2006 National Institute of Justice DNA Grantees Meeting

Program Abstracts

Plenary Session, Monday, 9:30 am – 11:30 am

Forensic DNA Research and Development: Tools for the Crime Lab I: Compromised DNA Evidence

Development, Characterization and Performance of New MiniSTR Loci for Typing Degraded Samples (on behalf of NIST)

Michael D. Coble, John M. Butler, Carolyn R. Hill, Peter M. Vallone

Forensic DNA analysts often perform short tandem repeat (STR) typing on highly degraded biological material and then turn to mitochondrial DNA testing if many or all of the STRs fail. The commercially available kits for multiplex amplification of the 13 CODIS STR loci usually exhibit allele or locus-dropout for larger sized loci with degraded DNA or samples containing PCR inhibitors. Recently, a number of studies have demonstrated an increased success for the analysis of degraded DNA specimens from mass disasters (or degraded forensic evidence) when smaller sized PCR amplicons are utilized. One advantage of generating smaller sized PCR products (miniSTRs) for the CODIS loci is that it is possible to obtain fully concordant results to the commercial kits while improving successful analysis of degraded DNA. However, many of the CODIS core loci have large allele ranges (e.g., D21S11 and FGA) or have "unclean" flanking regions for successful primer hybridization that make it impossible to create miniSTRs. The Human Identity Project Team in the DNA Measurements Group at NIST has examined a battery of new potential STR loci (called non-CODIS, NC) that can be made less than 150 bp in size (in most cases) and would therefore be helpful in testing highly degraded or low copy number DNA samples. Project scientists have also characterized these markers across more than 600 population samples. In this presentation, presenters will discuss the development, characterization, and performance of these new miniSTRs and their impact on the global forensic DNA community.

mtDNA SNPs and Control Region Databases to Increase mtDNA Utility in Forensics

Michael D. Coble, Toni M. Diegoli , Jodi A. Irwin, Rebecca S. Just, Thomas J. Parsons, Jessica L. Saunier, Katharine M. Strouss, Kimberly A. Sturk

Significant limitations in current mtDNA identification testing include (1) the lower power of discrimination when common mtDNA hypervariable region types (HV-types) are encountered, (2) the availability of high-quality, error-free mtDNA control region (CR) databases representing diverse United States subpopulations, and (3) an incomplete understanding of the magnitude and significance of regional mtDNA substructure among U.S. populations. Scientists have undertaken two projects designed to address these limitations.

Single nucleotide polymorphisms (SNPs) in the CR outside of hypervariable regions I and II (HVI and HVII) and in the coding region of the mtGenome can be used to provide additional discrimination in mtDNA identifications. Researchers sequenced more than 500

entire mtGenomes from the most common HV-types in the Caucasian, U.S. Hispanic, and African-American populations and identified phenotypically neutral SNPs that could provide additional discrimination in cases of matching HV-types. The SNPs were organized into multiplex panels, each targeting specific HV-types. SNaPshot™ (ABI) assays for the Caucasian multiplex panels were designed, optimized, tested and added to databases, and are currently being validated for use in forensic casework at AFDIL.

In an effort to increase the quantity, breadth and availability of mtDNA databases suitable for forensic comparisons, staff has developed a high-throughput process to generate approximately 3500 control regions per year from U.S. source populations, and U.S. populations currently under-represented in available databases. The system utilizes robotic instrumentation for all laboratory steps from pre-extraction through sequence detection, and a rigorous five-step, multi-laboratory data review process with entirely electronic data transfer. The high-quality control region data generated with this system will provide a framework within which to examine mtDNA substructure and admixture, and better interpret mtDNA evidence.

Forensic mtDNA Mixture Fractionation by Denaturing High-Performance Liquid Chromatography

Phillip B. Danielson, Richard Kristinsson

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.

Use of a Denaturing High-Performance Liquid Chromatography (DHPLC) system (WAVE® System, Transgenomic Inc.) 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. 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 mitotypes of individual contributors to a mixture.

Finally, the capability of DHPLC to simultaneously quantify and purify DNA makes it possible streamline the processing of all mtDNA samples. This improves efficiency by eliminating the need for separate yield gels or PCR clean up steps. Thus, DHPLC aids criminal investigations by making it possible to obtain conclusive mitochondrial DNA results from mixtures that would otherwise not be amenable to analysis by direct sequencing.

