## B185 Single-Step Photobleaching: A PCR-Free Alternative to Typing Low-Copy Number Templates

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After attending this presentation, attendees will better understand photobleaching, a single molecule technique that allows examination of the individual DNA molecules (rather than ensemble measurements) in a sample. In addition, attendees will realize that this strategy is advantageous for samples with too few molecules for Polymerase Chain Reaction (PCR) and that photobleaching experiments are not subject to the stochastic variation that is typical of PCR, particularly when Low-Copy Number (LCN) templates are amplified.

This presentation will impact the forensic science community by educating them on alternative strategies in dealing with LCN templates. Specifically, the goal is to stress that single molecule techniques, such as photobleaching, examine individual DNA molecules in a sample rather than making ensemble measurements as with PCR. In addition, the presentation emphasizes that by attaching genomic DNA to a glass surface, the same individual DNA molecule can be repeatedly measured. Finally, the forensic science community should realize that by using this technique, which is non-destructive, it should be able to recover the sample after performing these experiments, making it available for traditional PCR analysis if need be.

STR typing and next generation sequencing technologies rely upon PCR-based methods for the exponential amplification of DNA markers; however, the PCR process is known to have its limitations with regard to DNA that is degraded or is low in copy number. While the latter results in stochastic variations that are difficult for forensic investigators to interpret, the former can prevent PCR amplification altogether, particularly when targeting larger-sized fragments. Therefore, in this study, these issues are avoided by completely bypassing the PCR process. Instead, well-established single molecule-based approaches, which are sensitive enough and have the resolution (within one nm) to examine individual DNA molecules within forensically challenged samples, are utilized. The single molecule techniques also provide the added benefit of being reproducible, as long as the DNA of interest is anchored to the glass surface.

Specifically, single-step photobleaching was performed, a single molecule method that enables determination of the number of STR repeats at the TPOX locus by directly counting the number of steps in the fluorescence signal as the dye molecules are destroyed over time by exposure to light. The DNA molecules utilized for these experiments were generated by annealing primers and fluorescently labeled eight bp oligonucleotides to various single-stranded DNA templates, consisting of 6, 8, 12, and 16 tetranucleotide repeats flanked by two primer binding regions. The annealed double-stranded DNA construct was ligated using a previously published DNA ligation protocol. Upon completion, the size of each of the ligated DNA templates was confirmed by Capillary Electrophoresis (CE) on a 3700*xl* genetic analyzer. Following CE, the ligated products were diluted to single molecule concentration (10 to 100 picomolar) and then immobilized onto separate coverslips that were functionalized with poly-l-lysine. Each coverslip was subsequently examined by (TIRF), a popular single molecule technique that illuminates only those molecules present at the water-glass interface, and monitored until all the dyes in the region of interest had been bleached. Using this approach, this study was able to visualize two, three, five, and seven distinct steps when imaging DNA templates consisting of 6, 8, 12, and 16 tetranucleotide repeats, respectively. To confirm these results, the ligation reactions were performed again using eight bp oligonucleotides tagged with five nm gold nanoparticles and the products imaged via Transmission Electron Microscopy (TEM). As with the TIRF approach, the TEM images also revealed clusters of two, three, five, and seven beads using the abovementioned template sequences. Altogether, this data illustrates that the combined ligation/TIRF approach is a promising mechanism for determining the number of STR repeats at a particular locus; however, further work is required to be able to covalently (versus electrostatic interactions) attach the DNA to the surface of the glass. In doing so, this research will be able to examine genomic DNA, melt it, wash off the complementary strand, and repeat the experiment a number of different times for consistency. Although this process will enable generation of a genetic profile in high-profile cases where the DNA evidence is limited or compromised, the resolution will be lower than with traditional STR typing technologies. Additionally, it is hoped that by refining the attachment strategies the genomic DNA will be recoverable from the coverslip, making it available for traditional PCR analysis if so desired.

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**PCR-Free, Low-Copy Number, Single Molecule**

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