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Profiling DNA without PCR: A single molecule based approach

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In forensic casework, the DNA isolated from biological evidence is routinely amplified by Polymerase Chain Reaction (PCR) using commercially available STR kits which require ~1 nanogram (1,000 picograms) of template DNA. However, many samples collected from the crime scene and/or the victim, contain DNA that is degraded, low in copy number, or is contaminated from multiple donors. Consequently, stochastic artifacts including stutter, allele drop-out, allele drop-in, pull-up, and heterozygote peak imbalances are typically observed after PCR. The resulting STR profiles are therefore difficult to interpret. Although many forensic laboratories have begun implementing the use of mini STR kits for the amplification of degraded, low copy number (LCN) or touch DNA, 0.5 nanograms (500 picograms) of input DNA is still required. In our proposed study we avoid the PCR process, and its associated stochastic variation, by using well-established single molecule techniques that examine each DNA molecule individually in a sample. To do so, we attach an amino-modified DNA probe (containing the complete amount of STR repeats present at a particular locus) to a surface, add the complement and then label the double stranded DNA with a fluorescence marker. Alternatively, an amino-modified intercalator is tethered to a surface, followed by the STR repeat region and its complement. In both scenarios, the sample is genotyped by measuring the intensity of the fluorescence, which is proportional to the number of STR repeats present. It is important to note that this methodology is more time consuming and expensive than traditional PCR analysis but it should enable generation of an STR profile in high value cases, albeit at a lower resolution (±1 STR repeat), even where the DNA evidence is limited or compromised. In addition, we should be able to determine the number of contributors in a DNA mixture as well as genotype unidentified human remains.

Biography

Tanya M. Simms received her Ph.D. in Biology from Florida International University, Miami, FL, in April 2011. During her tenure at FIU she published a total of 8 articles, 6 of them as first author. She is currently a postdoctoral researcher in the Department of Physics & Astronomy at the University of Kansas, Lawrence, KS.

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