Program Abstracts

Plenary Session, Monday, 9:30 am - 10:50 am

R&D Deliverables for the Crime Laboratory

Duplex qPCR Assay for Quantifying Nuclear and Mitochondrial DNA in Forensic Samples: Implementation in the California DOJ Missing Persons DNA Program

Mark D. Timken, Martin R. Buoncristiani, Katie L. Swango

A duplex real-time qPCR assay for the simultaneous quantification of human nuclear and mitochondrial DNA in forensic samples was developed and validated for implementation in the California DOJ Missing Persons DNA Program (MPDP). This assay was designed to be of general utility for forensic DNA quantifications, but to be particularly useful for the analysis of samples that contain highly degraded DNA. Because the quality and/or quantity of extracted nuclear DNA in degraded samples can preclude successful short tandem repeat (STR) genotyping (resulting in partial or no STR profiles), such samples can require analysis by less discriminatory typing methods based on polymorphisms in hypervariable subregions I and II (HVI and HVII) of the human mitochondrial control region. The nuclear-mitochondrial duplex qPCR assay provides a tool to increase the efficiency of DNA analysis of these challenging samples. That is, a reliable estimate of the amounts of human nuclear and mitochondrial DNA aids in the selection of an optimal typing approach at the outset of analysis, leading directly to optimal genotyping or haplotyping results and to a concomitant savings in time, labor, reagent/kit costs and extracted DNA. The project's duplex qPCR assay, validated for use on the ABI 7000 SDS instrument, uses 5'-hydrolysis detection chemistry to amplify and detect a nominal 180bp nuclear target sequence that spans the TH01 CODIS STR locus, as well as a 69bp target sequence in the ND1 region of the human mitochondrial genome. Practical issues (for example, training, QA/QC, workflow) surrounding the implementation of this assay in the California Department of Justice MPDP will be discussed.

Applying Real-Time PCR DNA Quantitation to Casework Analysis

Eric Buel, Janice A. Nicklas

Determining the amount of human DNA extracted from a crime scene sample is an important step in DNA profiling. The forensic community is quickly turning to real-time PCR for the quantitation of DNA samples, abandoning the slot blot technique as the method of choice.

At previous NIJ meetings, presentations described methods for the quantitation of human DNA using real-time PCR instrumentation based on PCR amplification of a repetitive Alu sequence. Project staff has used this method for casework analysis for over a year and have found the method to be fast, sensitive, and able to yield reliable estimates of the amount of DNA required for useful STR profiles. The success with this method including some of casework results will be discussed. Researchers are also working on new assays that extend the linear

quantitation range for human DNA and a duplex assay to obtain both total human and male DNA in one assay. Other possible uses for real-time PCR assays that could streamline DNA analysis include screening methods to evaluate DNA quality and source. Outlines of these new assays will be discussed.

mtDNA Genome SNPs for Increased Forensic Discrimination in U.S. Caucasians, African Americans, and Hispanics

Thomas J. Parsons

In this NIJ-funded project, whole mtDNA genome sequencing has permitted identification of eight multiplex panels of SNP sites over the entire mtDNA genome that can aid in increasing the resolving power of mtDNA typing for U.S. Caucasians. Similar work is underway for identifying such sites for African Americans and Hispanics. Presenters will discuss the completion of whole mtDNA genome sequencing and will present panels of identification of SNP sites that offer more forensic discrimination of mtDNA within U.S. Hispanics and African Americans. Multiplex primer extension assays for the various panels will be presented, together with summary information on forensic applications and a discussion of the increased forensic utility of this approach.

Forensic mtDNA Mixture Fractionation by Denaturing High-Performance Liquid Chromatography

Phillip B. Danielson, Richard Kristinsson, Greggory S. LaBerge

Mitochondrial DNA (mtDNA) sequencing can provide crucial information to forensic investigators when the quantity and quality of DNA would otherwise be limiting. The difficulty of analyzing mtDNA mixtures, however, has been a significant obstacle to its broader use in forensics. The reason for this is that unlike short tandem repeats, mtDNA amplicons are identical or nearly identical in length and thus must be characterized on the basis of nucleotide sequence. What complicates the analyses of DNA mixtures is that the sequencing electropherogram with a mixture will contain multiple mixed base positions or regions of sequence that are out of register (and thus unreadable) due to subtle length polymorphisms.

Denaturing High-Performance Liquid Chromatography (DHPLC) in combination with linkage phase analysis is a promising approach for rapid, low-cost fractionation and analysis of DNA mixtures without secondary amplification or excessive sample manipulation. Using the same standard operating procedures that have been validated for use in forensic laboratories, this approach enables sequence-specific fractionation of natural (heteroplasmic) or situational (multi-contributor) DNA mixtures prior direct to sequencing. The robustness of the approach has been demonstrated using a population over 1200 mixtures of 88 distinct mitotypes. Collectively, these sequences represent 85 of 246 known variant sites in HV1 and 59 of 160 known variant sites in HV2. Based on the strong correlation between a change in DNA quantity vs. a change in electrophoretic peak height, as little as a 20 percent enrichment for either contributor to a mixture by DHPLC may make it possible to accurately determine the linkage phase and thus the true mitotypes in a mixture.

Finally, the capability of DHPLC to simultaneously quantify and purify DNA makes it possible to streamline the processing of all mtDNA samples. This will save time and money by eliminating the need for separate yield gels or PCR clean up steps. Thus, DHPLC makes it

possible for analysts to obtain conclusive mitochondrial DNA results from mixtures that would otherwise not be amenable to analysis by direct sequencing; thereby aiding criminal investigators.

Plenary Session, Monday, 11:10 am - 12:10 pm

R&D Deliverables for the Crime Laboratory, Continued

Miniplex Primer Sets for the Analysis of Degraded DNA

Kerry L. Opel, Bruce McCord

The goal of this project is to develop novel STR multiplexes with reduced amplicon sizes for the analysis of degraded and contaminated DNA. In such samples DNA template can become highly fragmented and the possibility of finding an intact target sequence for PCR is greatly reduced.

Project researchers are developing and validating the use of Miniplexes, STRs in which the primer sequences are moved as close as possible to the repeat region. A set of four Miniplex kits has been developed covering 12 of the 13 CODIS loci. In a series of studies with enzymatically degraded DNA we have demonstrated that reduced size STRs improve the success rate with degraded template. Investigators have also applied these primers in the analysis of human skeletal remains from variety of environmental conditions. In all cases, allele dropout is minimized and amplification efficiency improves. In a study of 532 samples, only 16 discrepant alleles were found, producing 99.77 percent concordance. Several of these samples were sequenced, and the results show effects due to deletions or substitutions at the primer-binding site.

Validation of four of the Miniplex kits for use on routine samples is currently under way. In these studies, tests have been performed to evaluate amplification performance including reaction volume, template concentration, Taq polymerase and magnesium concentration, cycle number and annealing temperature. These studies have been used to determine the optimal conditions for degraded DNA analysis. In addition, we have documented the effect of environment factors such as template length, inhibitor concentration, and sample matrix on amplification efficiency and peak balance. Overall our results show the Miniplex amplifications to be a robust and sensitive procedure for DNA typing of degraded samples.