Quantitative PCR (qPCR) Tools for the DNA Analysis of Challenging Samples

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

The forensic analysis of DNA extracted from casework evidence can be challenging. As examples, evidence samples that have undergone long-term environmental exposure often contain highly fragmented DNA, extracts from soil and bone often contain substances that inhibit PCR, and DNA extracted from archival (e.g., formalin-fixed paraffin) samples are typically degraded or chemically modified. We have developed and implemented two new real-time qPCR-based assays that can be used to assess both the quantity and quality of DNA in such samples. One assay simultaneously quantifies the amount of nuclear DNA and mitochondrial DNA in a duplex qPCR. The use of this assay to evaluate the quantity and quality of DNA extracts in missing persons' cases will be presented. The second assay is a triplex qPCR assay that simultaneously measures the quantity of a "long" nuclear target sequence (~180bp), a "short" nuclear target sequence (67bp), and an artificial target sequence that is spiked into each assay (an Internal PCR Control or IPC). A comparison of the quantity of long and short nuclear target sequences provides an assessment of DNA fragmentation, while the IPC assay provides an indication of the presence of co-extracted PCR inhibitors. The use of this assay for quantifying DNA in casework samples will be described.

Plenary Session, Monday, 11:30 am – 12:15 pm

Forensic DNA Research and Development: Tools for the Crime Lab II: Y-Chromosome Markers

Forensic Applications of Y-Chromosome STRs and SNPs

Michael Hammer

Project researchers identified and characterized 20 novel STRs on the non-recombining portion of the Y chromosome (NRY) that are robust and informative for forensic casework. These Y-STRs greatly improve resolution among paternal lineages above levels obtained with previously used Y-STRs. A total of 38 Y-STRs was typed in a panel of 2,517 U.S. samples representing African-Americans, European-Americans, Hispanics, Native Americans, and Asian Americans, as well as a large worldwide database. The entire U.S. Y-STR database is available for online searches to estimate frequencies of Y chromosome haplotypes determined from crime scene material. Comparisons of commercially available kits revealed that Applied Biosystems Yfiler©, which contains three of our novel Y-STRs, is superior to others. The 11 "core" Y-STRs recommended by the Scientific Working Group on DNA Analysis Methods were analyzed to estimate the extent of population structure within and among ethnic groups in the U.S. The analyses support the creation of separate African-American, European-American, Hispanic-American, and Asian-American databases in which samples of the same ethnic group from different geographic regions within the U.S can be pooled. Scientists recommend that separate databases be constructed for different Native American groups. A set of 61 Y chromosome single-nucleotide-polymorphisms (Y-SNPs) was also typed in the U.S. database to infer the geographic origins of Y-chromosomes in the U.S. and to test for paternal admixture among U.S.

ethnic groups. Admixture estimates vary greatly among populations and ethnic groups. A series of analyses was performed to test for the effects of inter-ethnic admixture on the structure of Y-STR diversity in the U.S. Results indicated that low levels of genetic heterogeneity between pairs of Hispanic-American populations disappear when African-derived chromosomes are removed from the analysis. This is not the case for an unusual sample of European-Americans from New York City when its African-derived chromosomes were removed, or for Native American populations when European-derived chromosomes were removed. We infer that both inter-ethnic admixture and population structure in ancestral source populations contributed to fine scale Y-STR heterogeneity within U.S. ethnic groups. Finally, empirical tests of association between Y-chromosome and autosomal markers are presented and a theoretical framework for determining a joint match probability is recommended. A conservative estimate of the joint probability is obtained by multiplying the largest value of the group autosomal match probabilities by the estimated matching probability for the Y-chromosome.

The Compilation and Management of a Comprehensive U.S. Y-STR Reference

Jack Ballantyne, Lyn Fatolitis

The establishment of a comprehensive national Y-STR reference database comprising an extended set of loci and compiling the existing database information maintained by agencies and corporations, is essential to facilitate the generation of reliable estimates of Y-STR multi-locus haplotype frequencies. Y-STR haplotype frequencies are required to provide a statistical estimate of the significance of a match. The National Center for Forensic Science is in the process of compiling and consolidating the existing Y-STR data from government, commercial, and academic resources throughout the U.S. to create the first comprehensive and expandable National U.S. Y-STR Haplotype Reference Database. The consolidated database will be made accessible to the forensic community via the World Wide Web. This presentation describes the project goals, what has been accomplished thus far, and highlights of the benefits that this project will afford to the forensic community.

