A24 Improving Traditional Multiplex STR Amplification of Low Template DNA Samples With the Addition of Proofreading Enzymes

Carey P. Davis, BS, Lynzee A. Chelland, María José Illescas, BS, and Tracey Dawson Cruz, PhD, 1000 West Cary Street, PO Box 842012, Virginia Commonwealth University, Richmond, VA 23284*

After attending this presentation, attendees will become aware of alternate less expensive methods for improving STR profiles from low template DNA samples using proofreading enzymes in combination with Taq polymerase for multiplex STR amplification.

This presentation will impact the forensic community by potentially providing a faster, cheaper alternative method for generating STR profiles from low template and/or challenged biological evidence samples. This approach includes the addition of no new analytical steps and could be accomplished with very little additional cost to the laboratory.

Touch or trace DNA evidence, including fingerprints, saliva, hairs, and miniscule drops of blood and other bodily fluids are sometimes the only evidence found on a crime scene. This type of evidence can often contain less than 100 pg of DNA (~15 diploid cells or less) and is referred to as low template DNA evidence. Because of the limited quantity of DNA available, these types of samples can become difficult to analyze and interpret with traditional STR analysis, preventing the acquisition of a full or even strong partial profile. With <100pg of template DNA, stochastic effects often prevail, including allele dropout, inter- and intra- locus peak imbalance, and high stutter. To overcome these limitations, some researchers have investigated preamplification methods that include the addition of proofreading enzymes to the PCR cocktail. Proofreading enzymes have 3'-5' exonuclease activity, allowing them to correct bases that were misincorporated by the traditionally used *Taq* polymerase. Typically, the addition of an enzyme that has proofreading capability results in longer fragments, although the exonuclease activity reduces the overall processivity of the reaction. Previous studies have shown that combining these proofreading enzymes with *Taq* polymerase for preamplification is the best approach for increasing fragment length *and* STR genome coverage, without compromising the speed of the reaction. However, preamplification techniques, such as whole genome amplification (WGA), are often labor intensive and more costly than traditional STR analysis. Thus, this project will seek to determine if combining proofreading enzymes with *Taq* directly into the standard STR amplification reaction mixture will improve the fidelity of the reaction when little template DNA is available. This is vital for STR multiplex reactions because if longer products can be obtained, then the number of STR copies generated would increase, decreasing allele drop out and increasing the probability of obtaining a complete STR profile.

For this project, a series of STR amplifications will be conducted using input DNA quantities from 7.5pg – 100pg and various ratios of *Taq*: proofreading enzymes. Two enzyme combinations were tested including a *Taq*:*Deep Vent* combination and a mixture of Taq Gold and an unknown proprietary enzyme(s). These enzyme mixtures were used in place of Taq Gold for multiplex STR amplification. Resulting STR products were separated and analyzed via capillary electrophoresis. STR success was measured by percentage of alleles present, intra-locus heterozygous peak balance, and the occurrence of other stochastic effects. STR data obtained using proofreading polymerase combinations will be compared to data obtained using traditional STR amplification (with Taq Gold alone) as well as to STR data obtained using other methods designed for low template DNA analysis. STR data is being accumulated for analysis and all results will be presented and discussed.

Low Copy Number DNA, Deep Vent, Proofreading Enzymes

 ** Presenting Author*