Genetic Database of Y Chromosome Markers for Forensic Analyses

Michael F. Hammer, Alan J. Redd, et al

To increase the forensic utility of human Y chromosome markers, this project identified and characterized 14 novel short tandem repeats (Y-STRs) and compared levels of variability with more than 20 commonly used Y-STRs in samples from different U.S. population groups. In our European-American sample, 74 percent of Y chromosomes were distinct using the minimal haplotype Y-STRs (DYS19-DYS385-DYS389I-DYS389(II-I)-DYS390-DYS391-DYS392-DYS393). The two most frequent haplotypes were 14-(11, 14)-13-16-23-11-13-13 (5.1%) and 14-(11, 14)-13-16-24-11-13-13 (4.4%). The latter haplotype is the most common haplotype in a large European database, found in 3.1 percent of 9972 individuals. One of the new markers alone (DYS464) distinguished 75 percent of these haplotypes in the European-American sample.

The remaining shared haplotypes were distinguished using only four additional Y-STRs (DYS 449, DYS458, DYS456, DYS447). Thus, these European-American Y chromosomes are not identical by descent, as the minimal haplotype suggested. To address the question of how differentiated U.S. population groups are with respct to Y-STRs, researchers determined the within- and among-group components of variance in four U.S. ethnic groups sampled from three locations in the U.S. (African Americans from Arizona, South Dakota, and Virginia; European Americans from Arizona, South Dakota, and Virginia; Hispanics from Arizona and South Dakota; Native Americans from Arizona and South Dakota). Analysts found that samples of the same ethnic group from different geographic regions were not statistically different, while different ethnic groups from the same region were highly differentiated. These results suggest that population specific databases should be built to test for heterogeneity in structure and in admixture proportions among U.S. ethnic groups. In sum, we found that the addition of the novel Y-STRs to the minimal haplotype greatly improved the ability to distinguish Y chromosomes in all tested U.S. population samples, and provided evidence for significant population structure among U.S. population groups. The project goal is to develop a kit comprised of the minimal number of Y-STRs to distinguish the maximum number of Y chromosomes in all U.S. population groups.

SpermPaints: Fluorescent Monoclonal Antibody Probes to Sperm Differentiation Antigens – Application in Sexual Assault Analysis

John C. Herr

The definitive microscopic identification of sperm is of critical importance in several clinical contexts. These include (1) the forensic analysis of sexual assault evidence to corroborate victim testimony; (2) semen analysis in cases of suspected male infertility or post-vasectomy; (3) post-coital testing of cervical mucus for assessing fertility in the infertility clinic; (4) determination of barrier integrity in contraceptive efficacy studies of diaphragms, caps or prophylactics; and (5) to monitor subject compliance in studies of contraceptives and microbicides, the latter of particular importance in HIV prevention. Collection of semen samples in many of the aforementioned cases is usually performed with a cotton swab, which is subsequently dried, cellular materials then eluted, and a microscope smear created.

If sperm are abundant a definitive identification is easily made based on the characteristic morphology of an intact sperm head with attached flagella. However, with increased time after coitus or sexual assault the sperm heads may separate from their tails and become difficult to distinguish from other cell types while the headless tails resemble fibers or debris. Another confounding variable is adherence to other cell types, often vaginal epithelial cells, which masks the presence of the sperm. With current methods, forensic practitioners may spend hours searching for a single intact sperm.

SpermPaints is a mixture of fluorescent dye conjugated monoclonal antibodies that allows definitive identification of human sperm using fluorescence microscopy. SpermPaints contains: (1) a monoclonal antibody directed to the sperm head antigen, equatorial segment protein [ESP] and (2) a monoclonal antibody directed to the sperm flagellar antigen, calcium binding tyrosine phosphorylated protein [CABYR]. Together, this mixture of monoclonal antibodies stains the sperm head with a characteristic band pattern through its central region corresponding to the equatorial segment and stains the majority of the sperm tail corresponding to the principal segment.

SpermPaints is anticipated to have several advantages for identifying sperm in forensic casework and post-coital testing. The ESP and CABYR target proteins are unique to sperm and testis, being differentiation antigens that arise during spermiogenesis. Due to this property, the biomarkers result in clear, bright, and selective fluorescent signals from both monoclonal antibodies for sperm heads and tails with no cross reactivity to other tissues. The target antigens ESP and CABYR are stable: SpermPaints has identified sperm in samples collected 1, 24 and 72 hours after coitus and from swabs that were stored at 4°C for two years. With these performance characteristics, SpermPaints can identify sperm in microscopic fields more quickly and conclusively, benefiting clinical and forensic settings.

Working Lunch, Monday, 12:30 pm - 2:00 pm

Objectively Proving the Value of Forensic DNA to Justify Additional Resources

Ray Wickenheiser

Using crime scene DNA coupled with a large DNA database to identify perpetrators has an immense potential positive impact on public safety and the protection of individual's rights. Elimination of innocent suspects prevents the violation of the rights of wrongfully accused persons. Recidivists repeat their crimes on new victims, often with increasing frequency and severity. Timely and objective identification of perpetrators permits early intervention, prevents future victims, and increases public safety.

Objective value comparison enables decisionmakers to judge competing interests and make better, more justifiable decisions. Calculations have been made using U.S. national sexual assault statistics to evaluate the potential cost impact of increasing the investment in forensic DNA to solve and prevent crime. Prior to the advent of forensic DNA, crimes with unknown suspects, such as sexual assaults committed by a stranger to the victim, have been very difficult if not impossible to solve. Crime laboratories historically see a small percentage of reported sexual assaults, including those committed by strangers. A two-pronged approach including the broad application of crime scene DNA to a large known offender DNA database has demonstrated the potential to solve and prevent many crimes of sexual assault. Analyzing all of the 366,460 sexual assaults reported annually in the U.S. costs an estimated $366 million. This represents an increase of $310 million over the estimated current spending level. Estimated savings resulting from apprehending serial offenders early in their "careers," and thereby preventing future crimes, is $12.9 billion. This figure represents cost of sexual assault to the victim alone and does not include investigative, legal, justice system, and preventative savings associated with the prevented sexual assaults. The potential cost benefit on additional investment in forensic DNA is over $35 for each $1 spent.

Actual forensic case results were used to verify the theoretical savings potential of using forensic DNA. A recent cold case study conducted at the Acadiana Crime Lab has demonstrated a $31 return in cost-of-crime saving for each $1 of investment in crime scene DNA. Of 317 no-suspect sexual assault cold cases, 77 were matched to known individuals in the NDIS (National DNA Index System) DNA database. Furthermore, as the size of the Louisiana DNA database grew, more hits were generated, increasing the return to $33 for each $1 invested to increase the size of the database.

A comparison with other forms of social spending, such as AIDS research, cancer research, and heart disease and stroke research, demonstrates the high relative worth of an

additional investment in forensic use of DNA. A straightforward forensic DNA case costs $1000, compared with annual per-incidence spending of $68,998 (AIDS), $3524 (cancer), and $3792 (heart and stroke) for research on the respective condition. With an increased investment in crime scene DNA and an expanded U.S. DNA database, an estimated 40 percent of future sexual assaults by strangers could be prevented by earlier apprehension of serial offenders.