Working Lunch, Monday, 12:15 pm – 2:00 pm

Identification Work on an Unknown Child from the 1912 RMS Titanic Disaster: DNA and Dental Discord

Ryan Parr

One of the persistent mysteries associated with the loss of the RMS Titanic is the identity of a small, male child, recovered in the Northwest Atlantic near the site of the sinking nearly one week following the disaster. The men of the victim recovery ship, Mackey-Bennett, estimated two years as the age of the child. Even though the child was not identified before burial in Halifax, Nova Scotia, the remains have been strongly associated with Gösta Leonard Pålsson, a 2-year-old Swedish child. According to the official White Star Line passenger list there are five additional male children who may also match the general description of the body, including both age and gender. Work to identify the remains results in the non-exclusion of two children, neither of which is Gösta Leonard Pålsson.

Plenary Session, Monday, 2:00 pm – 2:50 pm

Forensic DNA Research and Development: Tools for the Crime Lab III: DNA Quantitation

Quantitation of Human DNA in Forensic Samples and Improving the Efficiency of DNA Casework Analysis through Simple, Effective, PCR-based Screening Methods

Janice A. Nicklas, Eric Buel

This presentation will combine results from the just completed Quantitation grant and the just-beginning Improving Efficiency grant. During the Quantitation grant, a duplex total human DNA and male DNA quantitation assay was developed. This assay allows the analyst to quickly determine if a sample has sufficient male DNA for autosomal STRs or whether Y-STRs will be necessary, or whether the sample contains insufficient DNA for any analysis. This TaqMan-based assay uses the Alu sequence (VIC-labeled probe) to quantitate total human DNA and a Y-specific repeat (FAM-labeled probe) to quantitate male-specific (Y chromosomal) DNA. This assay has been validated on male and female samples of different types and is currently in beta testing in seven forensic laboratories. Difficulties encountered in assay development, characteristics of the assay, comparisons of different mastermixes and validation data will be presented.

The Improving Efficiency grant has two main goals: (1) development of a simple assay to determine if two samples are from a single donor and (2) development of an assay to determine the degradation state of the DNA in a sample. Both of these assays will streamline sample processes, allowing the analyst to pick samples most relevant to the case and most likely to yield DNA profiles. For example, if an analyst is presented with the suspect's shirt with 50 blood spots, using the sample differentiation assay, they could quickly and cheaply determine that 49 are from the suspect (i.e. probably not of interest) but the 50th is not from the suspect and could be from the victim. That 50th spot could then be profiled, saving the analyst performing STR analysis on 49 bloodspots. The degradation assay would determine if the DNA is too degraded for STR analysis and whether other analyses (or no analysis at all) would be possible.

For the sample differentiation assay, researchers chose to develop a six-plex (five-plex SNP plus gender assay) using FRET melting technology with the new six-color RG-6000 instrument from Corbett Research. Such an assay would allow samples from two different individuals to be successfully differentiated about 99.6 percent of the time. In melting FRET, real-time PCR is performed using two PCR primers and two probes (one hybridizing over the SNP with a dye and the other with a quencher). As PCR proceeds, an increasing percentage of the two probes hybridize to the PCR product and the fluorescence becomes progressively more quenched. After PCR, the probes are melted off. When the probes melt off, the dye is no longer quenched and fluorescence increases. This change can be detected as a peak. If the PCR product is a perfect match to the probe (allele A) then there is one peak at a high temperature, but if the product has the other allele which is not a perfect match (allele B) then there is one peak at a low temperature. A heterozygote will give both peaks; thus, the three genotypes (AA, BB, AB) can be detected.

For the DNA degradation assay, researchers are developing an assay based on three different-sized amplicons (about 65bp, about 123bp, about 250bp) of the Alu sequence. This

assay uses a fixed-forward primer and three different reverse primers. The ratio of the three products give the degradation state of the DNA, i.e. in degraded DNA, the amount of the larger product is decreased relative to the smaller products and in extremely degraded DNA only the smallest product will be obtained. Initial positive experiments using a gel-based readout have been performed in two labs. Scientists have moved to an assay that could be performed on a real-time instrument based on the Plexor™ method by Promega. This method uses dye-labeled primers with isodC and quenchers with isodG. As PCR proceeds, the quenchers are incorporated into PCR product and fluorescence decreases. Using a different dye on each of the Alu reverse primers should allow the amounts of the different-sized PCR products to be quantitated and ratios obtained.

