A114 High Sensitivity Detection and Typing of Mixed Contributor DNA Samples Using Massively-Parallel Deep Amplicon Pyrosequencing

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After attending this presentation, attendees will be introduced to an emerging massively-parallel DNA sequencing technology that is capable of detecting, typing, and resolving mixed contributor samples of human DNA. The presentation reviews the basic methods of preparing and analyzing samples using the Roche 454 sequencing system protocol.

This presentation will impact the forensic science community by demonstrating how massively parrallel deep amplicon pyrosequencing can be used to resolve complex mixtures of human DNA. This technology can be used to resolve STR and mtDNA mixtures of one to five individuals at low template DNA concentrations.

The project tests the hypothesis that 454 deep sequencing of individual polymerase chain reaction (PCR) amplicons yields far higher sensitivity of detection for minor contributor's short tandem repeats (STRs) and mitochondrial DNA in a mixed sample than conventional methods based on gel electrophoresis and dye terminator sequencing. Introduction of such technology thus has the potential to profoundly impact human DNA forensics, particularly in cases where the DNA of interest (e.g., perpetrator) is present in a mixed sample well below the detection threshold of conventional methods.

The presented data demonstrate highly sensitive detection of low copy contributor STRs well below the 1-10% level, which is the approximate limit for gel electrophoresis. Dilutions of human DNA in mixtures from one to five different contributors at different relative concentrations were prepared for PCR amplification and subsequent analysis by the 454 system. In addition, samples were also prepared after DNA extraction from objects touched by multiple individuals. Deep amplicon sequencing with the 454 system requires that the PCR primers used for loci of interest contain special fusion sequences appended to their 5' primer ends. The project used "fusion primers" for eight loci drawn from the standard CODIS STRs used in routine forensic casework. Following PCR amplification, a process known as emulsion PCR is performed, where individual amplicons in the sample are attached to a bead (one per bead) and clonally amplified in micro-reactors in a massively-parallel fashion. Each of the beads is then loaded onto a glass picotiter plate, where at most one DNA-linked-bead is captured into each well. Flow-based pyrosequencing is then performed to determine ~500 bp of the sequence of DNA coupled to each bead. Each bead sequence correlates to one single molecule of DNA, and thus to one individual contributor. The ratios of the major to minor contributor sequence reads at each locus enables linkage of detected alleles across different loci to form the respective genotypes. A typical 454 sequencing run yields up to one million reads, thus offering minor contributors detection thresholds greater than one part per thousand. Up to 16 different samples can be analyzed simultaneously in a single run, with trade-off between time/cost vs. sensitivity.

The demonstrated detection sensitivity is well below conventional methods using off-the-shelf Roche 454 protocols. Reagent costs are modest per sample (less than $500.00) when used in a multiplexed fashion. While pyrosequencing is not error-free, with an error rate of

~0.005 per base read, the impact of these errors on tandem repeat analysis is minimized through modest bioinformatics analysis of sequences against all known alleles. The system also offers the potential to both detect and sequence new alleles in the course of routine forensic casework

and thus can be an important future tool in the broader forensics community.

DNA Mixture Analysis, Pyrosequencing, STR Analysis

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