Design of an optimal justice system, to reap the benefits of this 3000-plus percent return in cost-of-crime savings, by incorporating DNA technology, requires accurate forecasting rates for crime relative to crime lab capacity and application of forensic DNA crime-solving tools. An optimal forensic laboratory model, independent of law enforcement, offers improved accountability to funding initiatives, avoids potential prosecutorial bias, and improves stewardship of the forensic sciences.

Plenary Session, Monday, 2:00 pm - 3:30 pm

Tools for Researchers and Practitioners

The Forensic Resource Network (FRN)

John Paul Jones

The Forensic Resource Network (FRN) was created by the National Institute of Justice as a mechanism to increase the capabilities and capacities of state and local forensic laboratories. The FRN includes:

- Marshall University Forensic Science Center, Huntington, West Virginia
- National Center for Forensic Science, Orlando, Florida
- National Forensic Science Technology Center, Largo, Florida
- West Virginia University Forensic Science Initiative, Morgantown, West Virginia

The FRN supports state and local crime laboratories in their efforts to establish and maintain the level of quality services demanded by the criminal justice system and the public. It provides innovative solutions to challenges facing the forensic science community though the delivery of technology based training tools, aid in systems support, and quality assurance products. This presentation offers information on the FRN members and the no-cost products and services they have developed for state and local forensic laboratories. Discussion highlights FRN successes in meeting the technology and training needs of the community and the development of model programs that can be transferred to state and local crime laboratories.

National Institute of Justice Expert Systems Test Bed Part I: Overview and Goals

Bridget Tincher

The National Institute of Justice's (NIJ) Convicted Offender DNA Backlog Reduction Program has made tremendous progress by outsourcing convicted offender samples, implementation of single amplification kits, and use of higher throughput instrumentation. However, bottlenecks for input of these profiles into the National DNA Index System (NDIS) are now occurring through the required technical review of data. Currently, for the submission of convicted offender profiles into NDIS, each sample profile must have two independent technical reviews and be approved by the state's administrator. Expert systems have been developed that

may address this bottleneck. The goal of the NIJ Expert Systems Program is to reduce the backlog in the review of data from convicted offender samples and to ensure timely submission to NDIS.

NIJ's program, or test bed, is designed to be an evaluation and thorough assessment of commercially available software programs to assist the forensic databasing community with the required review of the data. The objectives of the test bed are to evaluate expert systems as a mechanism for rapid, accurate technical review of convicted offender single-source samples; to hold workshops and training sessions for different systems; and to summarize features and limitations of each software package so that forensic scientists can make informed decisions on the purchase of such systems. The evaluation of various software packages is currently underway. Today's presentation will discuss the goals of the test bed program, the criteria for evaluation of the expert systems, the proposed timeline, and progress made to date.

Grant Progress Assessment (GPA) Program

Mark S. Nelson

The Grant Progress Assessment (GPA) program is a new service of the Forensic Resource Network (FRN) to the forensic community. This program will be delivered to the forensic community via the Free DNA Audit Program that NFSTC has been offering since 2002. The GPA program is designed to assess grantee progress in meeting program goals and objectives, to identify challenges faced by the grantee in achieving program objectives, and to identify successful programs. This program is also designed to assess vendor laboratory compliance with convicted offender delivery orders, to assess the impact of the grant funding, and to strengthen NIJ program management and oversight. The presentation will provide information on accomplishments of the GPA/DNA program to date, future plans, and deliverables. Information will also be provided on the personnel resources NFSTC has to staff these assessments, how to prepare for a GPA and/or DNA assessment, and resource information available to the community on the NFSTC website.

Plenary Session, Monday, 3:50 pm - 4:30 pm

Tools for Researchers and Practitioners, Continued

Development of a Speedy Rape Kit Screening Method

Jack Ballantyne, Ashley Hall

Every two minutes, someone in America is the victim of sexual assault. As recognized by the U.S. Department of Justice, forensic DNA evidence plays a critical role in the resolution of many of these cases. The current backlog of unexamined rape kits is estimated to be 180,000 but the true number may be as high as 500,000. This is a significant public health issue. Women are being raped and much of the evidence is either not examined or not examined in a timely manner. As a result, many rapists who may otherwise be identified by DNA and stopped are able to perpetrate additional crimes.

As one approach to reducing the backlog, project researchers have developed a speedy rape kit screening method. Briefly, a minute portion of the rape kit swab is cut and is subjected to a simplified nucleic acid extraction protocol, resulting in an admixed male/female sample.

The extract is then analyzed for the presence of Y-chromosome DNA using a real-time PCR assay or Y-STR haplotyping. Researchers have analyzed cervico-vaginal samples ranging from 0 hours to 4.5 days post-coitus using both our direct lysis procedure and a standard differential organic protocol, allowing us to assess accuracy of the former in predicting typing success in the latter. We were able to obtain both a positive real-time result and a Y-STR haplotype from the direct lysis extracts up to four days post-coitus. Analysis with an autosomal multiplex subsequent to an organic extraction proved to be less sensitive, with a male profile obtainable only up to 24 hours, but in each of these cases, a positive direct lysis result was predictive of typing success.

As an extension of this method, the project is developing a procedure for the rapid and direct co-extraction of both RNA and DNA. Nucleic acid extracts will be analyzed using a combination of methods that yield valuable information including the type of body fluid(s) present, the absolute quantity and relative proportion of male DNA in the sample, and the Y-chromosome haplotype. With these procedures, significant numbers of sexual assault swabs can be screened within six hours for processing via the standard autosomal STR analysis pipeline.

DNA.gov: The Face of the President's Initiative

Lee Mockensturm

DNA.gov is the official Website of the Presidential Initiative—Advancing Justice Through DNA Technology. Offering training, providing easy access to information, and allowing input and interaction from a variety of users and disciplines, the site helps the National Institute of Justice (NIJ) and the Initiative partners meet the goals of the Initiative. In this presentation, Mr. Mockensturm will give a brief overview of the site and coming improvements, including how NIJ is gathering input from real users and implementing open-source, Web-based tools that empower non-technical individuals to easily create, acquire, share, and control content in real-time.

Plenary Session, Tuesday, 8:30 am - 10:10 am

R&D New Projects

Development of Laser Microdissection to Separate Spermatozoa From Epithelial Cells for STR Analysis

Christine T. Sanders, Daniel A. Peterson

Short Tandem Repeat (STR) analysis has become a valuable tool in identifying the source of biological stains, particularly from the investigation of sexual assault crimes. Difficulties in analysis arise primarily in the interpretation of mixed genotypes when cell separation of the sexual assailant's sperm from the victim's cells is incomplete. The forensic community continues to seek improvements in cell separation methods from mixtures for DNA typing. The presentation describes the use of laser microdissection (LMD) for the separation of pure populations of spermatozoa from two-donor cell mixtures. In this study, cell separation was demonstrated by microscopic identification of stained sperm and female oral epithelial cell mixtures, and STR analysis of DNA obtained from the separated sperm cells. Clear profiles of the male donor were obtained with the absence of any additional alleles from the female donor. Five histological stains were evaluated for use with LMD and DNA analysis: hematoxylin/eosin, nuclear fast red/picroindigocarmine, methyl green, Wright's stain, and acridine orange. Hematoxylin/eosin out-performed all other stains however nuclear fast red/picroindigocarmine could be used satisfactorily with STR analysis. In addition, three DNA isolation methods were evaluated for LMD collected cells: QIAampÒ (Qiagen), microLYSISÒ (Microzone Ltd.) and Lyse-N-GoÔ (Pierce Chemical Co.). MicroLYSIS was not effective, yielding low levels of PCR product. Lyse-N-Go performed best when used for sperm cells, however, QIAamp performed best for epithelial cells. This research shows that LMD is an effective, low-manipulation separation method that enables the recovery of sperm while excluding epithelial cell DNA.