Progress Toward SRM 2372: Human DNA Quantitation

Margaret C. Kline, John M. Butler, Amy E. Decker, David L. Duewer, Janette W. Redman, Peter M. Vallone

The National Institute of Standards and Technology (NIST) is the National Metrology Institute for the United States of America. As such, NIST is responsible for the production of Standard Reference Materials (SRMs) for many different measurement systems. SRMs allow laboratories to establish traceability of a measurement to internationally recognized scales and units.

Quantitation of human DNA for STR typing can be a time consuming and sometimes frustrating operation. The NIST Quantitation Study 2004 (QS04) involved 80 participants who provided 287 independent sets of results from 19 different quantification methods. The results from QS04 indicated that the expected one standard deviation among-laboratory variability for sub-ng/µL DNA concentration samples is about a factor of 1.8. A factor-of-two uncertainty in the amount of template DNA is equivalent to ± a single PCR amplification cycle. QS04 also indicated that the ten quantitative PCR methods systematically gave somewhat different results. Follow-up studies indicate that use of this SRM 2372 Human DNA Quantitation Standard should assist in minimizing these biases.

Plenary Session, Monday, 2:50 pm – 3:30 pm

Forensic DNA Research and Development: Tools for the Crime Lab IV: Laser Microdissection Separation

Laser Microdissection for Low Copy Number Analysis of Sperm from Mixtures

Daniel A. Peterson, Kenneth Pfoser, Emily K. Reisenbigler, Christine T. Sanders

The goal of this presentation is to present the results of recent research in the area of mixture separation utilizing laser microdissection (LMD) for recovery of minute amounts of sperm cells from an epithelial cell mixture. STR analysis has become a routine tool in identifying the source of biological stains in the investigation of sexual assault crimes. Difficulties in analysis arise in the interpretation of mixed specimens or when only a small number of target cells are available for analysis. Development of improved methods for cell separation and tools for the recovery of limited amounts of available sperm cells in evidentiary samples are necessary

to overcome these current problems. In this study, LMD was employed on mixed sperm and epithelial cell smears to identify sperm while selectively dissecting and recovering the cells of interest for forensic DNA analysis.

The feasibility of applying LMD technology to precisely separate low numbers of sperm cells from sexual assault mixtures by visual inspection coupled with laser dissection was assessed through three experiments. First, collections of 5, 10, 20, 40, and 80 sperm cells were recovered from mixtures by LMD then amplified using increased PCR cycles. Second, sperm and epithelial cell mixtures were prepared at cell ratios of 1:5, 1:10, 1:20, 1:40, 1:80 and 1:160 then subjected to both the LMD method and a preferential lysis assay (PL). Finally, real casework evidence samples were subjected to LMD recovery. The results of AmpFlSTR® Profiler Plus typing show (a) Clear genotypes were observed from the male donor with alleles detected from as little as 5 sperm cells using LMD, (b) Detection and separation of male genotypes were greatly improved using the LMD over the PL method, and (c) LMD facilitated successful recovery and genotyping of the sperm donor from casework evidence samples.

The laser microdissection method physically dissects target cells avoiding contamination of adjacent foreign cells in a mixture, and then collects the target cells for direct DNA isolation and PCR. This bypasses the multi-step, high-manipulation process of a preferential lysis procedure and traditional human DNA quantification. Thus, LMD can facilitate the recovery of sperm cells from a mixture for low copy number analysis.

Application of Laser Microdissection Microscopy to Forensic Casework

Pat Wojtkiewicz

The purpose of this project is to develop laser microdissection (LMD) into a technique that will decrease sample analysis time, improve cellular identification methods, and open new ways to analyze mixtures of biological fluids from mixed-sex donors. There are three capabilities pioneered by microdissection microscopy, which we have incorporated into a novel, sequential process that can significantly reduce the usage of differential extractions as the methodology for mixed samples. Decreased analysis times, reduces casework turnaround times and can be accomplished by concurrent microscopic identification and isolation of epithelia and spermatozoa. Improvement in sperm identification by labeling spermatozoa with fluorescent-tagged antibodies can result in rapid detection and subsequent microdissection. Fluorescent in situ hybridization (FISH) labeling of sex-chromosomes in mixed-sex populations of nucleated cells (i.e., non-sperm) will expand the capabilities of analyzing inseparable mixtures seen in forensic samples. Finally, since the samples would not be destroyed by LMD microscopy, processing by conventional differential extraction, PCR, and genotyping with genomic and Y chromosome STRs would still be available as a last resort.

