Room Changes

Monday, June 20, 2011

10:30 a.m. – 12:00 p.m.

Indigent Defense: Research Needs and Alternative Practices Salon A
(Page 4)

Expert Systems, Databases, and Other Software-Based Methods to Improve Use of Forensic DNA Data Salon B
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Wednesday, June 22, 2011

1:45 – 3:15 p.m.

Familial DNA Searching: Issues and Answers Salon H
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Revised Panel Information

Monday, June 20, 2011

10:30 a.m. – 12:00 p.m.
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Winning the Future: Nationwide Broadband Communications for Public Safety Salon J

On February 10, 2011, President Barack Obama detailed his plan to "Win the Future" by catalyzing the build-out of high-speed wireless services that will enable businesses to grow faster, students to learn more, and public safety responders to access state-of-the-art, secure, and interoperable mobile communications. As part of this effort, the Administration has been working with State and local public safety officials, the vendor community, and Federal authorities to develop and implement a nationwide Public Safety Broadband Network. This network will allow public safety responders to send and receive critical voice, video, and data – thereby saving lives, reducing injuries, and preventing acts of crime and terror. Implementation of this strategy will likely have a significant impact on criminal justice and other public safety agencies with respect to operational capability and resource availability. The following federal, state and local representatives will discuss current and planned efforts in network development and evaluation:

■ **Anna Gomez,** Deputy Assistant Secretary for Communications and Information and Deputy Administrator, National Telecommunications and Information Administration
■ **Allan Sadowski,** Information Technology Manager, North Carolina State Highway Patrol
■ **Gregory Schaffer,** Deputy Under-Secretary (Acting), National Protection and Programs Directorate, U.S. Department of Homeland Security, Washington, DC
■ **Bill Schrier,** Chief Technology Officer, City of Seattle, Washington, Seattle, Washington
■ ***Moderator:*** **A. Marisa Chun,** Deputy Associate Attorney General, U.S. Department of Justice, Washington, DC

New Panelists

Monday, June 20, 2011

10:30 a.m. – 12:00 p.m.

Domestic Violence: What We Know From the Research and the Data Salon K

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■ **Jenna Truman,** Statistician, Bureau of Justice Statistics, U.S. Department of Justice, Washington, DC

Tuesday, June 21, 2011
3:00 – 4:30 p.m.

Pretrial Research and Practice Salon B
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■**Antoinette Davis,** Senior Research Associate, National Council on Crime and Delinquency, Oakland, California

Additional Biographies

James Arden Barnett, Jr., Rear Admiral (Ret.), is the Chief of the Commission's Public Safety and Homeland Security Bureau. He is responsible for overseeing Federal Communications Commission (FCC) activities for public safety, homeland security, emergency management, and disaster preparedness. Before serving at the FCC, Chief Barnett was a Senior Research Fellow at the Potomac Institute for Policy Studies. From 1984 to 2001, he was a senior partner at Mitchell, McNutt and Sams, P.A., in Tupelo, MS. Chief Barnett served 32 years in the U.S. Navy and Navy Reserve, retiring in 2008. His previous Active Duty assignments included Director, Naval Education and Training at the Pentagon and Deputy Commander, Navy Expeditionary Combat Command at Little Creek, VA.

Robert J. Casey is the Section Chief of the Law Enforcement Support Section (LESS), Criminal Justice Information Services (CJIS) Division. As the LESS Chief, he is responsible for more than 300 FBI personnel and contractors who support a variety of criminal justice information programs and systems, including the National Crime Information Center, Law Enforcement Online, and the Uniform Crime Reporting Program. Mr. Casey is also responsible for the CJIS Division's Contract Administration Office, the CJIS Audit Unit, and the CJIS Advisory Group Management Unit. Before coming to the FBI, he served as a Second Lieutenant in the U.S. Army. Mr. Casey has a master's of management degree from Webster University and a bachelor's degree in accounting from Old Dominion University.

A. Marisa Chun was appointed U.S. Deputy Associate Attorney General on May 4, 2009. Her responsibilities include a range of the Department's civil and criminal law enforcement issues, including intellectual property rights, antitrust, telecommunications, and financial fraud issues. Previously, she was a Litigation Partner at Coblentz, Patch, Duffy & Bass, LLP, in San Francisco, CA, a Court-appointed Mediator for the U.S. District Court for the Northern District of California, a Senior Trial Attorney at the U.S. Department of Justice, and a Law Clerk on the U.S. Court of Appeals for the Ninth Circuit. Ms. Chun is a graduate of Harvard Law School, where she served as Developments Editor of the *Harvard Law Review* and Yale University (*summa cum laude*).

Anna Gomez is the Deputy Assistant Secretary for Communications and Information and Deputy Administrator of the National Telecommunications and Information Administration (NTIA).Prior to joining NTIA in February 2009. Previously, Ms. Gomez was Vice President of Government Affairs at Sprint Nextel. She served for 12 years in various management positions at the Federal Communications Commission (FCC), including Deputy Chief of the International Bureau and Chief of the Network Services Division in the Common Carrier (now Wireline) Bureau. Ms. Gomez also served as Senior Legal Advisor to former FCC Chairman William Kennard; Deputy Chief of Staff in the National Economic Council during the Clinton Administration; Staff Counsel in the

U.S. Senate for the Subcommittee on Communication, Committee on Commerce, Science and Transportation.
Ms. Gomez earned her juris doctor degree from the George Washington University and is a graduate of Pennsylvania State University.

Chuck Heurich is a Program Manager with the National Institute of Justice (NIJ). He manages the Solving Cold Cases with DNA program, the NIJ Forensic Technology Center of Excellence (National Forensic Science Technology Center), the Using DNA Technology to Identify the Missing and National Missing and Unidentified Persons System programs and other projects for the Office of Investigative and Forensic Sciences. He worked as a Crime Scene Technician in Baltimore City, MD, a Forensic Scientist in the Forensic Biology Unit with the Montgomery County Crime Lab, and as a Forensic Specialist 2 in the Forensic Services Section. He received his master's degree in forensic science from the George Washington University and his bachelor's degree in biology from the University of Pennsylvania, Slippery Rock.

