleles of the same length.

This presentation will impact the forensic community and/or humanity by providing an automated mechanism capable of screening a large collection of samples and derive maximal information from partial STR profiles.

In cases where nuclear DNA is damaged or limited, standard STR typing is often difficult and may yield partial profiles. Short STR loci offer an advantage over longer STR loci for PCR amplification in such cases due to increased likelihood that the shorter DNA stretch will be unbroken. Moreover, several of the common CODIS STR loci display polymorphisms between alleles of the same length that are not differentiated with the current standard STR typing technologies. In cases where a limited number or only short STR loci are efficiently amplified, or when comparing profiles of relatives across generations (such as a missing persons case), the ability to differentiate equal-length alleles with polymorphisms could provide the information to differentiate samples not discriminated by standard STR typing.

STR typing involves the PCR amplification of multiple short repetitive DNA units that display a collection of alleles in the human population that differ in the number of repeats. Typically, the products are analyzed in polyacrylamide gel or capillary electrophoresis using fluorescent detection methods. Different alleles for each locus are distinguished from each other based on PCR product length. Because multiple markers are utilized that are not genetically-linked, the product rule can be applied to estimate the probability of a random match to any STR profile where population allele frequencies have been characterized for each locus. This leads to extremely high levels of discrimination with potential random match probabilities of less than one in the human population. STR analysis has become the standard DNA analysis used in cases where sufficient nuclear DNA is obtainable to provide an STR profile.

In certain situations, such as mass disaster victim identification, a large number of samples with varying DNA quality can be produced, many of which may produce only partial STR profiles. In such cases, the ability to exploit polymorphisms in alleles can increase the observed allelic variation for several common STR loci. In addition, the development of an automated platform capable of high-throughput sample processing would provide a mechanism to process a large number of samples produced simultaneously or over a short time period, such as during a mass disaster or wartime. ESI-MS provides a platform capable of automated sample processing and analysis that will resolve polymorphisms between STR alleles. Accurate measurement of the mass of each product produced in a standard PCR reaction allows the determination of the base composition (number of A, G, C, and T bases) of each PCR product. This not only provides the length of each allele, but also identifies polymorphisms within alleles. Moreover, high- resolution characterization of “off-ladder” alleles that sometimes complicate standard STR analysis is an inherent part of the ESI-MS approach. Importantly, the base composition of each STR allele can be associated specifically to existing allelic nomenclature and can be further annotated with observed polymorphisms.

Preliminary studies have been performed using DNA extracted from buccal and blood samples. Multiplex PCR reaction sets have been developed that amplify nine core CODIS STR loci and the sex marker, Amelogenin. Correct allele assignments were confirmed by comparison of ESI-MS analysis results from blinded samples to truth data produced using standard STR typing techniques. A number of allelic variants that differ only in sequence polymorphisms were revealed. Moreover, several examples of heterozygous individuals were observed where standard STR typing labeled them homozygous because the alleles were the same length. The ESI-MS platform will provide an automated mechanism capable of screening a large collection of samples and derive maximal information from partial STR profiles.

Mass Spectrometry, Human Short Tandem Repeats, Base Composition Analysis

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