Y16 Generating Artificially Degraded Human DNA in an Environmental Chamber

Natalie Rivera Cardenas, BSc, Arizona State University West Campus, Glendale, AZ 85306; Natalie Damaso, PhD, Oak Ridge Institute for Science and Education, FBI Laboratory, Woodbridge, VA 22191; Patrick Rydzak, PhD, Stafford, VA 22554; James M. Robertson, PhD, Reserve Support Unit, Quantico, VA 22135*

Learning Overview: After attending this presentation, attendees will better understand the mechanisms of DNA degradation on various substrates when exposed to environmental conditions, such as Ultraviolet (UV), humidity, and temperature over time and their effect on obtaining a full Short Tandem Repeat (STR) profile.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing experimental methods that can be used in preparing artificially degraded DNA over time.

Environmentally damaged samples contain either degraded DNA fragments or contain somewhat intact DNA with single-stranded nicks and DNA lesions that can hinder acquisition of a complete DNA profile. As a result of this, there is a need in forensic research to generate artificial degraded DNA samples that can be used to improve and test STR typing protocols.

Within the field of forensic science, the testing and analysis of DNA has become notably important and contributed in solving crimes over the years. However, the issue on whether or not it is possible to retrieve an accurate DNA profile from degraded blood left behind at a scene is a recurring concern in the criminal justice system. Often, DNA left at crime scenes is scarce and highly degraded due to various environmental exposure conditions, such as heat, humidity, and UV irradiation. Environmentally damaged DNA may result in failure of amplification, and samples may not have a sufficient number of loci, which are needed for match comparisons. Thus, the purpose of this project was to optimize a method to artificially degrade control DNA and blood stains on different substrates (i.e., jeans and cotton shirt) using controlled environmental conditions (i.e., temperature, humidity, UV). This was done to mimic a "casework type" degraded sample that can be later used to: (1) test novel, enhanced methods (e.g., extraction kits, enhanced polymerase or STR kits) for obtaining a complete STR profile; and (2) better understand the mechanisms of degradation on various substrates and conditions.

To accomplish this, either control DNA (e.g., 9947A) or blood was applied to 1" x 1" squares of cotton and jeans fabrics. Next, the fabric squares were placed in the Environmental Chamber (Q-Sun Xe-3 Test Chamber) under conditions to imitate an outdoor environment (i.e., 15 hours of 71% RH, 0.21–0.25 W/m^2, 35°C, followed by 9 hours of 60% RH, no UV, 35°C) and fabrics were recovered at various time points across 8 days. DNA extraction was performed for the blood samples using the QIAamp® DNA Investigator Kit and the EZ1™ Advanced XL instrument and all samples were quantified using Quantifiler® Trio for all of the time points to obtain the DNA concentration (ng/ul) and level of Degradation (DI). STR profiles were obtained using the Identifiler® Plus and the THERMO FISHER™ CE 3130XL to determine the level of degradation (e.g., ski slope effect, locus drop out, and decrease in Relative Fluorescent Units [RFU]) at the various time points.

Analysis Of Variance (ANOVA) and Tukey's post-hoc statistical analysis revealed evidence of the ski-slope effect (i.e., a decrease in the RFU of larger loci targets compared to smaller ones; p=0.001) and a significant decrease ($p<0.001$) in the concentration of DNA recovered from pant samples (avg 5.96±1.70) when compared to shirt samples (avg=8.71±1.95). Furthermore, significantly higher DNA concentrations were recovered from samples at the 72hr time point than samples at 192hr time point (p=0.003). However, no significant difference in the degradation index was found for any sample at any time. Overall, results illustrated a promising method that decreased DNA recovery and illustrated a ski-slope effect indicative of typical degraded DNA STR results.

DNA Degradation, STR Ski-Slope Effect, Environmental Chamber

*Presenting Author