Development of an Automated System to Detect Spermatozoa on Laboratory Slides to Increase Productivity in the Analysis of Sexual Assault Cases

Eric Buel, Janice A. Nicklas

The analysis of a sexual assault case by the forensic laboratory is a multi-step procedure. One step in this process is an often lengthy microscopic examination of slides of vaginal smears or smears from other crime scene evidence to determine the presence or absence of spermatozoa. This manual search for sperm can take considerable time depending upon the nature of the slide. Since the identification of sperm and the number available is a good indicator of the potential success of a subsequent STR analysis, there is need to develop a procedure that allows a swift analysis of these slides. This project explores the possibility of using off-the-shelf hardware and modifying existing software to develop an automated sperm searching system. The system would allow the analyst to load multiple slides into the device for un-attended analysis. Verification of the computer-identified spermatozoa could be performed through the inspection of captured images or through a computer directed review of the slides.

Developing an SNP Panel for Forensic Identification

Kenneth K. Kidd

There are clear advantages to using SNPs for forensic identification because the typing can be automated. However, compared to STRPs, each SNP is much less informative. The allele frequencies can show greater variation, making the statistical evaluation of a match much less certain. To overcome this problem, researchers are working to identify SNPs with high average heterozygosity and low allele frequency variation among populations. The project uses a three-stage screening strategy starting with SNPs that meet criteria based on three populations (European, African American, and East Asian), testing those markers on seven populations, keeping those that continue to meet criteria and testing those that are kept on 40+ populations. To date only about 10 percent of the initial SNPs continue to meet criteria when typed on the 40+ population sample. The markers identified to date and their statistics will be presented.

Pairwise Relatedness Estimation: Accounting for Evolutionary Effects

Amanda Hepler

The amount of relatedness between two individuals has been widely studied across disciplines. There are several cases in which accurate estimates of this quantity are important in the forensic arena. Perhaps the most common application is in the area of remains identification. In addition, there are several scenarios in which pair-wise relatedness estimates may be required in the courtroom. For example, the defense may suggest that a relative of the suspect is the culprit of the crime.

Many estimators of pair-wise relatedness have been proposed over the years, however none account for the potential effects of evolution. Populations tend to have subpopulations within them, and individuals typically mate within their own subpopulation. A small amount of inbreeding will result, which in turn introduces an additional amount of relatedness between random individuals within a subpopulation. This extra amount of relatedness should be taken into account when estimating pair-wise relatedness.

The objective of this research is to develop a new maximum likelihood estimator of pair-wise relatedness that accounts for evolutionary effects. The project builds upon the foundation provided by earlier work in the area. A simulation study compares this new estimator to previous approaches, using simulated populations with and without inbreeding. Researchers also evaluate the new estimator using a real data set, obtained from the FBI.

Miniaturized Forensic DNA Analysis System for SNP-based Detection

Carl A. Batt, Nathaniel Cady, Scott Stelick, Xin Yang

A field-portable system for forensic DNA analysis is being developed for SNP-based analysis of forensic samples. This self-contained system, the microFLUIDICS DESKTOP, integrates sample preparation, DNA extraction/purification and real-time PCR analysis into a 30cm x 25cm x 15cm unit, weighing approximately eight pounds. Included in the system is a disposable microfluidic chip that incorporates DNA purification and real-time PCR amplification along with on-board fluid and temperature control. Analysis is carried out using an integrated fluorescence detection system. Using this system, researchers have been able to purify DNA from cheek swab samples and analyze SNPs for several alleles including the autosomal targets CYP3A56 and TSC0820041/9. In addition, the project has begun to develop assays that

distinguish biological samples as originating from males or females using a locus in the amelogenin gene on the X and Y chromosomes. Current improvements being built into the system include multi-color fluorescence detection system for multiplex real-time PCR. The multi-color system makes it possible to perform multiplex 5' nuclease (TaqMan)-based PCR for detecting multiple alleles at a single locus. The system is also being modified to include on-board data analysis as well as wireless data transmission for teleforensics applications.

Plenary Session, Tuesday, 10:30 am - 12:10 pm

R&D New Projects, Continued

Microfabricated Capillary Array Electrophoresis Genetic Analyzers for Forensic Short Tandem Repeat DNA Profiling

Richard A. Mathies, Jeffrey D. Ban, Cecelia A. Crouse, Susan Greenspoon, Amy McGuckian, George F. Sensabaugh, Stephanie H. I. Yeung

This project is developing microfabricated capillary array electrophoresis (μCAE) instruments and methods that integrate amplification, product clean-up and multiplex STR electrophoretic analysis of up to 96 samples on a single-wafer system. To begin this work researchers have benchmarked the 96 channel μCAE breadboard system at Berkeley using PowerPlex® 16 labeled samples. Studies of the accuracy, microvariant resolution, sensitivity, mixture analysis and the analysis of non-probative test samples will be presented. Similar μCAE validation studies have been successfully performed using the ABI AmpFLSTR® Profiler Plus® kit. The project will prepare improved energy-transfer cassette labeled primers for the loci used in the PowerPlex® 16 kit. These enhanced intensity labels will be evaluated compared to the conventional dye labels to quantitate the improved sensitivity and the concomitant capability to amplify from lower copy number samples or with fewer PCR cycles. A next-generation, commercial version of our μCAE instrument has also been developed and is in the process of being evaluated and optimized at Berkeley for forensic typing. Once this evaluation is complete, this μCAE instrument will be installed and used at the VaDFS later this year for routine high-throughput analyses. More advanced analysis systems that include sample cleanup are also in development. In these studies, a linear acrylamide gel is conjugated with capture oligos designed to hybridize to the various STR products in an amplified sample [see Paegel et al., *Anal. Chem*. **7**4 , 5092-5098 (2002)]. This solid-phase capture approach permits the various allelic products to be concentration normalized and desalted for effective electrophoretic analysis. This approach will be particularly useful for low copy number amplification and degraded DNA amplification.

Generating More Precise Postmortem Interval Estimates With Entomological Evidence: Reliable Patterns of Gene Expression Throughout Calliphoroid Larval and Pupal Development

David Foran

Insects, especially flies (Diptera), can be important tools in forensic investigation of deaths, as many insect species eat, breed on, or are otherwise associated with decaying remains. Calliphoridae, Sarcophagidae, and Muscidae flies are the most useful insects in this type of investigation because they are capable of locating and ovipositing (egg laying) on a corpse within hours or even minutes of death. Based on an approximation of the age of immature flies (larvae or pupae) associated with the body, an estimate of post mortem interval can be made. Presently, length measurements of fly larvae or qualitative analysis of the color of pupae are the most common estimators of immature fly age. The former is useful in age estimates as fly larvae increase linearly in length throughout most of their immature life cycle. However, towards the end of larval development flies shrink as they prepare to pupate, meaning age estimates based on length can be inaccurate. Likewise, it is difficult for investigating forensic entomologists to age pupae due to a dearth of information on pupal development. Consequently, the most useful characteristics to age pupae are color and basic morphological structures in dissected pupae that indicate only what stage they are in, i.e., just entering, in the midst of, or about to emerge from pupation.