Linda Mellgren is a Senior Social Science Analyst in the Office of the Assistant Secretary for Planning and Evaluation, Department of Health and Human Services (HHS). Her areas of policy and research work include child support, fatherhood, marriage and the intersection of human services, and criminal justice populations. Currently she is managing the National Center for Family and Marriage Research, the evaluation of the family strengthening grants for fathers and their partners, and HHS activities related to the Interagency Reentry Council. Ms. Mellgren has a master's degree from the Maxwell School of Citizenship and Public Affairs at Syracuse University.

Stephen Mercer is the Chief Attorney of the Forensics Division at the Maryland Office of the Public Defender, and an Adjunct Professor at the David A. Clarke School of Law, where he teaches an advanced evidence course on forensic science. He is actively involved in litigation and public commentary on issues arising at the intersection of forensic science and civil liberties, for example, leading the successful effort before the Maryland General Assembly to ban familial searching of the State DNA database; and appearing on the CBS broadcast, *Sixty Minutes*, focusing on the emerging practice of familial searching of DNA databanks.

Jay F. Nunamaker, Jr., is the Regents and Soldwedel Professor of Management Information System, Computer Science and Communication. He is Director of the National Center for Border Security and Immigration at the University of Arizona. Dr. Nunamaker was inducted into the Design Science Hall of Fame, May 2008, and received the LEO Award for Lifetime Achievement. He is widely published in the fields of collaboration technology and deception detection. He was a Research Assistant at the University of Michigan and an Associate Professor at Purdue University. Dr. Nunamaker received his doctorate degree from Case Institute of Technology, master's and bachelor's degrees from the University of Pittsburgh, and a bachelor's degree from Carnegie Mellon University. He received his professional engineer's license in 1965.

Daniel Olmos is Senior Counsel at the Access to Justice Initiative at the U.S. Department of Justice. Prior to joining the Justice Department, Mr. Olmos was a Criminal Defense Attorney at a Palo Alto, CA, law firm where he defended clients charged with a variety of offenses, including murder, narcotics trafficking, trade secret theft, and economic espionage. Before going into private practice, Mr. Olmos was a Deputy Public Defender at the Contra Costa County Public Defender's Office. He served as Clerk for Judge Claudia Wilken of the Northern District of California and Judge Stephen Reinhardt of the Ninth Circuit Court of Appeals. He received his law degree from the University of California, Berkeley.

Paul Allan Sadowski is the North Carolina State Highway Patrol transmission Control Protocol/Internet Protocol (TCP/IP) Communications Lead for the Voice Interoperability Program for Emergency Responders, providing technical leadership for legacy and trunked voice radio communications, interfacing, and infrastructure. He also served as Manager for conversion of a

statewide law enforcement SNA WAN to a TCP/IP WAN. For the State Interoperability Executive Committee, he served as Subject Matter Expert for Digital Communications and Project Lead for the first North Carolina Statewide Interoperability Communications Exercise COMEX09. He served as Consultant to the National Institute of Justice, the Kentucky and Maryland State Police Departments, and several local police agencies for data networking. He was an Originator of a U.S. Department of Defense joint secondary image dissemination system (5D).

Gregory P. Schaffer is the Acting Deputy Under Secretary for the National Protection and Programs Directorate, where he leads the U.S. Department of Homeland Security's (DHS's) efforts to reduce physical and cyber infrastructures risk. Prior to this, DHS Secretary Janet Napolitano appointed Mr. Schaffer Assistant Secretary for Cybersecurity and Communications. Before joining DHS, Mr. Schaffer served as Senior Vice President and Chief Risk Officer for Alltel Communications, LLC. He was also a Director in PricewaterhouseCoopers, LLP, Cybercrime Prevention and Response Practice, and a Computer Crime Prosecutor in the Computer Crime and Intellectual Property Section at the U.S. Department of Justice. He holds a juris doctor degree from the University of Southern California Law Center and a bachelor's degree from the George Washington University.

Karen Stern is a Social Science Analyst in the International Center (IC) at the National Institute of Justice (NIJ), where she studies the feasibility of transferring innovative foreign technologies and practices to the United States. Dr. Stern also serves on the Suicide Prevention Task Force for Youth in Contact with the Juvenile Justice System, and is NIJ's representative on the Office of Justice Programs Working Group on Identity Theft. Her current projects involve human trafficking, community management of sexual and/or violent offenders, and crime scene investigation. Dr. Stern received her doctorate and master's degrees in clinical psychology from the University of North Carolina at Chapel Hill and a bachelor's degree in psychology from the University of Rochester.

Eugene Tan is the Vice President of Product Development at Network Biosystems. He is responsible for the development and manufacture of the instrumentation and biochips for microfluidic biochip-based DNA analysis. He has spent the last 12 years developing products and commercializing technology, including a microfluidic chip-based high throughput DNA sequencer, sunlight legible flat-panel displays for aircraft applications, and highly tunable lasers for telecommunications. Prior to joining Network Biosystems, he was employed by Nortel Networks in a variety of senior engineering and manufacturing positions. He received his doctorate degree in engineering physics from McMaster University, Canada.

Melissa Taylor is a management and program analyst with the Law Enforcement Standards Office (OLES) at the U.S. Department of Commerce's National Institute of Standards and Technology. She works within the OLES Forensic Science Program, focusing primarily on fingerprint-related research and integrating human factors principles into forensic sciences. Ms. Taylor currently serves as a member of the INTERPOL Automated Fingerprint Identification System (AFIS) Expert Working Group, Associate Member of the International Association of Identification, and Executive Secretary of Subcommittee on Forensic Science's Latent Print AFIS Interoperability Task Force. Ms. Taylor has a bachelor's degree from the University of Maryland.

