Annual Meeting
Association of Forensic DNA Analysts and Administrators

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Program

Analysis of DNA from Post-blast Pipe Bomb Fragments for Identification and Determination of Ancestry

Presenter: Esiri Tasker, Doctoral Student, Sam Houston State University

Challenges with Analyzing Complex DNA Mixtures Utilizing STRMIX™- From Casework to the Courtroom

Presenter: Rachel Oefelein, MSc, DNA Labs International

An Examination of Improved Methods for DNA Extraction of Skeletal Remains

Presenter: Joseph Warren, Ph.D, UNT Health Science Center

Abstract pending

Comparative Tolerance of Inhibited Samples for Short Tandem Repeat and Massively Parallel Sequencing Chemistries

Presenter: Kyleen Elwick, Sam Houston State University

How infectious are forensic specimens in sexual assault and rape cases? Safety and legal considerations.

Presenter: M. AL Salih, BVSC, MT (ASCP),MS,PhD, President, Medical Laboratory Director & Forensic Technical Leader, DNA REFERENCE LAB

Application of Massive Parallel Sequencing in Forensics Using Custom Panels including Markers Potentially Linked to Human Behavioral Traits

Presenter: Elizabeth Chesna, BS, EMT-B, President, Society of Forensic Science, Sam Houston State University
Tales from the Darkside: The DNA Defense Expert Perspective

**Presenter:** George Schiro, Scales Biological Laboratory, Inc.

FBI Quality Assurance Standards Updates

**Presenter:** Douglas Hares, PhD, NDIS Custodian, FBI Laboratory CODIS Unit

NDIS/CODIS Updates

**Presenter:** Douglas Hares, PhD, NDIS Custodian, FBI Laboratory CODIS Unit

DNA Legislation Update

**Presenter:** Lisa H. Hurst, Sr. Government Affairs Consultant, Gordon Thomas Honeywell

Texas CODIS Update

**Presenter:** Gary J. Molina, CODIS Program Manager, Texas Department of Public Safety Crime Laboratory Service - CODIS Lab

That’s not my gun! Case studies in firearms possession involving close relatives and probabilistic genotyping.

**Presenter:** Angela Tanzillo-Swarts, M.S, Forensic DNA Specialist, DNA Technical Leader, Cayman Islands Forensic Science Laboratory

Technical Note: The importance of Y-intercepts in Quantitative PCR

**Presenter:** Vanessa Nelson, PhD, F-ABC, Forensic Scientist - DNA, Texas DPS Crime Lab

Case Study: Canine Sperm

**Presenter:** Andrea Ormos, M.S., DNA, Forensic Scientist - DNA, Texas DPS Crime Lab
Abstracts and Summaries

Analysis of DNA from Post-blast Pipe Bomb Fragments for Identification and Determination of Ancestry

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Improvised explosive devices (IEDs) such as pipe bombs are weapons used to detrimentally affect people and communities. A readily accessible brand of exploding targets called Tannerite® has been identified as a potential material for abuse as an explosive in pipe bombs. The ability to recover and genotype DNA from such weapons may be vital in the effort to identify suspects associated with these devices. While it is possible to recover DNA from post-blast fragments using short tandem repeat markers (STRs), genotyping success can be negatively affected by low quantities of DNA, degradation, and/or PCR inhibitors. Alternative markers such as insertion/null (INNULs) and single nucleotide polymorphisms (SNPs) are bi-allelic genetic markers that are shorter genomic targets than STRs for amplification, which are more likely to resist degradation.

In this study, we constructed pipe bombs that were spiked with known amounts of biological material to: 1) recover "touch" DNA from the surface of the device, and 2) recover traces of blood from the ends of wires (simulated finger prick). The bombs were detonated with the binary explosive Tannerite® using double-base smokeless powder to initiate the reaction.