Research in developmental genetics has shown that in the fruit fly Drosophila melanogaster, these stages require up- and down-regulation of thousands of genes. The research introduced here is designed to increase the accuracy of aging entomological evidence, and thus PMI, through isolation and characterization of developmentally regulated genes in the forensically useful species Lucilia sericata (the green blowfly). These genes will be selected based on homologous genes that are known to change their expression levels through this period, from either the closely related sheep blowfly (L. cuprina) or D. melanogaster. Genes regulated in late and early third instar larvae, as well as in pupae, will be specifically targeted. Isolation of genes will be based on PCR amplification using primer regions conserved across multiple species, or through cDNA generation from genes differentially expressed. Following isolation, the genes will be characterized throughout early fly development, at multiple developmental temperatures. The method will then be applied to flies in a blind study, with comparison to length and color data. Flies from other regions of the country will be examined as well. If successful, this methodology is amenable to miniaturization and analysis by means of array technology.

mtDNA Reference Database for the Domestic Dog

Marc W. Allard, Kristen M. Webb

Dog hair and thus dog mitochondrial DNA (mtDNA) is an additional source of mtDNA evidence that is present at many crime scenes. Taking advantage of this evidence is relatively straightforward, as human hair has commonly been used in many investigations. The ability to identify canines from biological samples found at crime scenes could prove invaluable in terms of convicting or eliminating potential suspects. While canine mtDNA from dog hair has been used successfully in some criminal investigations, comparing the hair found at the crime scene to potential suspects in the case is the extent of its current capabilities. Project researchers are in the process of creating a reference database that is widely available to the forensic community.

Development and Validation of a Standardized Canine STR Panel for Use in Forensic Casework

Sree Kanthaswamy

With 40 percent of U.S. Households having at least one dog as a companion animal, canine biological material is abundant and is frequently collected along with human evidence at crime scenes. However, despite its tremendous potential for facilitating forensic investigation, canine DNA evidence still remains seldom used. Testimony based on short tandem repeats (STR) analysis of animal DNA evidence has been used in a number of court cases worldwide. Casework performed by the Veterinary Genetics Laboratory Forensics has been used to successfully prosecute individuals by linking a perpetrator to a crime scene in instances of murder, burglary, sexual assault, and cases of animal abuse and theft. The few laboratories that currently perform forensic analysis of canine DNA use a variety of marker sets with little overlap among them. Recent court challenges to canine DNA analysis have demonstrated a need for a standard canine STE panel that has been validated according to human forensic guidelines.

In collaboration with the California Department of Justice, the National Institute of Standards and Technology, the National Institutes of Heath, and the Federal Bureau of Investigation, researchers propose to develop and validate a canine forensic STR panel. The fluorescent-based multiplex will include 16 independently-segregating tetranucleotide markers that are robust, reliable, and informative for all dog breeds. The panel will also incorporate the gender identification amelogenin gene. This multiplex will be used to profile our Pit Bull terrier and Rottweiler samples (two breeds frequently involved in forensic cases); the mixed-breed samples, and the collection of pure-bred dogs (over 1,000 unrelated individuals representing 28 American Kennel Club- recognized breeds). These subpopulations will form the foundation of the canine DNA database.

An additional 13,000 dog buccal samples originally submitted for parentage verification are also available for databasing. Familial samples from the Cornell Canine Reference Family will be used to analyze linkage disequilibria and mutation rates among the candidate loci. With permission from submitting agencies, non-probative samples from completed casework will be available for panel validation. Genotyping will be performed on a capillary format (ABI 3730 DNA Analyzer). The resulting data will be assessed for estimating inbreeding coefficients, genetic and geographic substructures, and recombination ratios.

Progress: so far the project has developed a canine-specific qPCR assay using a Taqman probe. The sequencing of the canine genome last year has provided a wealth of information. Analysts have identified over 300 primer pairs that meet criteria for forensic markers. Those markers will be evaluated for incorporation into the current panel. Recently published panels of canine markers have several shortcomings: unmapped or highly mutable markers with many off-ladder alleles, proprietary markers, markers with low PIC values, and markers with undefined repeat regions. Project investigators originally intended to incorporate some of the better markers from those panels to facilitate acceptance by others in the canine typing community. Now, however, the project focuses on making the best panel possible within the proposed time frame.

Forensic Stain Identification by RT-PCR Analysis

Janice A. Nicklas, Eric Buel

With the advent of innovative molecular biological techniques becoming the norm in the forensic laboratory, it is plausible to imagine the eventual replacement of serological testing methods with molecular biological techniques. New fast, definitive tests that could identify the tissue or fluid of origin and could potentially be multiplexed and yield rapid results on a minimal amount of sample would be a boon to the forensic laboratory. mRNA profiles are different for each cell type and often the production of certain mRNAs (such as hemoglobin in red blood cell precursors) are definitive markers of a certain cell type. While mRNA lore suggests that mRNA is ephemeral, in fact, it is quite stable in a dried stain. Thus, specific forensic assays could be developed employing mRNA as the tissue-specific determinant. The nature of this research is to identify mRNA transcripts that will definitively identify tissue of origin, determine if such transcripts survive the typical environmental insults that forensic samples may potentially encounter, and develop rapid assays to assess these molecules using small amounts of sample. The project describes successes (and failures) to date in using real-time PCR (TaqMan) to determine the presence of mRNA transcripts for beta-hemoglobin, kallkrein3 (PSA), histatin3 and uroplakin2, which are specific for blood, seminal fluid, saliva and urine, respectively. Researchers will also discuss future plans for assay development.

Working Lunch, Tuesday, 12:30 pm - 2:30 pm

R&D Demonstrations and Practitioner Posters

Multiplex_QA and MixSTR: Excel-Based Tools for STR Multiplex Measurement Quality Assessment and for Mixture Evaluation

John M. Butler, David Lee Duewer

Two Excel-based software tools for the examination and evaluation of forensic STR multiplex data have recently been developed at National Institute of Standards and Technology (NIST). The Multiplex_QA system works in conjunction with the NIH-developed BatchExtract system to visualize short- and long-term changes in ABI 310 and 3100 electrophorogram quality. The BatchExtract system converts the ABI binary *.fsa files into readily usable text data; Multiplex_QA uses these files to estimate quality metrics, mostly using internal sizing ladder results, that capture changes in electropherographic resolution and efficiency. Several different graphical displays enable identifying unusual events over time scales from a single *.fsa file to all *.fsa files of a given system. While primarily intended to investigate the utility of the various quality metrics, the current Multiplex_QA system is sufficiently robust for use by forensic scientists with an interest in data analysis and access to a reasonably fast PC. The MixSTR system, developed in collaboration with the West Palm Beach Sheriff's Office, helps identify reference profiles in sets of mixed-source samples. Given allele lists for one or more evidential samples and all relevant suspect and control reference profiles, the MixSTR system provides detailed lists of the match and miss-match alleles among all samples and profiles. Sorted summaries of the percent of included alleles and loci for all comparisons are also provided. This tool is intended for use with mixed-source samples, by all forensic scientists.