Jennifer Truman is a Statistician in the Victimization Statistics Unit at the Bureau of Justice Statistics. Her current research and work focuses on victimization patterns and trends, student victimization, and intimate partner victimization using the National Crime Victimization Survey. She received her doctorate degree in sociology from the University of Central Florida in 2010. Her dissertation focused on examining intimate partner stalking and the use of technology in stalking.

Monday, June 20, 2011, 3:45 p.m.
Forensic Science Poster Session

Researchers: Titles and Abstracts

Abigail Bathrick and Robert Bever

FISH Probe Design and Development for a SNP-Based Screening System for Cellular Mixtures of the Same Gender and Morphology

Laser microdissection (LM) has proven to be an effective method for cell mixture separations in the forensic laboratory. While sperm and epithelial cell sexual assault mixtures can easily be separated based upon morphological differences, mixtures of the same cell type are more difficult to separate. During our work on National Institute of Justice Contract #2006-DN-BX-K032, we developed the capability to successfully separate male/female cellular mixtures of similar morphology using chromosome X/Y fluorescence *in situ* hybridization (FISH) probing. In the cases of cellular mixtures of the same gender, we believe that developing fluorescent probes based upon human genetic single nucleotide polymorphisms (SNPs) to create additional FISH screening methods could provide a basis for separation of these samples with LM instruments. Screening panels of FISH SNP probes could effectively visually detect individual contributors of sample mixtures, while LM technologies could physically separate the cells for further STR processing. Development of a successful SNP screening system is highly dependent on FISH probe design and signal amplification techniques. Traditional FISH probes typically target areas which span from 1 kilobase (kb) to millions of bases. The hybridization of many probes to these regions results in the generation of crisp, bright signals. However, because SNP probes are designed to hybridize to sequences that display one base pair difference, it is necessary to drastically decrease the length of the probe to discourage nonspecific binding. Signal amplification techniques must be employed to increase the sensitivity of FISH when detecting the short FISH probes. Tyramide signal amplification (TSA) utilizes the activity of horseradish peroxidase to generate increased signal amplification of the target DNA sequence of interest in situ. The use of efficiently labeled short oligonucleotide probes combined with TSA techniques may ultimately be used to develop a FISH-based SNP screening panel.

Mark Wilson and Brittania Bintz

Development of a Novel Human Mitochondrial DNA (mtDNA) Amplification Method for use with Illumina® Next-Generation Sequencing Instrumentation

Challenging forensic DNA samples, including bones and hair often contain DNA that is degraded and/or is present in very low concentrations. Mitochondrial DNA analysis is often utilized on these sample types. Studies employing newly emerging DNA sequencing technologies have been designed to interrogate targets down to the single molecule level. While these technologies are capable of producing large quantities of usable sequencing data, they are laborious and peripheral instrumentation can be costly. For example, typical library preparation for the Illumina® GA_{IIx} platform includes gDNA fragmentation (often using an expensive Covaris® DNA-shearing instrument), end-repair and addition of A-overhang, adapter ligation, and DNA selection. We have developed a novel method for human mtDNA amplicon generation for single-read DNA sequencing on the Illumina® GA_{IIx}, using a traditional amplification step employing a TaKaRa™ high-fidelity polymerase enzyme. Adapters and multiplexing indices are included on the 5' end of the mtDNA hypervariable (HV) region-specific primers, and are incorporated into the amplicon during PCR. We have shown that this amplification strategy produces higher concentrations of amplicons than the current strategy used in forensic laboratories. Further, these amplicons can also be sequenced using Sanger methods, without any apparent hindrance from the extended primer sequences. Thus, this method enables forensic laboratories to adopt one mtDNA amplification protocol for multiple downstream sequencing technologies. Additionally, this library

preparation proves to be more efficient, and more cost effective than methods recommended by Illumina.®

Christopher C. Cooney, Alexander Kukhtin, Steve Garber, Nitu Thakore, Peter Qu, Maria Belgrader, Jennifer E. Reynolds, and Phillip Belgrader

A Low Cost Microfluidic Microarray System to Type SNPs for Physical Appearance
The purpose of this project is to design, build, and test an integrated microfluidic-controlled microarray platform to type SNP markers for physical appearance. Generating key phenoytpe information from crime scene DNA evidence can aid in rapidly apprehending a perpetrator or identifying a victim. The platform requirements being targeted include:

- rapid processing (sample to answer in 2 hours or less)
- walk-away push-button operation (including the sample preparation)
- small-footprint (2 cubic feet)
- processing up to four samples per run
- portability (field or mobile lab use)

The current prototype system consists of the instrument (i.e., liquid handling, bladder thermal cycler, reader, and cartridge interface) and a disposable cartridge (i.e., TruTip, PCR/TruArray flow cell chambers, microfluidic circuits, and microfluidic valves). The work performed to date has demonstrated: 1) a multiplex PCR and isothermal Allele-specific Arrayed-Primer Extension (AS-APEX) test for eye color, 2) positive results from blood samples processed on the integrated system, and 3) positive results using lyophilized assay reagents.

Mark Powell, Michael Donley, and Roger Kahn

Genetic Markers Associated with Sudden Unexplained Death or Sudden Infant Death
We are developing and will validate and implement a DNA sequencing array system specific for genes linked to Sudden Infant Death Syndrome (SIDS) and Sudden Unexplained Death (SUD) in children and young adults. In the United States each year, there are thousands of sudden deaths of infants and young adults for which the cause of death is listed as undetermined, that is, no cause is identified during autopsy. The Harris County Institute of Forensic Sciences has classified 750 unexplained deaths as SIDS or SUD since 2000. It is estimated that as many as 10 percent of the SIDS cases and approximately 30 percent of the SUD cases can be linked to mutations in genes of cardiac function. In collaboration with the Baylor College of Medicine Human Genome Sequencing Center, a tool based on second generation Illumina sequencing will be developed and validated. This tool will simultaneously sequence DNA from 65 genes implicated cardiac metabolism associated with SIDS or SUD and, upon validation, be implemented to test the Harris County Institute of Forensic Sciences cohort. Traditional sequencing methods are available to sequence these genes; however, it is prohibitively expensive (e.g., approximately $19,000 per sample for sequencing only 19 of the genes) and time consuming, requiring hundreds of PCR and sequencing reactions per case. Applying second generation DNA sequencing tools will reduce the cost per sample to less than $1,000, reduce the processing time, and allow the addition of additional genes at very little cost.