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

DNA Demonstration/Evaluation Projects

A Randomized Control Trial Testing the Cost- Effectiveness of Collecting DNA in High Volume Crimes

John Roman

The Justice Policy Center at the Urban Institute is conducting a two-year evaluation of the cost effectiveness of collecting and processing DNA evidence for high volume crimes on behalf of the National Institute of Justice. The evaluation will measure costs and outcomes of expanded DNA evidence collection using a random controlled trial in five demonstration sites (Denver, Los Angeles, Orange County (California), Phoenix and Topeka (Kansas). Each of the demonstration sites received funding to expand their capacity to collect and analyze biological evidence in high-volume crimes. The five demonstration sites will collect biological evidence from residential burglaries and other high-volume crimes and will randomly assign 250 cases to each experimental condition. Treatment cases will undergo immediate DNA processing. Control cases will be held for 60 days before evidence can be processed (some of the sites will not process control samples ever, or only when an arrest is made for reasons other than a DNA match). Cases in both conditions will be tracked for up to six months to determine whether the DNA evidence processing yields differential case-level outcomes. Outcomes will include: suspect identification from a reference sample or CODIS (the Combined Digital Index System); suspect arrest; and, whether the case is accepted for prosecution. The study will simultaneously collect cost data on program inputs. The price and quantity of each resource used in the case investigation, including the processing of DNA, will be measured to develop estimates of the differential costs of processing. At the conclusion of the demonstration, Urban Institute researchers will compare treatment and control group costs and case outcomes to test whether DNA evidence collection at high volume property crime scenes is cost-effective.

Evaluation of Impacts of Federal Casework DNA Backlog Programs

Heather J. Clawson

NIJ has a contract with Caliber, an ICF International Company to assess the impacts of the Federal Casework DNA Backlog programs over time and to diagnose performance problems, as well as best practices in current casework programs. With an estimated 80 to 90 crime labs supported each year through NIJ grants and approximately 97 unique law enforcement agencies receiving grant funds to support casework programs, the field is rich with information and ready for such an assessment. This presentation will provide grantees with an overview of the study, including a discussion of each phase, project timeline, possible role of the grantees, and implications for the field.

Plenary Session, Monday, 4:30 pm – 4:50 pm

Legislative Updates

Legislative Updates

Lisa Hurst

More than any other forensic science, the quantity, and sometimes quality, of forensic DNA analysis is affected significantly by public policy-makers in Congress, state legislatures, and even city and county councils. These legislators control who can (and cannot) be placed into DNA databases. They can set policy for collection or elimination DNA samples used in investigations. They determine who receives post-conviction DNA tests, and what circumstances must be met. They establish time limitations on the prosecution of crimes. And they certainly control the purse strings of most labs, thereby establishing the amount of DNA analysis that can be conducted as well as other ancillary DNA needs such as training. This presentation will present an overview of state legislative efforts at expanding DNA databases and the ever-increasing role that victims are choosing to play in advocating for broader statutes. The presentation will also discuss other legislative forays into the forensic DNA realms, with statute of limitations issues or John Doe warrants, creation of missing persons programs, and developments in local, state, and federal funding sources.

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

Forensic DNA Research and Development: New Projects

Degenerate Oligonucleotide Primed-PCR: Thermalcycling Optimization for Forensic DNA Analysis