Forensic Botany Applications in Casework and Use as Tracers in Drug Distribution Networks

Heather Miller Coyle, Albert Harper, Ian C. Hsu, Cheng-Lung Lee, Henry C. Lee, Timothy M. Palmbach

Forensic botany is still an under-utilized form of evidence for many forensic cases. Often it is collected as trace evidence but is not further analyzed due to lack of training etc. However, plants have played a key role in some very important cases involving the estimation of time of death from mass graves or from stomach contents, as evidence in the wood chipper homicide, identification of locations of bodies, and for cases with historical interest such as the Shroud of Turin. An overview of such cases and the focus of current research involving using plant DNA to trace marijuana distribution patterns will be presented. The consideration of the genetics and breeding history of a plant species and its importance for construction of a plant reference database will be discussed.

SpermPaint: Fluorescent Monoclonal Antibody Probes for Sperm Identification

John C. Herr, Linda Gilmer, Kenneth L. Klotz

SpermPaint is a mixture of fluorescent dye conjugated monoclonal antibodies that allows definitive identification of human sperm using fluorescence microscopy. The SpermPaint formulation reported in this report contains: (1) a monoclonal antibody directed to the sperm head antigen, equatorial segment protein [ESP]; (2) a monoclonal antibody directed to the acrosomal matrix protein, SP-10; and (3) a monoclonal antibody directed to the sperm flagellar antigen, calcium binding tyrosine phosphorylated protein [CABYR]. Together, this mixture of monoclonal antibodies stains acrosome intact sperm heads with a characteristic cap pattern; acrosome reacted sperm heads with a characteristic band pattern through the mid portion of the head corresponding to the equatorial segment and stains the majority of the sperm tail corresponding to the principal segment.

SpermPaint is anticipated to have several advantages for identifying sperm in forensic casework. The ESP, SP-10, and CABYR target proteins are unique to sperm and testis, being differentiation antigens that arise during spermiogenesis. This property of these biomarkers results in the fluorescent signal from both monoclonal antibodies being clear, bright, and selective for sperm heads and tails with no cross reactivity to other tissues. The target antigens ESP, SP-10, and CABYR are stable: SpermPaint identified sperm in samples collected 1, 24 and 72 after coitus and from swabs that were stored at 4°C for four years. Significantly, the SpermPaint reagent: (1) identified sperm heads and tails when they were detached from one another, (2) identified sperm heads and tails that were masked by adherence to vaginal epithelial cells, and (3) identified sperm hidden within cellular debris. These performance characteristics of SpermPaint are anticipated to allow more rapid identification of sperm in microscopic fields and increase the number of conclusive identifications.

Evaluation of Track-Etch Filters for Isolating Sperm DNA in Rape Kits

Carll Ladd, Eric J. Carita, Alex Garvin, Henry C. Lee, Elaine M. Pagliaro

The large number of unprocessed sexual assault cases nationwide constitutes an ongoing concern for the forensic community. Many of these cases have sufficient numbers of sperm to generate DNA profiles that could be used to query the CODIS database and identify rape suspects. The project has evaluated a vacuum driven filtration method as an alternative approach

to the standard differential extraction protocol for separating sperm from digested epithelial cells that is more easily automated in a 96 well format. First, the sample is digested with proteinase K + RNase for two hours at 56°C. Sperm are collected on two micron track-etch filters, while the epithelial cell DNA is collected in the filtrate (vacuum pressure = 300 torr). The filters are then washed, and the sperm DNA is solubilized with a reducing agent and collected in the filtrate.

Mock body fluid sample mixtures (5,000-100,000 sperm per swab) and non-probative case samples were processed by the vacuum filtration method and the standard differential extraction procedure to determine the ability to separate male from female profiles using the Profiler Plus and COfiler STR kits from ABI.

Research has shown that the ISOPORE™ track-etch filter can be effective for identifying the DNA profile of a semen donor from mixed body fluid samples. The efficiency of the separation using the track-etch filter is consistently equal/superior to the standard differential extraction procedure. Furthermore, more than two hours of hands-on bench time per experiment was saved using the track-etch filters, and the method is more amendable to automation.

Prototype Polymeric Microfluidic DNA Analysis System for Forensic Analysis

Laurie E. Locascio, Michael Gaitan, John Kakareka, Nicole Morgan, Tom Pohida, David Ross, Paul Smith, Wyatt Vreeland

The presentation describes the development of a prototype polymer-based microfluidic system for the rapid, multiplexed analysis of forensic short tandem repeat (STR) DNA samples. The microfluidic system being developed for this project is an eight-channel system fabricated poly(methylmethacrylate). Devices are fabricated in polymeric materials in order to decrease the fabrication and materials costs, and to increase the future commercial potential of this technology. Further, since the device cost is low, polymer microfluidic systems can also be employed as single-use devices to prevent issues of cross-contamination. In this presentation, results of DNA separations performed in polymer microfluidic systems will be discussed, emphasizing improvements in low-cost device fabrication and improved speed-of-analysis. Other critical aspects of this project include development of new optical detection systems optimized for DNA detection in polymer devices (with P. Smith, N. Morgan, NIH); testing of new higher performance DNA separation polymer matrices (with A. Barron, Northwestern University); and development of software analysis tools and control systems for electronic interfacing and microfluidic switching (with T. Pohida, J. Kakareka, NIH). In this 16-month project, the key accomplishments included fabrication of the eight-channel device, optimization of DNA genotyping separations in a polymer microfluidic device, development of a sensitive and flexible miniaturized optical detection system, and development of software analysis tools.

Quantitation of Human DNA in Forensic Samples

Janice A. Nicklas, Eric Buel

This demonstration will have two parts running concurrently. The first part consists of a step-by-step tutorial demonstrating quantitation of human DNA using real-time Alu PCR. This presentation includes a computer MS PowerPoint© show giving a background explanation of the assay, demonstrating and comparing two real-time PCR instruments (the 72 tube Corbett Rotorgene 3000 and 96 well plate Stratagene MX3000P), showing how to setup real-time PCR and performing software analysis of the data to determine human DNA concentration. There will also be handouts available with protocols, reagent lists, and sample data. Presenters will be

able to answer questions about assay design, setup, or instrument acquisition. The second part of the demonstration will highlight the current NIJ-funded research projects of the Vermont Forensic Laboratory. This includes assays on gender identification, quick sample identification, and human DNA quantitation for short (mini) STRs. There will be explanation of the real-time probe based methods used for these assays (TaqMan® and molecular beacons) as well as presentation of data.

Multiplex qPCR Assays for the Quantification of DNA in Forensic Samples

Mark D. Timken, Martin R. Buoncristiani, Mavis D. Chong, Katie L. Swango

Two human-specific multiplex qPCR assays for the quantification of DNA in forensic samples will be described. The assays were developed on an ABI 7000 SDS instrument using 5'-hydrolysis detection chemistry and are currently being ported to the ABI 7500 instrument platform. One is a duplex nuclear-mitochondrial assay that was developed for implementation in the California Department of Jusice Missing Persons DNA Program. This assay amplifies and detects a ~180bp nuclear target sequence that spans the CODIS TH01 STR locus and a 69bp mitochondrial target sequence at ND1. The other is a triplex assay that was developed to assess both the quantity and quality of DNA in forensic samples. This assay includes a "long" nuclear target sequence (~180bp at TH01 as described above), a "short" nuclear target sequence (77bp in the flanking region of the CODIS CSF1PO STR locus), and an internal PCR control (IPC) target sequence (an artificial target designed to be non-homologous to commonly encountered genomes). Data will be presented to show that the ratio of DNA quantities determined by the short and long nuclear amplifications provides an assessment of DNA fragmentation and that the IPC allows for an assessment of the presence of DNA inhibition in forensic samples.