Erin Finehout, Weston Griffin, Philip Shoemaker, Zaeem Khan, Xuefeng Wang, Scott Duthie, and Pierino Bonanni

Automated Processing of FTA Samples
The number of DNA samples being submitted into forensic labs for short tandem repeat (STR) analysis and databanking is increasing every year. As such, there is a growing need in forensics

labs for automated sample handling and processing for a variety of sample types. One commonly used material for the collection and storage of these samples is the FTA card, which is effective for long term room-temperature storage and stabilization of DNA. In this project, a prototype automated system was designed which allowed a user to load in a stack of buccal swabs on FTA EasiCollect cards, and then get out a 96-well plate of punches ready for PCR. Our goal is to be able to show improved throughput and decreased risk of sample loss. This project was supported by Award No. 2009-DN-BX-K187, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this poster are those of the authors and do not necessarily reflect those of the Department of Justice.

Christian Carson, Alex Garvin, and Kim Gorman

Single Source Male Profiles from Sexual Assault Evidence: *Erase* – Sperm Isolation Kit
Obtaining a single source male profile from a sexual assault is often very difficult or impossible using standard differential extraction methods. Alternative extraction methods may improve separation but a significant portion of the sperm sample may be lost in the process. The *Erase* – Sperm Isolation Kit provides a single source male profile with minimal loss of sperm cell DNA in both single tube and 96 well platforms. Using this method, epithelial cells are differentially lysed and a single centrifugation step is performed to pellet the intact sperm cells. After transferring an epithelial cell DNA fraction into a second tube, the epithelial DNA remaining in solution with the sperm pellet is destroyed using a DNA nuclease. Once the solubilized epithelial DNA is degraded, nuclease activity is stopped and the sperm cells are lysed. Using the *Erase* – Sperm Isolation Kit, it is possible to obtain a clean male profile, with minimal hands-on time, even when the ratio of epithelial cells to sperm cells is very high. *Erase* has been validated with multiple DNA purification methods.

Thomas A. Hall[1], David D. Duncan[1], Maria A. Tobar[1], Kristin Sannes-Lowery[1], Sheri M. Manalili[1], Jessica E. Paulsen[1], Kristen Boles[1], John Planz[2], and Steven A. Hofstadler[1]
[1]*Ibis Biosciences, a subsidiary of Abbott Molecular, Carlsbad, California*
[2]*University of North Texas Health Sciences Center, Fort Worth, Texas*

Analysis of DNA Forensic Markers Using High-Performance Mass Spectrometry
A DNA forensics platform based on PCR followed by fully automated high throughput electrospray ionization mass spectrometry (PCR/ESI-MS) has been developed. Forensic DNA markers are “weighed” with sufficient accuracy to provide base compositions (the number of A’s, G’s, C’s, and T’s). Base compositions for multiple makers provide a DNA profile for an individual that can be referenced to existing forensics databases. Assays for short tandem repeat (STR) and Y-chromosomal STR (Y-STR) analysis have been developed. These assays are run on the same automated Ibis T5000™ and PLEX-ID™ platforms as the existing Ibis mitochondrial DNA profiling assay. The PCR/ESI-MS system is capable of identifying and storing polymorphic alleles while maintaining backwards-compatibility with current databases. Because an intrinsic property of each amplification product (i.e., its mass) is measured, allelic ladders are unnecessary. The masses of the forward and reverse strands of each product directly identify the allele and reveal polymorphisms relative to the nominal allele of the same length. For autosomal STR loci, polymorphisms have been observed in 11 of 13 core CODIS loci, with six loci displaying polymorphisms in greater than 20 percent of alleles observed in 863 samples (D13S317, D21S11, D3S1358, D5S818, D8S1179 and vWA). Moreover, instances of heterozygous loci displaying alleles of the same length (one or both alleles being polymorphic) have been observed in 9 of the 13 core CODIS loci, with five loci (D13S317, D3S1358, D5S818, D8S1179 and vWA) displaying frequencies of greater than 5 percent of individuals being heterozygous with same-length alleles. STR and Y-STR assays are constructed with eight reactions per sample in 96-well assay plates, such that 12 samples can be run per assay plate. All reaction components are pre-fabricated in kit plates so only 5 □l of template needs to be added per well prior to thermocycling,

and all subsequent steps through data processing are fully automated. Assay sensitivity is 125-250 pg of DNA per reaction (1-2 ng per sample, 4 ng recommended). With a nested PCR approach in development, sensitivity for the CODIS STR panel down to 62-125 pg total DNA per sample has been demonstrated while maintaining the ability to resolve polymorphisms in STR alleles.

Micah Halpern[1], John Gerdes[2], Jack Ballantyne[3], Erin Hanson[3], and Anahita Kiavand[2]
[1]*Gensol Diagnostics*
[2]*Micronics, Inc.*
[3]*National Center for Forensic Science*

Rapid STR Prescreening of Forensic Samples at the Crime Scene
The goals of this project were to continue development of a unique melt-based approach to STR genotyping, integrate and test that approach for compatibility with existing microfluidic extraction, amplification, and melt subcircuits, and determine applicability for forensic applications. The benefit to this approach is the simplification of converting laboratory protocols for portability, mainly by eliminating the many challenges associated with capillary electrophoresis (CE) size-based analysis. These can include biological/technological artifacts, added sample processing steps, and complex equipment demands required for portability. Some of the advantages not possible with CE-based protocols demonstrated through the validation phase of this project include: higher sensitivity (5-25 picograms); elimination of pre-quantification; minimal or no apparent biological artifacts including stutter; the ability to detect microvariants (SNPs); and transfer compatibility to a microfluidic platform.