DNA extracted from the post-blast fragments was quantified with the Quantifiler® Trio DNA Quantification Kit. STR analysis was conducted using the GlobalFiler® Amplification Kit, INNULs were amplified using an early-access version of the InnoTyper™ 21 Kit, and SNP analysis via massively parallel sequencing (MPS) was performed using the HID-Ion Ampliseq™ Identity and Ancestry panels using the Ion Chef and Ion PGM sequencing system.

The results of this study showed that INNUL markers resulted in the most complete genetic profiles when compared to STR and SNP profiles. The random match probabilities calculated for samples using INNULs were lower than with STRs when less than 14 STR alleles were reported. These results suggest that INNUL analysis may be well suited for low-template and/or degraded DNA samples, and may be used to supplement incomplete or failed STR analysis. Human identification using SNP analysis via MPS showed variable success with low-level post-blast samples in this study (<150 µL). While neat DNA samples (6 µL input as recommended) resulted in <50% of SNP calls, samples that were concentrated from 15 µL to 6 µL (15 µL was added to the STR and INNUL typing) resulted in more complete SNP profiles. Five out of six blood samples recovered from the wires attached to the pipe bombs resulted in the correct ancestry predictions.
Challenges with Analyzing Complex DNA Mixtures Utilizing STRMIX™- From Casework to the Courtroom

**Presenter:** Rachel Oefelein, MSc, DNA Labs International

Forensic science is on the cusp of change across not only the United States but also the world as more and more laboratories transition to reporting DNA mixtures as a likelihood ratio (LR) utilizing probabilistic genotyping. Since January of 2016 DNA Labs International (DLI) has been reporting LRs from STRmix™ analyses in cases covering 22 jurisdictions in four countries. As time passes these cases are now making their way through the courtroom. Challenges encountered with adjustments to report writing, transitions to the expanded short tandem repeat (STR) commercial kits, courtroom testimony and Daubert/Frye challenges will be discussed. Additionally, several recent landmark cases regarding probabilistic genotyping and the PCAST report will be examined.
An Examination of Improved Methods for DNA Extraction of Skeletal Remains

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Presenter: Joseph Warren, Ph.D, UNT Health Science Center

DNA analysis is often an essential component in the identification of human skeletal remains recovered in missing person, mass disasters or unidentified human remain cases. Conventional DNA extraction methods were developed and optimized to remove DNA from bacteria, tissue or body fluid samples, not bone. DNA obtained from bone is typically more aged, has a lower yield and contains more PCR inhibitors than does DNA from body fluids or tissue samples.

Studies were performed to develop a DNA extraction procedure that was optimized to improve the yield and purity of DNA obtained from human bone. The two main areas of investigation were to determine if using the enzyme Collagenase Type II (CLII) as the primary digestive enzyme, or in conjunction the Proteinase K (Pro K) would increase the yield of the isolated DNA when compared to Pro K by itself.

The second study was to determine if the effects of an anionic detergent would increase the quality of an STR profile by improving the removal of PCR inhibitors. Differing concentrations of SDS, SLS, Triton X100 and Buffer ATL were compared and analyzed by using Peak Height Ratios (PHR) and the percent of alleles recovered. The data appears to indicate that a combination of Collagenase Type II and 1% SDS increases PHR from full STR profiles obtained from bone samples.
Comparative Tolerance of Inhibited Samples for Short Tandem Repeat and Massively Parallel Sequencing Chemistries

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Presenter: Kyleen Elwick,

Victim identification is one of the most important goals after a mass disaster event or in a missing persons case. Often times these human remains are very challenging samples to identify as they may be highly degraded and fragmented, burnt, decomposed, or containing inhibitory substances. CE-based STR markers and mitochondrial analyses are traditionally used for DNA identification, but MPS has more recently emerged as an alternative approach for identifying human remains. The purpose of this study is to compare the tolerance of a commercial STR kit and a MPS-based system to common inhibitors that are frequently encountered in skeletal and decomposed tissue samples requiring identification in forensic and missing persons’ casework.

