B53 Single Molecule Forensic DNA Characterization With Laser-Induced Nanopore Heating

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After attending this presentation, attendees will recognize that pre-analysis sample characterization could be a useful tool in the forensic workflow. Attendees will be briefed on a novel method of DNA analysis using nanopore sensing and its potential applications to forensic science.

This presentation will impact the forensic science community by proposing a novel method for pre-analysis mixture deconvolution and promote more research toward that goal.

Forensic DNA analysis relies on techniques that can yield incomplete or very complex results, based on the number of people contributing to the sample. This creates a need to develop rapid screening methods that provide previews of the genotype(s) in the sample, especially given the impending costly and time-intensive high-throughput sequencing revolution of forensic human identification. Determining the number of amplified fragments in solution from a single amplified Short Tandem Repeat (STR) locus can indicate the number of individuals contributing to the evidence. A new approach has been identified that could provide this "preview genotype" of the sample, utilizing laser heating in conjunction with nanopore-based resistive pulse sensing to identify the number of differently sized DNA fragments in a given sample.

Nanopore sensing is a well-established technique that utilizes the Coulter-counting principle to detect single molecules. Briefly, an applied transmembrane voltage drives ionic current through an isolated pore and individual DNA molecules are driven into the pore, thus reducing the flow of current and giving rise to short-lived current blockades. The magnitude and duration of these blockades can be analyzed to infer information about the size of the DNA molecules. Nanopore-DNA studies showed that the time for a single stranded (ssDNA) molecule to move through an alpha hemolysin pore scales linearly with the number of bases. Given the short time that ssDNA spends moving through the pore (»2-3µs/base), it is difficult to distinguish between different, but similarly sized, DNA molecules.

This presentation will describe the efforts taken to address this issue through modification of the nanopore sensor with the addition of infrared laser heating and the study of double stranded DNA (dsDNA). Studies showed that heating the solution in and around a pore facilitates unzipping of the dsDNA and this process yields DNA pore transit times that scale exponentially with the length of the DNA molecules. This phenomenon was consistent over hundreds of replications. This suggests a way to clearly distinguish between current blockades from different-sized molecules, which could lead to a rapid and accurate technique for prioritizing samples for further forensic analysis. The results show that heat-induced residence time discrimination was enhanced over a range of DNA sizes up to 70nt. This discrimination also enables the relative proportion of different-sized DNA fragments in a binary mixture to be distinguished. Future efforts will expand these results to DNA sizes on the order of the commonly used forensic STR "mini" loci (60-100 nucleotides) with the goal of demonstrating mixture ratio analysis on samples containing alleles differing in size by 4nt-8nt.

Mixture Deconvolution, Nanopore, Forensic DNA

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