Through development and testing of the optimized assay with a microfluidic platform, successful sample extraction from blood, PCR amplification and melt-based allele detection was demonstrated using a microfluidics-based subcircuit design approach. It is the transfer of this approach to a fully integrated microfluidic lab-on-a-card format that will permit processing and analysis of samples in an enclosed environment; thereby minimizing the chances for cross contamination and more importantly providing a means for post-analysis archiving of DNA extracts for follow-on laboratory testing of probative samples. Results from this project have demonstrated successful generation of a classic CE profile 18 months following archiving of extracted DNA. This will not only provide a preliminary profile at the scene to determine victim versus non-victim samples but will also permit post-scene laboratory analysis of the probative samples without the need for additional sample extraction. Successful microfluidic processing of 5-10 µl blood spots dried on a subcircuit surface was also demonstrated, providing high confidence for application to crime scene samples. Collaborative efforts during this project produced convincing evidence that with further development and validation, a platform for on-site STR-based prescreening of probative samples in a rapid cost-effective manner is possible.

Katherine Butler, Michelle Peck, Jessica Hart, Moses Schanfield, and Daniele Podini

Molecular "Eyewitness": Forensic Prediction of Phenotype and Ancestry
When a STR DNA profile obtained from crime scene evidence does not match identified suspects or profiles from available databases, further DNA analyses targeted at inferring the possible ancestral origin and phenotypic characteristics (i.e., hair color, skin color and eye color) of the possible perpetrator could yield valuable information. Such a tool would aid in prioritizing suspect processing, corroborating witness testimony, determining the relevance of a piece of evidence to a crime, and ultimately increase the ability to identify individuals related to the crime scene. Single nucleotide polymorphisms (SNPs), the most common form of genetic polymorphisms, have alleles associated with specific populations and/or correlated to physical characteristics. We have used single base primer extension (SBE) technology to develop panels which include 100 ancestry and phenotype markers selected from recent literature. Over 250 DNA samples, along with corresponding ancestry/phenotype survey information, and spectrophotometric skin color

data, have been collected from anonymous volunteers of varying ethnicity, gender, and age. These DNA samples, and additional samples of known ancestry, have been screened with the SBE panels to assess the predictive value of the candidate SNPs, with the goal of identifying the optimal panel of SNPs to efficiently assess an unknown individual's characteristics. Different statistical approaches are being evaluated for best ancestry and physical trait inference. Preliminary results show good correlation between a small set of SNPs and eye color making it a highly predictable trait, whereas hair and skin pigmentation together with ancestry are more complex characteristics to prognosticate.

Lisa Hebda

Trace DNA from Fingernails: Increasing the Success Rate of Widely Collected Forensic Evidence

Forensic nurses, other emergency personnel, and pathologists often collect material beneath fingernails from surviving or deceased assault victims for DNA testing. Unfortunately, very little is known about the utility of such collections, including if the existing methods for obtaining and testing fingernail/DNA evidence are optimal for producing probative evidence. In consultation with several forensic practitioners, a research regimen is being conducted to address these questions in an objective and statistically reliable manner. Multiple methods for collecting nail evidence for subsequent DNA analysis are being compared. Along with this, the various DNA analysis procedures widely used in crime laboratories (STRs, miniSTRs, Y-STRs) are being considered, producing an optimal collection and analysis strategy for exogenous nail DNA. The ultimate goals of the proposed research are to: 1) determine the best method for isolating foreign DNA from nail evidence, 2) determine what type of genetic testing of nail evidence is most informative, and 3) determine if the way nail evidence is currently treated can be improved. The research is designed to be thorough, efficient, and informative, and should be of broad, practical use to the great number of forensic scientists who encounter fingernail evidence every day.

Diane J. Rowold and Michael Jablecki

CODIS STR Template Enrichment by Affinity Bead Capture and Its Application in Forensic DNA Analysis

Short tandem repeat (STR) profiling is a workhorse in forensic analysis; however, investigations involving sub-optimal evidentiary DNA samples are often hampered by incomplete and/or ambiguous combined DNA index system (CODIS) STR profiles arising from a number of factors. Especially challenging are cases in which evidentiary DNA is degraded and/or co-extracted with polymerase chain reaction (PCR) inhibitors. In this study we investigate an affinity capture technology using streptavidin-coated beads and biotinylated CODIS STR primers to isolate and purify relevant STR templates for subsequent amplification and detection. This strategy is applied to obtain unambiguous CODIS STR profiles from challenging degraded DNA samples. The developed affinity capture process may also show promise as a cleanup technique for PCR inhibitor and bacterial contamination removal. In addition, we evaluated this procedure and demonstrated preservation of source DNA proportions in human DNA mixtures.

Milko B. Kermekchiev, Zhian Zhang, and Wayne M. Barnes
DNA Polymerase Technology, Inc., and Washington University Department of Biochemistry, Saint Louis, Missouri

Novel TAQ Mutant Enzymes and PCR Enhancers Designed for Forensics

Major problems with PCR-based forensic tests of samples containing blood, soil, or other inhibitory substances are false negative results and low sensitivity caused by such inhibitors. The effect of the PCR inhibitors is primarily associated with inactivation of Taq DNA polymerase. Therefore, various protocols and DNA extraction procedures are being used to purify DNA prior to

PCR. These extra steps add to cost, are time-consuming, may not completely remove inhibitors, or may lead to losses of target DNA. As a novel alternative to these pre-PCR steps, we have engineered mutants of Taq polymerase (OmniTaq and CesiumTaq) highly resistant to PCR inhibitors and possessing a built-in hot-start feature. We also developed novel PCR enhancer cocktails (PECs), which further improve the performance of the mutant enzymes in crude samples, and increase the specificity and sensitivity of DNA detection.