DNA Chip Instrument for Automated Human Identification

Ronald Sosnowski

Training of technical staff to perform DNA analysis for human identification is expensive and time consuming. Also, procedures requiring numerous manipulations increase the probability that mistakes will be made. While there is significant effort to utilize robotics and Lab Management Systems to automate tests in DNA identification labs, these systems are large and expensive and therefore most suitable to high throughput applications. The NanoChip 400 is the second-generation commercial instrument from Nanogen. It features an internal robot for handling fluidics from 96- or 384- well microtiter plates and Nanogen's microelectronic chip for DNA analysis.

An application has been developed for analysis of selected autosomal, Y chromosome and mitochondrial SNPs. This protocol includes online, in situ amplification of the loci in conjunction with genotyping at the amplification site. Features include a highly multiplex assay and automated genotyping calls. This demonstration displays the NanoChip 400 as well as Nanogen's new active 400-pad chip. Its features will be discussed, and the steps for doing DNA analysis will be demonstrated.

Plenary Session, Tuesday, 2:30 pm - 2:50 pm

NDIS Updates

Update on the FBI's CODIS Program

Thomas F. Callaghan

An idea conceived 15 years ago, the Combined DNA Index System or CODIS as it is known in the forensic DNA community, has matured into a program supporting DNA databases in over 200 forensic laboratories around the world. As the DNA database programs evolve, the CODIS software has accommodated the changing needs of the DNA community and will continue to do so. A major component of the CODIS program is operation of the National DNA Index System (NDIS). All 50 States as well as the FBI Laboratory and United States Army Crime Laboratory participate in the National DNA Index System. Changes made by the Justice for All Act of 2004 will affect the administration and operation of the National Index. Supporting its many contributors and ensuring compliance with the recent statutory changes has resulted in the establishment of new procedures governing NDIS. Changes to NDIS procedures that may be of interest to the DNA community will be discussed, such as such as the new requirement that NDIS participants be accredited by October 30, 2006, and new criteria for one-time searches of the National Index System. Many challenges await the CODIS program in the coming year, including an expected increase in DNA data contributed by laboratories receiving Federal funding. Priorities of the CODIS program as well as recent statistics and trends will be presented.

Plenary Session, Wednesday, 8:30 am - 9:50 am

R&D Ongoing Projects

Increasing the Predictability and Success Rate of Skeletal Evidence Typing: Using Physical Characteristics of Bone as a Metric for DNA Quality and Quantity

David Foran

Obtaining genetic results from biological samples has become routine for forensic laboratories. In pristine condition, biological evidence will generate results that effectively identify the person who left it. In contrast, evidence that is compromised due to age, environment, and other factors, begins to degrade, as does the DNA within it. The ability to effectively predict what genetic results are likely to be obtained from compromised biological evidence would help the DNA analyst choose the best technique for its analysis, potentially saving large amounts of time, effort, and materials.

The most common aged forensic material encountered is bone. In spite of this, little objective research has been conducted comparing the outward appearance of skeletal material (how weathered it is) and the quality or quantity of the DNA found within. Research presented here addresses this topic, beginning with a set of skeletal remains obtained from the Voegtly Cemetery near Pittsburgh. The small cemetery was used for a short period of time; the remains had little variation in age or environment, yet they contained a wide range of degradation levels. This study examines how and if skeletal weathering correlates with DNA quality and quantity. Specific bones within a skeleton and localized soil conditions were also assayed to see if either influences observed genetic material and bone degradation. Finally, bone samples from other locations were tested to deduce the generalization of the findings.

Assessment and In Vitro Repair of Damaged DNA Templates

Jack Ballantyne, Ashley Hall

DNA extracted from biological stains is often intractable to analysis. This may due to a number of factors including a low copy number (LCN) of starting molecules, the presence of soluble inhibitors or damaged DNA templates. Remedies may be available to the forensic scientist to deal with LCN templates and soluble inhibitors but none presently exist for damaged DNA. In fact, only recently have analysts begun to examine the biochemical nature and the extent of DNA damage in physiological stains and the point at which the damage inflicted upon a particular sample precludes the ability to obtain a genetic profile for purposes of identification. The primary aims of this work were first to ascertain the types of DNA damage encountered in forensically relevant stains, correlating the occurrence of this damage with partial or total loss of a genotype, and then to attempt the repair of the damage by means of in vitro DNA repair systems.

The initial focus of the work was detection of damage caused by exogenous, environmental sources, primarily UV irradiation, but also factors such as heat and humidity. We found that the primary causes of the damage that resulted in profile loss were strand breaks, both single and double stranded, as well as modifications to the DNA structure that inhibited its

amplification. Armed with this knowledge, the next focus was repair of the damage by means of in vitro DNA systems. Efforts have been concentrated on single strand break/gap repair and translesion synthesis assays. By modifying the assays and employing various combinations of the systems, a genetic profile has been obtained from previously intractable samples.

Gene Polymorphism and Human Pigmentation

Murray H. Brilliant

The overall goal of this research project is to determine with a high degree of accuracy, the pigmentation phenotype (skin, hair, and eye color) of an individual subject from a forensic DNA sample. Human pigmentation is programmed genetically. Among the candidate genes mediating pigmentation variation are P, MATP, MC1R, ASIP, and DCT, each previously associated with mouse and/or human hypopigmentation disorders that exhibit a spectrum of phenotypes. The study involved 800 participants who were phenotyped for pigmentation attributes and genotyped at 52 polymorphisms in 16 genes. For skin pigmentation, the project found five polymorphisms in four genes (two in P and one each in MATP, DCT, and ASIP) account for 60 percent of the phenotypic variation. For total hair pigmentation, five polymorphisms in five genes (MC1R, MATP, DCT, P, and ASIP) account for 73 percent of the variance. Additionally, five polymorphisms in four genes (two in MC1R and one each in MATP, P and ASIP) account for 80 percent of the ratio of red to black hair pigment. For eye color five polymorphisms in four genes (two in P and one each in MATP, DCT, and ASIP) account for 39.2 percent of the variance in the sample (when blue eyes and green eyes are binned together), demonstrating that eye color is genetically the most complex pigmentation trait. Thus, it is possible to predict the skin and hair color of an individual by genotyping only a few polymorphic loci from a DNA sample.

Developing Methods for Bone Sample Preparation and DNA Isolation Using Proteinases

Richard C. Li

Bone tissue is often used for recovering DNA samples for the purpose of human identification. However, forensic science laboratories are challenged to determine identification when the evidence remaining is a compromised skeletal fragment. The identification of partial DNA profiles or a failure to obtain a DNA profile has been reported when such samples were analyzed. Also, in some cases the amount of sample is recovered in limited quantities, which makes DNA isolation more difficult. This study will develop methods for bone sample preparation and for improvement of the yield of DNA isolation, thereby creating a higher success rate in generating genotype profiles for DNA analysis.

