B184 Increasing Discrimination of Degraded DNA Using Quantifiler® Trio With the Ion Personal Genome Machine® Sequencer

Joseph P. Chang, BS, Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Allison Holt, PhD, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Narasimhan Rajagopalan, MS, Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Robert Lagace, BS, 850 Lincoln Centre Drive, Mail Stop 404-1, Foster City, CA 94404; Sharon Wootton, PhD, 850 Lincoln Centre Drive, Foster City, CA 94404; Sheri Olson, MS, Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, CA 94080; Nnamdi Ihuegbu, PhD, 180 Oyster Point Boulevard, Rm 258, South San Francisco, CA 94080; Reina Langit, MS, 850 Lincoln Centre Drive, Foster City, CA 94404; and Chien-Wei Chang, PhD, 850 Lincoln Centre Drive, MS 404-1, Foster City, CA 94404*

After attending this presentation, attendees will learn how Single Nucleotide Polymorphisms (SNPs) and Next Generation Sequencing (NGS) technologies can improve the analysis and identification of degraded DNA samples.

This presentation will impact the forensic science community by presenting a possible complementary solution on the use of NGS in degraded DNA casework analysis. Attendees will observe that the Probability of Identity (PI) values provided by partial STR profiles will be further augmented by PI values from SNPs.

Forensic casework analysis that involves degraded or compromised DNA samples in trace amounts often shows allele dropout at larger molecular weight loci, which results in partial profiles that may have limited or no probative value. It is beneficial to have accurate DNA quantification data to enable forensic analysts to critically evaluate how to select the appropriate downstream STR technologies in order to obtain the most probative results with challenging casework samples. At the threshold that STR kits cannot provide complete profiles anymore, forensically informative SNPs can provide the additional discriminatory power to complement partial or no STR profiles produced by Capillary Electrophoresis (CE). The HID-Ion AmpliSeq™ Panels were developed to provide an alternative and complementary approach to current human identification STR technologies, as well as utilize NGS high-multiplexing capabilities.

The HID-Ion AmpliSeq™ Identity Panel consists of 90 autosomal, of which 85 are unlinked, and 34 upper Y-clade markers. These Identity Informative SNPs (IISNPs) were selected due to their high heterozygosity and low population heterogeneity. Using an input of pristine DNA, the combined 85 unlinked IISNPs provide a random match probability of 10^{-33} in most parts of the world. The 34 upper Y-Clades were selected from the Y-Phylogenetic Tree.[1]

The HID-Ion AmpliSeq™ Ancestry Panel consists of 55 Kidd and 123 Seldin markers.[2,3] These Ancestry Informative SNPs (AISNPs) were selected to infer ancestry from the major eight global regions. These AISNPs are highly supported by Kidd's ALFRED database, which contains a considerable amount of allele frequency variation and population statistics.[4]

This study intends to evaluate data to determine how to proceed with sample processing for degraded DNA samples. Using a human male genomic DNA, PB001, varying degrees of degradation were produced with a combination of both mechanical and enzymatic shearing techniques. Then, utilizing the Quantifiler® Trio DNA Quantification Kit, a Degradation Index (DI) was produced to evaluate the integrity of the degraded PB001. The DI is a ratio of the Small Autosomal (SA) target concentration to the Large Autosomal (LA) target concentration in the stock. The SA locus is 80bp in length while the LA locus is 214bp in length. For four levels of PB001 degradation — none (control), low, medium, and high — the observed DIs were 0.9, 3.4, 37.3, and undefined, respectively. The DI for the highly degraded sample was undefined because there wasn't enough LA.

A correlation was observed between DI and probative value obtained from CE STR and Ion PGM™ System SNP results. Using the GlobalFiler® kit, the PIs for Africans, Asians, Caucasians, and Hispanics for the sample whose DI was 37.3, were $7.14x10^{-3}$, $1.01x10^{-2}$, $9.201x10^{-3}$, and $1.22x10^{-2}$, respectively. For the SNP panels for the same sample, the PIs for Global, Asian, Ad-Mixed American, African, and European were $2.21x10^{-14}$, $1.89x10^{-13}$, $2.41x10^{-14}$, $3.42x10^{-13}$, and $1.35x10^{-14}$, respectively. The control, low, and medially degraded samples did not lose any SNPs. A plot of PI vs. DI was prepared to illustrate the best downstream sample processing for samples identified with varying levels of degradation.

This work demonstrates that the HID-Ion SNPs offer complementary discrimination power to CE STR data for samples that would be considered "poor" quality for STR processing in a casework laboratory based upon their DI value.

References:

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Degraded DNA, Next Generation SNP Sequencing, Quantification