We present data on direct STR genotyping of human DNA from a variety of crude samples, relevant to the forensic practice, including blood, soil, humic acid, semen, bile, indigo dye, tannins, cigarette butts, aged buccal swabs, soda can swabs (some of them kindly provided by BODE Technology), showing that our genetically engineered enzymes OmniTaq and CesiumTaq, supplemented with the PEC enhancer formulations, can outperform the two top commercial kits available today, PowerPlex 16 HS and AmpFlSTR Identifiler-Plus, generating complete allele profiles with 17 crude samples tested, skipping the DNA extraction. In comparison, out of the 17 crude samples, the PowerPlex 16HS kit generated 12 full and 5 partial profiles, and the Identifiler-Plus kit, with 14 of these 17 samples attempted, generated 6 full profiles, 2 partial profiles, and no profiles were generated in the other 6 samples. The mutant enzymes generate full STR profiles with blood on FTA cards, regardless of whether the blood was untreated or treated with the common anticoagulants EDTA, heparin or citrate.

Our novel enzyme-enhancer systems could eliminate, in many cases, the need to purify DNA prior to PCR and speed up, lower the cost, and increase the efficiency of forensic DNA testing.

This work was supported by National Institute of Justice grant number 2008-DN-BX-K299.

Kenneth K. Kidd, Judith R. Kidd, Andrew J. Pakstis, William C. Speed, and Michael P. Donnelly

Progress in SNP Resources for Ancestry Identification

Virtually all of the sets of SNPs for ancestry inference (AISNPs) published in the scientific and forensic literature do not function well within geographic regions because their selection was based on a limited number of populations representing major continental regions of the world. Our recent research has begun to identify SNP sets that can begin to refine the estimates of ancestry within some of these continental regions. Whether or not we can achieve a large likelihood ratio for distinguishing ancestry within a geographic region like Europe or the Native populations of North America with only a few hundred SNP markers remains to be demonstrated—this is a research project. For discrimination among Siberian and East Asian we have identified a subset of 57 SNPs out of the 128 SNPs identified by Seldin's group that functions as well as the entire 128 SNPs; however, there is still no differentiation among Southeast Asians, Han Chinese, Koreans, and Japanese. This is clearly a distinction of forensic relevance and better SNPs are needed. We will present an overview of our recent work studying such existing panels and identifying new AISNP sets to identify those that are best for each geographic region. Our studies are defining the utility of diverse SNPs by typing them on the large numbers of populations necessary as references when an unknown sample is examined for assignment of probable ancestry. Our presentation will provide an overview of our project thus far. We are already sharing intermediate results via the ALFRED database.

Vitali Sikirzhytski, Aliaksandra Sikirzhytskaya, Greg McLaughlin, Aliea Afnan, and Igor K. Lednev

Raman Spectroscopy for a Confirmatory Identification of Body Fluid Traces: Multidimensional Spectroscopic Signatures, Interference of Substrates and Contaminants

Currently, unknown biological stains revealed at a crime scene are identified through a series of chemical and biochemical tests, which are destructive and require typically toxic reagents. The

ultimate goal of our research is to develop an easy-to-use, portable instrument for rapid, nondestructive, confirmatory identification of body fluid traces. Phase 1 of this project, the development and evaluation of the proposed novel methodology under controlled (laboratory) conditions is currently supported by Award No. 2009-DN-BX-K196 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice.

Here we report on the results of the investigation focused on three objectives of the award. Multi-dimensional Raman spectroscopic signatures were obtained for vaginal fluid and sweat (blood, semen, and saliva were reported previously). The developed method was extended to body fluid stains on solid substrates including glass and floor tile. Body fluid traces contaminated with non-biological components including dust, sand, soil, and cleaning fluid (bleach) were also investigated. We demonstrated that body fluid traces could be detected and identified by means of Raman spectroscopy combined with advanced statistics in all cases above.

Kevin Legg and Phillip B. Danielson
University of Denver, Department of Biological Sciences, Denver, Colorado

Validation of Highly Specific Protein Markers for the Identification of Biological Stains
While DNA profiling makes it possible to individualize biological stains, the identification of the stain itself can present forensic serologists with a significant challenge. For example, there is no reliable test for the presence of vaginal secretions, and tests for blood do not allow the practitioners to distinguish between peripheral and menstrual blood. Using highly-reproducible protein separation technology (multidimensional HPLC), a custom bioinformatics software suite, and tandem mass spectrometry, the proteomes for six body fluids with clear forensic relevance (i.e., peripheral and menstrual blood, vaginal secretions, semen, urine, and saliva) have been mapped and compared for five individuals. Based on these analyses, a database of more than 30 candidate protein biomarkers for the identification of biological stains has been built. The current research objective is to validate the specificity of the most promising candidate biomarkers for their target body fluids across multiple humans. Validation assays are being conducted using high-sensitivity targeted mass spectrometry on a quadrupole time-of-flight LC/MS instrument. This system employs a multiplex assay which can simultaneously detect and quantify all target biomarkers in approximately 25 minutes of instrumentation time. This research will provide forensic practitioners with a panel of validated biomarkers for the confirmatory identification of biological stains. These markers can be used to screen fluids on the mass spectrometry based platform or to develop antibody based technologies similar to the ABAcard and Seratec® kits.

Richard Li

Application of Proteinase for DNA Isolation of Bone Specimens
Forensic analysis of DNA from bone can be important in a variety of situations, including cases involving the identification of skeletal remains in criminal matters and mass fatality incidents. However, bone is difficult to process for isolating DNA. Thus, we have been developing a simple trypsin method for processing bone specimens prior to DNA isolation. Trypsin is utilized in enzymatic maceration methods for processing bone samples in anthropological laboratories. In our previous study, the trypsin-based maceration technique was adapted to the sample processing method prior to DNA isolation from bone samples. By incubating bone samples with the trypsin solution, the outer surface of the bone samples was removed. The trypsin-processed bone samples could then be used for DNA isolation. Our previous results, tested on fresh human bone samples, have suggested that the trypsin method can be potentially useful for forensic DNA analysis. In this study, additional experiments will be done on samples that are more typically encountered in actual forensic cases. In particular, the study will consist of two parts: 1) to characterize the effect of trypsin treatment on the yield and the quality of DNA isolated, and 2) to characterize the effect of trypsin treatment on the quality of DNA profiling.