Tracey Dawson Cruz, Denise N. Rodier

Degenerate Oligonucleotide Primed-PCR (DOP-PCR) has been shown to provide high quantities of DNA from low copy number templates in various clinical analyses. It is suspected that DOP-PCR product DNA may also be suitable for achieving high-quality multiplex STR profiles from low copy DNA obtained from evidence samples. In this procedure, a degenerate primer is coupled with low annealing temperatures to theoretically amplify overlapping fragments of the entire genome. Preliminary studies show that while DNA yield is dramatically increased after DOP-PCR, downstream STR analysis provides only partial profiles, most likely due to incomplete genomic coverage. Optimization of thermalcycling conditions may help by improving genomic coverage, thereby improving STR profiles. Currently, the DOP-PCR reaction includes five non-specific amplification cycles with three minutes each of ramping and elongation. By increasing the length of the ramping and elongation times, longer fragments should be achieved, thus ensuring better genomic coverage. By increasing the number of cycles, the DNA fragments should be created in higher numbers for subsequent specific cycling rounds. In the first experiment, the non-specific ramp and elongation times were simultaneously modified ranging from one to 12 minutes. By increasing the ramp/extension time, the average fragment length did increase from 2.7kb to 6.3kb (for 12 minute extension), as expected, without further yield increases. However, at extension times beyond 10 minutes, DNA product was observed in negative control samples. In the second experiment, the cycle number of the non-specific amplification step was modified ranging from three to 15 cycles. As expected, the total yields did increase as cycle number increased. For DNA inputs of 0.125ng, yields increased by 173 fold (for five cycles) to 580-fold (for 15 cycles). The success seen with increasing cycle numbers was even more significant with lower input amounts. However, increasing cycle numbers did not change the average fragment length observed (about 3kb). Future studies will include STR amplification of all DOP-PCR products.

A SNP-based Microarray Technology for Use in Forensic Applications

Giulia C. Kennedy

The use of forensic DNA analysis tools for human identity testing has revolutionized the criminal justice system. Most forensic DNA tests utilize short tandem repeat (STR) genetic marker systems. While STRs have been effective for human identity testing, they provide limited phenotypic information, such as geographic ancestry, about DNA contributors. Therefore, a need exists for the characterization of additional genetic marker systems to augment STR-based analyses of DNA samples. Single nucleotide polymorphisms (SNPs) are the most frequent form of DNA sequence variation in the human genome. Analysis of SNP genetic marker systems can provide additional information about the source of DNA samples, such as hair-color, eye-color, and ancestry. Therefore, a growing need exists for SNP-based analysis tools and technologies that can be appropriately validated and implemented for forensic use. The

goal of this project is to develop an accurate, affordable, microarray-based forensic DNA assay, which utilizes the SNP genetic marker system. The project will utilize the Affymetrix GeneChip® microarray platform to enable highly sensitive, rapid, parallel, accurate, and reproducible SNP genotyping for forensic applications. A forensic assay will be developed for the analysis of three specific forensic sample types: (1) single donor samples, (2) mixture analysis of multiple DNA sources in one sample, and (3) forensically useful sample quantities. Preliminary data will be presented that demonstrates the utility of mitochondrial resequencing and SNP genotyping microarrays for use in forensics applications.

The Determination of the Physical Characteristics of an Individual from Biological Stains: Age Determination

Jack Ballantyne, Michelle Alvarez

It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. However, in those instances where there is no developed suspect as yet or there is no match with any database sample, the DNA profile per se presently provides no meaningful information to investigators, with the notable exception of gender determination. To aid in these investigations another useful biometric that could provide important probative information is the biological age of an individual. For example, the ability to provide investigators with information as to whether a DNA donor is a newborn baby, an adolescent teenager or an elderly individual could be useful in certain cases, particularly those such as kidnapping, involving young children. Currently no reliable validated molecular tests are available for age determination.

Two approaches have been evaluated for their ability to determine biological age: messenger RNA profiling and telomere length analysis. As humans proceed through the biological process of aging there are a number of developmentally recognized stages, each requiring genes to be turned on and off. RT-PCR candidate genes can be tested to determine if their expression patterns are restricted to a single developmental stage. Another approach using real-time PCR evaluates the length of chromosome telomere ends to determine if the repeat number decreases as biological age increases.

This presentation will describe the project goals and experimental design and will provide details of our progress so far.

DNA-based Identificiation of Forensically Important Diptera

Ronald W. DeBry

Dipteran larvae can provide information useful for estimation of PMI. This tool is currently underutilized because of the specialized expertise required to identify larvae to species. Species identification is a fundamentally different problem from identification of a specific individual as the source of a piece of DNA, because a single species will encompass both geographic and individual variation. The appropriate approach to DNA-based species identification is phylogenetic analysis based on DNA sequence data. The project will construct a DNA sequence database that will allow species identification without the need to rear larvae to adulthood. The project will proceed in 2 phases. Phase 1 includes a search for a well-defined mitochondrial locus that provides robust species discrimination. Phase 2 will be construction of

the database, incorporating a large number of samples from the entire continental U.S. The database and a phylogenetic analysis engine will be made available via a World Wide Web interface.

