A117 Effects of Degradation on Single-Source and Mixture Profiles Generated Using Traditional and Mini STRs

Jonathon S. Dunn, BS, Ruiyi Ren, MS, Robin W. Cotton, PhD, and Catherine M. Grgicak, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806B, Boston, MA 02118*

After attending this presentation, attendees will become aware of the effects of degradation on mixture interpretation when utilizing both traditional- and mini- STRs.

This presentation will impact the forensic science community by characterizing the way in which degraded samples amplify in mixtures. This will be accomplished by determining important mixture characteristics such as drop-out rates and heterozygous imbalance for degraded mixture samples.

Many samples submitted for DNA analysis from crime scenes are often degraded or contain mixtures from two or more individuals. Degradation of DNA in these samples presents a challenge as larger molecular weight STR markers are inefficiently amplified, if they're amplified at all, resulting in a loss of information when attempting to analyze and interpret samples of interest. Additionally, complexities associated with mixture interpretation can be further exacerbated when one or more of the contributors of a mixture exhibit degradation.

Commercially available multiplex amplification assays utilizing primers that amplify mini-STRs have been developed in order to gain information from degraded or inhibited samples that may otherwise be lost.

For example, the AmpFℓSTR MiniFiler™ kit allows for the amplification of the eight largest loci from the AmpFℓSTR® Identifiler® kit (plus the Amelogenin locus) and has been shown to increase the likelihood of obtaining genetic information from compromised samples.[1] The goal of this research is to evaluate the amplification success of both types of assays by comparing the MiniFiler™ and AmpFℓSTR® Identifiler® kits when amplifying both degraded and non-degraded single source samples and mixtures. The objectives of this work include: (1) comparing peak height ratios of heterozygous markers and drop-out rates of the single source samples between kits; (2) determining whether these

ratios are maintained in mixtures, and, (3) to examine whether the DNA within a mixture amplifies independently.

DNA from one male and one female was degraded by incubating the sample at 37°C for ten minutes in the presence of 15 units/ml of DNase I. The DNase I was then inactivated by adding EDTA and incubating at 75°C for ten minutes. Agarose gel electrophoresis with GelStar® staining was used to visualize the level of degradation which ranged from non- degraded (>1500 b.p.'s) to very degraded (<100 b.p.'s). Both the male and female samples were run as single-source samples in both the degraded and non-degraded forms in quadruplicate using both kits with targets of 2, 1, 0.5, 0.25 and 0.125 ng. Mixtures of the two samples where both were non-degraded and both were degraded were then run using each kit with female to male ratios (in ng) of 2:0.125, 2:2, 1:0.125, 1:1, 0.5:0.125, 0.5:0.5, 0.25:0.125, 0.25:0.25, and 0.125:0.125, respectively.

Finally, mixtures using the same female to male ratios (with the exception of the 2:2 ratio) were run using the degraded female sample and the non- degraded male sample. All mixture samples were also run in quadruplicate.

This represents a detailed study aimed at determining the effects of degradation on mixture interpretation for both traditional- and mini- STRs. Emphasis will be placed on determining whether drop-out rates, heterozygous imbalance and amplification anomalies increase with degradation, and if so what laboratory tools may be used to determine which downstream PCR-STR kit to utilize prior to amplification. **Reference:**

1. Mulero, Julio J., et al. "Development and Validation of the The AmpFℓSTR® MiniFiler™ PCR Amplification Kit: A MiniSTR Multiplex for the Analysis of Degraded and/or PCR Inhibited DNA." Journal of Forensic Sciences 53 (2008): 838-52.

DNA Mixtures, Degraded DNA, Mini-STRs

 ** Presenting Author*