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Development of a Microfabricated Filter Device to Isolate Sperm From a Mixed-Cell Population

When analyzing sexual assault cases in a crime laboratory, relatively pure sperm DNA must be isolated from vaginal swabs that initially contain a mixed male-female cell population. The primary method in forensic laboratories involves preferential female epithelial cell lysis, but the sperm pellet obtained is often contaminated with female cell DNA, complicating the sperm STR analysis. In addition, this method needs multiple centrifugation and washing steps that are both time and labor-intensive, and can result in sperm loss and low sperm DNA yields. In this project we are engineering parylene-based microfilter with uniform pores and high density to achieve a rapid isolation of sperm within a 25mm^2 area by utilizing the size difference between sperm and epithelial cells (approximately 5µm vs. approximately 30–50µm, respectively). Currently, we are using a differential extraction buffer as the initial step for lysing epithelial cells to circumvent the potential problem of clogging from epithelial cells and converting to using single layer of 1µm filter for capturing sperm. In a side-by-side comparison test using a model system, filter-based sperm enrichment yielded higher DNA recovery rate and cleaner STR profile with minimal carryover from the female. Further device characterization with side-by-side comparison utilizing real forensic samples is underway to demonstrate utility in expediting the backlog of DNA typing rape cases.

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Improved Tools for Examining Low Copy Number DNA Obtained From Challenged or Degraded Samples

There are limits to forensic DNA analysis. One important parameter is the amount of template DNA used in the polymerase chain reaction (PCR). When the amount of DNA is below a certain quantity, the results obtained from current forensic DNA typing methodology generally are not reproducible because low copy number (LCN) typing is not sufficiently robust. In order to improve LCN typing, several approaches were taken, including: 1) improvements to the robustness of the amplification through the use of PCR enhancers; 2) increasing DNA recovery using pressure cycling technology (PCT), improved silica columns, or synchronous coefficient of drag alteration technology (SCODA); and 3) more efficiently reducing inhibition. The data illustrate that each of these approaches can contribute to improving the efficacy of analysis either by increasing yield of sample, more effectively purifying a sample, or by increasing amplification efficiency (e.g., decreased stutter). The impact is that some samples that traditionally yield too little DNA for typing may become suitable for routine analysis or a more effective methodology may be developed that will enable analysis of samples that typically have not been typeable. Moreover, more challenged samples may be analyzed by combinations of better purification columns, PCT, SCODA, and PCR enhancement.

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Overcoming PCR Inhibitors of Degraded DNA Using Various Thermostable Polymerases
Numerous substances can inhibit PCR and they are routinely encountered in forensic DNA investigations. The presence and concentration of PCR inhibitors have major consequences for downstream applications and can lead to underestimated DNA concentrations, allelic drop-out, and/or false-negative results. A number of studies have shown that *Taq* polymerase is highly susceptible to inhibitory substances in contrast to polymerases such from *Pyrococcus furiosus* (*Pfu*), *Pyrococcus woesei* (*Pwu*), *Thermus flavus* (*Tfl*), and *Thermus thermophilus* (*Tth*). This investigation evaluated the overall effectiveness of 10 different thermo-stable polymerases and polymerase blends in amplifying DNA extracted from inhibited skeletal remains (Monroe, Kemp- unpublished data) that vary in age and locality. Samples were amplified with various polymerases in order to evaluate their respective efficiencies in overcoming a wide range of inhibitors, their overall fidelity, and selectivity. Quantitative PCR was used to measure DNA yield and quantify the effects of inhibition.

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Developing an Empirically Based Ranking Order for Bone Sampling: Examining the Differential DNA Yield Rates Between Human Skeletal Elements Over Increasing Post Mortem Intervals
The identification of skeletal remains often challenges forensic investigators. Ante mortem records, such as fingerprints or dental x rays, are not always available. This is particularly true during identification projects tied to mass grave excavations, or disaster projects where entire families or communities have been killed. In such cases, the only means of identification may lie in the bones' DNA. As remains decompose, skeletal elements including bones and teeth yield higher levels of DNA than muscle. Additionally, skeletal material often survives long after muscle tissue has decomposed. While bone protects DNA better than muscle, all bones are not equal and some yield DNA at higher rates than others.

Currently, the selection of skeletal elements for DNA testing is based on the collective wisdom of practitioners. Recent evidence questions the accuracy of this collective wisdom (Mundorff et al. 2009). A subset of remains from the World Trade Center Identification Project was used to measure differential DNA preservation by skeletal element. Interestingly, they found that along with weight-bearing long bones, bones generally bypassed in DNA sampling yielded DNA at surprisingly high rates. The better yielding bones included several smaller elements, such as the patellae, metatarsals, and foot phalanges. Similar retrospective studies measuring differential DNA yield rates between skeletal elements for both mitochondrial DNA (Edson et al. 2004, Leney 2006) and nuclear DNA (Milos et al. 2007) have also found weight-bearing long bones to be most successful, although often the smaller elements were not tested and therefore not considered in their ranking.

This will be the first study to prospectively measure DNA yield rates from each skeletal element to establish a comprehensive ranking according to each bone's potential to provide usable genetic material for DNA identification. It will replace intuition with empirical data and provide investigators with a clear DNA sampling strategy.

This research project will also develop empirically based bone-sampling standards to maximize the success rate of identifications from bone of varying post mortem intervals. DNA yields of different skeletal elements from three recently skeletonized individuals are being analyzed first. This will allow us to rank the most successful elements. Next, skeletons from increasing post mortem intervals (the period of time from the individual's death to the present time) will be tested

to assess whether the same bones successfully yield sufficient DNA for identification 0–3, 3–10, 10–20, and 20+ years post mortem.