Dielectrophoretic (DEP) Separation of Sperm and Epithelial Cells for Application to Sexual Assault Case Evidence

Mark D. Timken, Martin R. Buoncristiani

A critical step in the successful DNA analysis of most sexual assault cases is the effective separation of male sperm and female epithelial cells. This separation is commonly performed using a two-step differential extraction that exploits chemical differences between sperm and epithelial cells, specifically the greater preponderance of membrane proteins with disulfide bonds in sperm cells. Although the differential extraction procedure is, by and large, effective for a large number of cases, it suffers from drawbacks including loss of sperm DNA to the female fraction in the initial lysis step and loss of sperm cells in subsequent washing steps. In addition, the differential extraction is labor intensive and not especially amenable to automated or microfluidic-based procedures. To address these drawbacks, researchers are investigating the use of a cell separation procedure based on dielectrophoresis (DEP). DEP is the motion of a particle (cell) caused by its dielectric polarization in a non-uniform electric field. The DEP polarization induces an effective electric dipole onto the cell, causing it to move under the influence of the non-uniform electric field. The DEP response of a cell depends very sensitively on such factors as the cell's size and shape, the composition and surface morphology of its membrane, and the ratio of lipid to other molecular components in the cell. Presenters will describe initial experiments to demonstrate that populations of sperm and epithelial cells can be separated using DEP on a microfluidics platform.

Development of a Highly Informative Multiplex PCR and Linear Array Typing System Targeting Variation in the Mitochondrial Genome

Cassandra D. Calloway, Henry Erlich, Sarah Stuart

Mitochondrial DNA, particularly the hypervariable regions I and II (HVI and HVII), has proven to be a useful target for the forensic genetic analysis of limited and/or highly degraded samples. However, there are some inherent limitations to targeting only the HVI and HVII regions independent of the method of analysis. Although the HVI/II regions are highly polymorphic, hence informative for individual identification and the overall distribution of mtDNA HVI/II sequences is highly skewed towards rare types, there are some common types among all populations. Seven percent of Caucasians share the same common HVI/II sequence (differing from the Anderson reference sequence at 263G) and 13 additional sequences are shared among >0.5% of the population. Therefore, to overcome the limited power of discrimination that results from a few relatively common HVI/HVII sequences, additional sequence polymorphisms in the variable and coding regions need to be targeted to increase the power of discrimination of mtDNA analysis.

Scientists proposed to develop a highly sensitive multiplex PCR system that would target the most informative polymorphic sites within the mtDNA genome (coding and VR regions, in addition to the already targeted HV regions). These sites would be typed simultaneously with a panel of 85 probes immobilized on a nylon membrane (linear array). To achieve this goal, primers and probes targeting informative sites outside the HV regions will be added to our current HVI/HVII linear array system. Progress on the multiplex amplification and typing system will be presented here.

Plenary Session, Tuesday, 10:50 am – 12:30 pm

Forensic DNA Research and Development: Ongoing Projects I

Forensic Stain Identification by RT-PCR Analysis and Consequent Development of a New DNA Extraction Method

Trisha L. Conti, Eric Buel

This presentation will combine the latest results from the Forensic Stain Identification grant and describe a new DNA extraction method that arose during the search for an efficient RNA isolation technique. In an age of countless scientific advances in molecular biology, DNA profiling has proven to be an invaluable tool in solving crimes. The potential exists, however, for the tissue origin of the suspect DNA to be called into question. For example, a semen stain containing suspect DNA can have far more serious consequences than a saliva stain. Any cell produces a collection of mRNAs unique to that cell type. A differentiation could be made using mRNA as a fluid- or tissue-specific determinant. This presentation describes results to date using real-time PCR to determine specificity, sensitivity and discriminatory limits of real-time assays, as well as the stability of mRNA over time. A major aim of this grant is to work towards multiplexing the real-time PCR assays once mRNAs are identified in order to clearly define specific types of stains. The study utilizes is the PlexorTM System from Promega. This system allows the multiplexing of four to six mRNAs in one assay, thus reducing the amount of sample needed and time of analysis. The initial focus was to design a blood-semen stand alone assay, since initial studies have identified mRNAs that are specific for these fluids. Therefore, scientists have designed PlexorTM primers sets that detect two blood-specific and two semen-specific mRNAs, in order to ascertain whether blood and/or semen is present in a stain. In the future, researchers hope to greatly increase mRNA multiplexing using a Luminex™ bead-based assay system. This approach allows multiplexing of up to 100 different gene expression assays, which will support the detection of mRNAs from collected fluids or tissues that might be brain, heart, liver, intestine, kidney, skin, muscle, or adipose.

