B57 Development of Two Mini-X Chromosomal Short Tandem Repeat Multiplexes

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The goal of this presentation is to describe the development of two multiplex polymerase chain reaction (PCR) assays for the detection of fifteen different X chromosome short tandem repeat (STR) markers and the sex-determining region of the Y chromosome. How these assays might be utilized at the Armed Forces DNA Identification Laboratory (AFDIL) to aid in identity testing will be demonstrated. The attendee will also learn about developmental considerations necessary when working with reduced-size STR assays.

This presentation will impact the forensic community by introducing X-STR markers for identity testing, as well as providing a suitable context for their use.

The multiplex detection and analysis of STR markers is a common tool used for genetic identity testing in the forensic setting. Numerous published assays describe a number of potential markers located throughout the autosomes and male-specific Y chromosome. Commercial kits are available, allowing routine use in crime laboratories. Markers located on the X chromosome, however, have thus far been relatively rarely used in forensic identity testing within the United States. At AFDIL, kinship testing is routinely used to identify skeletal remains. In cases where maternal reference individuals are unavailable or where the unidentified individual has one of the most common mitochondrial DNA (mtDNA) haplotypes, mtDNA testing is insufficient for establishing human identity. Sufficient statistical power must then result from fewer, smaller STR loci or low copy number analyses. In such cases, markers on the X chromosome may provide additional information. This is especially true for situations where a daughter is utilized as the reference individual for her father.[1] Consequently, the selection of candidate X chromosomal markers and the development of these markers into mini-STR multiplexes offers the potential to supplement both traditional STR testing and mtDNA sequencing.

STR loci are chosen for forensic use based upon their discriminatory power, repeat size, and observed heterozygosity. Additionally, because DNA templates encountered in the forensic setting, and at AFDIL specifically, are often degraded, amplicon size should be considered in selecting potential markers. In such cases, shorter amplicon sizes are favored with the goal of recovering the maximum number of alleles. Each of these factors were taken into consideration in the design of the two multiplexes presented here; markers were selected to maintain high heterozygosity (>0.630) while limiting the largest amplicon size to under 180 base pairs. Another criterion for selecting candidate STR loci is pairwise linkage; in order to take advantage of the product rule in the calculation of random match statistics, markers should not be linked to one another. Marker selection restricted to a single chromosome, then, introduces an additional challenge because some loci will necessarily be linked. Based on a review of published X chromosome mapping studies[1], four linkage groups have been identified. The markers present in the two nine-plexes represent all four linkage groups, affording maximum discriminatory power.

In this presentation, data illustrating both marker selection and multiplex development for two nine-plex X chromosomal STR assays will be presented. So-called “X-plex 1” consists of markers DXS7424, DXS6789, DXS7130, DXS9902, DXS7423, DXS9895, GATA165B12, DXS101, and SRY; “X-plex 2” consists of markers DXS7133, GATA172D05, DXS8378, DXS7132, DXS6803, HPRTB, GATA31E08, DXS9902, and SRY. SRY and DXS9902 were included in both multiplexes for concordance and gender confirmation (SRY).

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.

Reference:

[1] R. Szibor, M. Krawczak, S. Hering, J. Edelmann, E. Kuhlisch, D. Krause, Use of X-linked markers for forensic purposes, *Int J Legal Med* 117 (2003) 67-74.

X Chromosome, Mini-STRs, Identity Testing

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