## A21 Sequencing of Select Novel X Chromosomal Short Tandem Repeat Alleles

*Toni Marie Diegoli, MFS*, and Mike Coble, PhD, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Rockville, MD 20850*

The goal of this presentation is to describe allele sequencing results from a select set of short tandem repeat (STR) markers located on the X chromosome. The presence of new alleles and microvariants previously not observed in published population data will be presented. The attendee will also learn about the process of allele sequencing, from sequencing primer design to sample selection, and data analysis.

This presentation with impact the forensic community by presenting data illustrating both the sequences of the new alleles in comparison to published allele sequencing data as well as information on the prevalence of these novel alleles in the U.S. populations examined.

The multiplex detection and analysis of STR markers is a common tool used for genetic identity testing in the forensic setting. Numerous publications have characterized genetic markers located throughout the autosomes and male-specific Y chromosome. More recently, markers located on the X chromosome have emerged as additional tools in this forensic arsenal. X chromosomal STRs can be used to supplement traditional kinship testing due to their unique inheritance pattern and, correspondingly, the breadth of published literature on the subject has expanded greatly in recent years due to this increasing interest in their utility. Numerous X STR markers have been characterized and a variety of multiplexes proposed. Currently only one commercial kit for X chromosomal STRs is available, and it is in use predominately throughout Europe. The process of allele sequencing is a necessary part of this growth in available information because it is able to reveal the molecular basis for the variation seen in these markers and aid in understanding the observed STR results.

Because DNA templates encountered in the forensic setting, and at the Armed Forces DNA Identification Laboratory (AFDIL) specifically, are often degraded, amplicon size should be considered in selecting potential markers for use in the forensic laboratory. In such cases, shorter amplicon sizes are favored with the goal of recovering the maximum number of alleles. Here, two reduced amplicon size multiplex STR (or mini-STR) assays were developed to type a total of 14 markers for 800 U.S. population samples from the five major subgroups: African American, Asian, Western European Caucasian, Hispanic, and Native American. Through this databasing process, the authors noted several novel variants that were present in these population samples and not previously reported in the literature. In most cases, published primer sequences were used in these two multiplexes, but in two instances, alternative amplification primers were designed to achieve the smaller amplicon size desired. Sequencing of these new alleles was performed to verify concordance of the repeat structure with that of the published data obtained using larger amplicons. Additionally, the sequencing process was used to investigate the presence of potential microvariants observed in the data. For seven markers in particular – DXS9902, DXS7423, DXS7424, DXS7130, DXS7132, DXS7133, GATA31E08 – new alleles
and/or microvariants were confirmed.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

**Mini-STRs, X Chromosome, Allele Sequencing**

 ** Presenting Author*