The ability of DNA testing to aid identification is directly related to the ability to obtain sufficient DNA. This project will allow us to determine which skeletal samples are most likely to provide both the quantity and quality of DNA needed to produce DNA profiles from increasing post mortem intervals. This will provide much needed guidance on which samples are best suited for DNA testing.

Amritha Premasuthan, Jillian Ng, Chris Johnson, Jessica Satkoski Trask, and Sree Kanthaswamy

Multi-Species Determination and DNA Quantification Assays

We have developed a fluorescence-based quantitative PCR (qPCR) assay to detect and measure trace amounts of nucleic acids from homogenous or mixtures of human, dog, and cat DNA samples. Our assay targets the human THO1 locus, and the dog and cat Melanocortin 1 Receptor (MC1R) gene. The developmental validation of this triplex assay included mixture and minimal studies as well as the evaluation of the effects of inhibitors and DNA degradation on the assay's sensitivity. We are also designing and validating another qPCR assay for species-determination and DNA quantification of samples from common poultry and hoofed mammals including chicken (*Gallus gallus*), cow (*Bos taurus*), duck (*Anas platyrhynchos*), goat (*Capra hircus*), goose (*Anser anser*), horse (*Equus caballus*), pig (*Sus scrofa*), sheep (*Ovis aries*), and turkey (*Meleagris gallopavo*). This assay will be designed to comprise two separate qPCR reactions, one for hoofed animals and the other for poultry. We also present data from a third assay that was designed and developed for the species identification of *Cannabis sativa* trace and particulate materials including seeds and other plant parts. This assay targets the trnL and trnL 3' exon-trnF intragenic spacer regions within the *C. sativa* chloroplast. This assay is capable of identifying trace amounts of DNA from a sample as small as a single *C. sativa* seed.

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Nucleic Acids, Nails, and the Post Mortem Interval

Although the post mortem interval (PMI) is important in the investigation of criminal cases, there are few available methods for accurately estimating PMIs out several years. This is because decomposition is heavily influenced by factors, such as environmental conditions (moisture, temperature, ecosystem, insects, animals, and season), the circumstances of the death, and the location of the body.

The utilization of nucleic acid degradation (messenger RNA, ribosomal RNA and DNA) from bones, teeth and nails is likely to lead to the development of PMI indicators for longer time periods. These tissues are more stable against environmental conditions so the rate of degradation of nucleic acids will be less influenced by environmental conditions.

With a focus on using methods that are currently in forensic practice, we have successfully modified the Promega DNA IQ™ method to co-extract RNA and DNA from nail clippings. We are taking the time since the nails were clipped as being the PMI for this initial work. We are also currently optimizing methods for co-extracting RNA and DNA from bones and teeth.

Using reverse transcriptase PCR (RT-PCR), we have amplified four keratin mRNA transcripts and 18S rRNA from nail clippings. Placing nail clippings in different environments, including

submerged in water, soil, and at room temperature, we have found that all four keratin mRNA transcripts are stable under different environmental conditions over a week, and we are currently investigating longer time periods. Using these results, a statistical model is being developed to correlate the rate of degradation of the different keratin mRNA transcripts and 18S rRNA with the time since the nails were clipped (PMI).

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A DNA Database for Species Identification of Forensically Important Carrion Flies in the Continental United States

We have developed a DNA database for rapid, accurate identification of forensically important Diptera in the continental United States. This database will improve both the speed and precision of postmortem interval (PMI) estimation using dipteran larvae. The database comprises a 900 base-pair locus within the mitochondrially encoded Cytochrome Oxidase I (COI) gene. We have sequenced this segment for greater than 500 specimens collected from 19 different States. These samples represent approximately 100 distinct species of Diptera, including all of the species that are of primary forensic interest in the continental United States. The 900 base-pair segment allows identification of nearly all of these forensically most-important species with high support. The single exception is that the pair *Lucilia coeruleiviridis / Lucilia mexicana* cannot not be resolved with the present data. For species that are both widespread and most commonly found in association with human remains, we provide data for specimens collected from a range of localities across North America. Although all species show some degree of sequence variation within the 900 bp segment, no geographic structure is apparent, suggesting that the database will be suitable for use throughout the continental United States.

Bo Zhou, Heyi Yang, Donald Siegel, Yingying Tang, and Mechthild Prinz

Development of a Proteomic Assay for Species Identification from Blood

(NIJ Forensic DNA R&D Grant: 2010-DN-BX-K192 – Development of a Proteomic Assay for Menstrual Blood, Vaginal Fluid, and Species Identification)

Species identification from blood can provide valuable information in forensic investigations especially when samples may reasonably be attributed to domesticated animals, such as livestock (dinner) or pets. Conventional forensic methods for blood testing typically do not provide species information. Methods currently in use for species identification include immunological assays and mtDNA sequencing for either cytochrome *b* gene and cytochrome *c* oxidiase gene, or the 12s ribosomal RNA coding region. Both methods have drawbacks, such as the lack of appropriate species-specific antibodies and the requirement of large amount of sample in an immunological assayand time consuming and labor intensive in sequencing of mtDNA targets. Potential RNA-based methods would require complicated multiplexing assays with carefully designed species-specific primer pairs based on prior knowledge of the sequences unique for candidate species. An ideal test would be capable of identifying species simultaneously in a single assay without having to select candidate species for testing.

The goal of this work was to develop a single, sensitive, proteomic assay for species identification from blood using mass spectrometry. The test was based on the principle that the same body fluid specific protein(s) that can identify blood (e.g., hemoglobin) can also be used to distinguish species because of the unique peptides sequences. Hemoglobin was chosen as a target protein because its abundance, meaning 1) increased assay sensitivity, and 2) high sample throughput as it can be detected without prior protein purification. Here we demonstrated a rapid species identification test that can be performed using whole blood in a single mass spectrometry assay. Of the 11 species tested, including human and domesticated and wild animals, all were correctly

identified. This assay is efficient, confirmatory, and sensitive, and would be a useful addition to the tools available in forensic investigations.