B157 Validation of a New and Improved Human Genotyping Multiplex Containing the 13 CODIS Loci, Amelogenin and Three Additional New Loci

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After attending this presentation, attendees will see the utility of using an alternative human genotyping multiplex, which is potentially cheaper and more efficient than commercially available kits. This new method uses locus-specific DNA molecules to bracket the true alleles. These act as both a size standard and as an allelic ladder, with each bracket containing DNA molecules that are one repeat shorter and one repeat longer than the amplified alleles. Therefore, when co- electrophoresed with the true alleles, their sizing has potential to be more accurate. The new kit includes the 13 CODIS loci, the gender- identification locus amelogenin as well as 3 new loci (SE33, D2S1772 and D7S3048).

This presentation will impact the forensic community and/or humanity by offering a cheaper alternative to human genotyping, and this is especially important with the backlog of DNA samples to be done and the financial burden on crime labs, as well as an alternative to costly commercial kits for paternity testing organizations.

Short tandem repeat (STR) characterization has established itself as the principal method for human forensic DNA typing. STRs are distributed throughout the human genome, and are useful because of their conformance to Hardy-Weinberg equilibriums, and have a relatively low mutation rate. Newer methods are being developed which may be advantageous, but because of the current database of convicted offenders which have been typed using the 13 CODIS (Combined DNA Index System) loci, a method which is improved over current ones but still uses the same loci is needed. Commercially available human identity and paternity kits such as Applied Biosystems Identifiler® or Promega's PowerPlex® are validated, but costly. In addition to being patent protected dye sets, they require an allelic ladder, which reduces the number of lanes or injections that can be used for samples, and an internal size standard, which increases the number of dyes required for a kit. A new kit, which uses locus-specific brackets (LSBs) to bracket the amplified alleles, are an improvement over existing kits. Because the internal size standard molecules can form a different secondary DNA structure, and carry a different dye than the sample alleles, their electrophoretic mobility responds differently from the sample alleles to a temperature shift. This can lead to a miscalled allele, especially with a microvariant. This new kit is less susceptible to this problem because it is sized using locus specific brackets, which serves as both a size standard and as an allelic ladder. Since each bracket is composed of DNA with the same repeat structure and dye as the locus of interest, it responds more like a sample allele to electrophoretic conditions.

A validation study was performed of an LSB multiplex kit including the 13 CODIS loci plus 3 additional STRs. DNA from blood and buccal swab contributed by 100 human subjects was amplified using the new kit and the Identifiler® multiplex. Amplified product was analyzed using Applied Biosystems 310, 3100-Avant, and 3130 genetic analyzers. There was complete agreement in the genotypes produced using either kit. In addition, the three new loci have been sequenced to verify their repeat number.

Human Identity Testing, Locus-Specific Brackets, DNA

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