DNA (1ng and 0.1 ng) was spiked with various concentrations of five inhibitors (humic acid, melanin, hematin, collagen, calcium). Samples (N = 150) were amplified with the GlobalFiler® PCR Amplification Kit (Thermo Fisher Scientific) and genotyped on the ABI 3500 Genetic Analyzer (Applied Biosystems). A subset of samples (N = 25) was also sequenced using the HID-Ion AmpliSeq™ Identity Panel (Thermo Fisher Scientific) with the Ion PGM™ (Thermo Fisher Scientific).

In general, STR results showed a decrease in the number of alleles being amplified and detected as inhibitor concentrations increased. As expected, the average peak height and average heterozygote peak height ratios showed a decreasing trend as inhibitor concentration increased. Samples with 0.1 ng DNA input resulted in considerably poorer STR profiles than 1 ng DNA samples at all inhibitor concentrations, suggesting that samples amplified with less DNA template are more susceptible to the effect of PCR inhibition.

MPS sequencing results suggest that the HID-Ion AmpliSeq™ Identity chemistry may not be as tolerant to PCR inhibitors as STR amplification kits. Samples with the same inhibitor concentrations generated considerably worse results via MPS. The lowest inhibitor concentrations for humic acid, melanin, and hematin resulted in complete STR profiles but performed poorly when the samples were sequenced via MPS. However, the highest inhibitor concentrations for collagen and calcium resulted in poor STR profiles but performed well with MPS resulting in complete SNP profiles.

Data also showed that when highly inhibited samples were pooled for library amplification and loading onto the same chip for sequencing, carry-over inhibition from highly inhibited samples affected the performance of samples with little or no inhibition. In fact, when samples with the highest amounts of inhibitor were processed, library amplification failed completely. Overall, the chemistry in commercial STR kits is more tolerant to most of the common inhibitors found in biological samples than the MPS sequencing chemistry tested in this study.
Application of Massive Parallel Sequencing in Forensics Using Custom Panels including Markers Potentially Linked to Human Behavioral Traits

Elizabeth Chesna, BS, EMT-B, Sam Houston State University

Massive parallel sequencing (MPS) is a new technology that provides an opportunity to analyze a large number markers simultaneously. A custom primer panel can be comprehensively designed in order to include markers (in genes of receptors, transporters, or metabolic enzymes) targeted for a specific analysis. While this technology has previously been used to analyze ancestry and potential for genetic diseases and cancer, this study aims to use MPS to determine possible associations between single nucleotide polymorphisms (SNPs) and behavior.

Imbalanced levels of OXT and 5-HT are known to correlate with social behavior. Furthermore, the expression of specific alleles may be related to the regulation of these neurotransmitters. Previous studies have shown that various SNPs located within genes associated with OXT, 5-HT and their receptors, transporters, and related metabolic enzymes correlate with behavioral traits. Understanding the influence of OXT and 5-HT on behavior may help explain the etiology of social behaviors including aggressive and antisocial behavior. This study analyzed two single nucleotide polymorphisms (SNPs) located within the intron region of OXT gene (rs877172 and rs4813625) and three SNPs located within the serotonin transporter gene (5-HTT) (rs25531, rs6314 exonic, and rs6311) using single base extension (SBE) and MPS with a custom designed panel of SNPs linked or related to genes of neurotransmitters. A student sample set (N=100) was genotyped and individuals participated in a survey designed to assess behavioral traits. Two SNPs linked to OXT (rs877172 and rs4813625) were analyzed using SBE and MPS in order to compare methods. It was found that for all samples, the alleles called were 100% concordant indicating MPS with this custom panel was comparable to the robust conventional SBE method. Furthermore, these results indicate that a custom panel may be used to assess a large panel of behavioral markers at once. It was also found that OXT and 5-HT may have an impact on social behavior. Statistically significant associations were found between two SNPs (rs25531 and rs877172) and behavioral traits including antisocial behavior, drug use/distribution, and property crimes.