Plenary Session, Wednesday, 10:10 am - 11:30 am

Programs of the DNA Initiative

NIJ's DNA Training Initiative

G.A. Redding

Training is the underpinning of any successful program. The criminal justice workforce must possess the skills and competencies needed to exploit DNA technologies. As DNA-related technologies have advanced, in the field and in the lab, so have instructional technologies in fixed and remote environments. The National Institute of Justice (NIJ) envisions a distributed and highly adaptive learning infrastructure blending Advanced Distributed Learning techniques and technologies. Advanced means a technology and common framework provide reusable, platform-independent learning software. "Distributed" means it will be delivered, updated, and managed using network technologies. Learning, in this sense, encompasses education, training, and aids to performance. This effort recognizes shrinking resources – allowing for pooled resources rather than competing demands. Organizations learn to reassess imperatives to reduce duplicity and shift from "what we have always done" to "what we can do best." They come to rely on the strengths of key partners. This session will describe the NIJ direction and outline projected outcomes.

Solving Cold Cases Through the Identification of Missing Persons and Unidentified Human Remains

Arthur J. Eisenberg

Each day in the United States, there are nearly 100,000 active missing persons cases. Tens of thousands of individuals, both children and adults vanish each year under suspicious circumstances. Preliminary studies have indicated that across the U.S. there may be as many as 40,000 remains that cannot be identified and as few as 5,500 have NCIC entries. In the vast majority of these cases where a physical examination of the unidentified remains has been performed and a cause of death has been determined, death was the result of a violent crime. There are currently only three operational programs in the U.S. (the University of North Texas Health Science Center (UNTHSC), the California Department of Justice, and the Federal Bureau of Investigation) that are capable of analyzing, uploading, and searching both STR and mtDNA profiles for missing persons and unidentified remains. The UNTHSC DNA Identity Laboratory has received three grants from NIJ in 2004 under the President's DNA Initiative related to missing mersons. Award 2004-DN-BX-K212 provides funds for the DNA analysis of unidentified human remains and family reference samples for law enforcement, medical examiners, and coroners throughout the United States. Award 2004-DN-BX-K213 provides funds for the development, purchase and distribution of standardized kits throughout the U.S. for the collection of unidentified human remains and family reference samples. Award 2004-DN-BX-K214 provides funds to field test new technologies such as mini STRs for the analysis of unidentified human remains. The progress and recent Cold-Hits obtained as a result of these grants will be discussed.

Demonstration Projects of the DNA Initiative: Overview

Edwin W. Zedlewski

Property crime offenders have high recidivism rates, their crime and violence can escalate, and property crime cases often go unsolved. Several police departments in the United States are finding that when analyzing DNA from a burglary, they get evidence that solves several other cases as well. And they are finding that biological evidence collected from property crime scenes can prevent future property crimes and more serious offenses. The Miami-Dade Police Department, Palm Beach County Sheriff's Office, and the New York City Police Department are solving high-volume property crimes (burglary and auto theft) and violent crimes (sexual assault and murder) using funds they received from NIJ. They are discovering that analyzing DNA from property crimes can have major public safety benefits. This presentation will focus on the results from these agencies, and will additionally provide an overview of NIJ's demonstration projects on the use of DNA evidence under the President's DNA Initiative.

Prioritizing Minor Crime DNA Analysis to Yield Major Results: TwoYear No-Suspect Grant Summary

Dawn M. Hicks, Cecelia A. Crouse, Melanie McElroy, Tara L. Sessa

The Palm Beach County Sheriff's Office (PBSO) Serology/DNA Section has designed and implemented a successful DNA Backlog Reduction model to research every no-suspect case from all 32 agencies and conduct DNA analysis on all viable no-suspect cases with the help of a two-year National Institute of Justice (NIJ) grant. The grant emphasized testing evidence from minor crimes. The project was divided into three phases: (1) A retired experienced law enforcement detective was contracted to assist the DNA Section in identifying and submitting No-Suspect cases. (2) All qualifying no-suspect cases were screened for biological material, followed by outsourcing of the appropriate cases. (3) Staff review and enter all qualified DNA profiles into CODIS within five days of receiving vendor data, followed by research from an experienced retired prosecutor to determine if a judicial filing was appropriate. This three-phase program yielded unprecedented cooperation among Palm Beach County law enforcement agencies. PBSO also implemented many protocols to increase efficiency of internal testing capabilities, including the validation and implementation of an extraction robot, electronic capture of evidence, video screening of sexual assault items, rt-PCR methodology, and hiring of an Evidence Coordinator. In summary, PBSO has submitted 690 no-suspect cases in 24 months. Of these, 590 cases have been returned and reviewed; from these, 420 qualifying DNA profiles (71%) were entered into CODIS. There have been 192 hits (46%) to date. Over 18,000 cases have been reviewed by the contracted ex-detective and the State's Attorney's Office has filed three John Doe warrants based on burglary cases.

Working Lunch, Wednesday, 11:45 am - 1:15 pm

Evidence Collection From a Living Crime Scene: SANE Practice and DNA

Patricia M. Speck

Crying, disheveled, and smelling of alcohol a 19-year-old tells the officer that when she left the local tavern, she was abducted and raped by a stranger. Once removed from the place where the alleged act occurred, the real crime scene sits in the SANE's office and… she is a living breathing person with her boyfriend and mother standing by for support. Nursing process demands that the SANE consider the biological, psychological, social, and spiritual issues that are faced by all patients, but this patient is unique and suffering from an acute traumatic reaction that requires special forensic nursing skills to complete the physical evaluation and evidence collection, hopefully producing a DNA sample that will yield the identity of the offender. Discover the knowledge and skills necessary in the forensic nurse SANE, to help the patient who has been victimized to actively participate in the evidence collection while being assessed for health and treatment options.

Plenary Session, Wednesday, 1:15 pm - 2:20 pm

Overview of Federal DNA Programs

NIST On-Going Projects to Aid the Human Identity Testing Community

John M. Butler, Michael D. Coble, Amy E. Decker, David L. Duewer, Carolyn R. Hill, Margaret C. Kline, Janette W. Redman, Peter M. Vallone

The project team at the National Institute of Standards and Technology (NIST) is funded by the National Institute of Justice (NIJ) to conduct research that benefits the human identity testing community and to create tools that enable state and local DNA laboratories to be more effective in analyzing DNA. Researchers conduct inter-laboratory studies, produce new assays to enable improved recovery of information from degraded DNA, evaluate new loci for potential future use in human identity applications, and generate standard information and training materials that are made available on the NIST STRBase website. In addition, all publications and presentations that result from our work are all made available on the STRBase website: http: //www.cstl.nist.gov/biotech /strbase/NISTpub.htm. New genetic markers and assays involving STR and SNP loci are examined in a U.S. reference population data set involving approximately 650 samples that are of Caucasian, Hispanic, and African American origin. A portion of this presentation will also be devoted to discussing the results from the mixture interpretation inter-laboratory study (MIX05) conducted in early 2005 where over 50 different laboratories returned interpretation results on the same DNA samples.