One of the goals of the Forensic Stain Identification grant is to improve RNA extraction so that minimal amounts of sample are used, as well as potentially to extract both DNA and RNA, eliminating the need for two separate extractions. The project has compared RNA yields from numerous commercially available RNA extraction kits, as well as several “home-brew” protocols published in various journals. During this process, staff found a method reported for plant DNA/RNA extraction that was very proficient for DNA extraction. Although this technique does not yet extract ample amounts of RNA, researchers felt it was important to investigate and optimize as a fast and inexpensive DNA extraction protocol. They are in the

process of validating the method for CODIS samples and standards (blood and buccal cells on FTA paper) in order to switch from the current automated DNA-extraction system, which is time-consuming and costly in reagents and supplies. Additionally, the alternative, phenol/chloroform extraction, is not only laborious, but involves hazardous chemicals that require numerous safety precautions and special disposal. Although the new approach is not automated at this point in time, scientists hope to be able to automate or streamline it in the future. It will decrease the time it takes to extract samples and significantly reduce the cost and safety concerns associated with DNA extraction. Studies are currently underway to determine whether the new method can be further refined for simultaneous DNA and RNA extraction, as well as to validate it for casework samples.

Gene Polymorphism and Human Pigmentation

Murray Brilliant

The overall goal of this research project is to determine with a high degree of accuracy, the pigmentation phenotype (skin, eye, and hair color) of an individual subject from a forensic DNA sample. Human pigmentation is programmed genetically. Among the genes mediating pigmentation variation are P and SLC45A2 (also known as AIM1 and MATP), associated with albinism with a spectrum of hypopigmentation. Others include MC1R and ASIP, both associated with differences in the type of melanin pigment in hair (red to black). An additional gene has recently been identified, SLC24A5 (NCKX5 or golden), which plays a role in human pigmentation and correlates with ethnicity. The study involved 800 participants who were phenotyped for pigmentation attributes and genotyped at multiple polymorphisms in these and other genes. Using these data, project scientists have developed a model that uses a relatively small number of SNPs to account for the majority of the phenotypes of four pigmentation aspects: skin color, eye color, total hair melanin, and the ratio of red to black melanin in the hair. Thus, a small number of SNPs can be multiplexed and used to predict a person's hair, eye, and skin color from a forensic DNA sample.

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

Sree Kanthaswamy

With dogs as household pets in at least 40 percent of U.S. homes, canine bio-material, particularly hair and dander, represent abundant and commonly collected evidence at crime scenes. Despite this, canine DNA evidence remains largely under-utilized in forensic investigations. Available canine PCR kits and protocols have been neither optimized with the appropriate validation studies nor furnished with standard nomenclature and macros for the ease of allelic designation. Testimony based on STR analyses of canine DNA evidence by QuestGen Forensics, LLC, in Davis, California, and the Veterinary Genetics Laboratory Forensics Unit at the School of Veterinary Medicine, University of California, Davis, have been used successfully to prosecute individuals by means of linking a perpetrator to a crime scene in instances of murder, burglary and sexual assault and in cases of animal abuse and animal theft. Among the few local and international laboratories that perform canine forensic DNA analyses different STR multiplex panels are being employed. Recent court challenges to canine DNA analysis have demonstrated a need for a standard canine STR panel that has been validated according to human forensic guidelines.

The project proposes to develop and validate a canine-specific 5-flurorescence dye-based STR multiplex with 16 to 20 independently segregating tetranucleotide markers that are robust, reliable, and informative for regionally representative dog breeds including mixed-breeds (or mongrels). The panel will also incorporate the sex identification Amelogenin gene. Research staff also propose to develop the components needed for broader use of the canine STR typing by the forensic community.

Generating More Precise Post Mortem Interval Estimates With Entomological Evidence: Reliable Patterns of Gene Expression Throughout Calliphorid Larval and Pupal Development

David R. Foran, Aaron M. Tarone

Insects, especially flies (Diptera), can be important tools in the investigation of deaths, as they eat and breed on decaying remains. Blow flies (Diptera: Calliphoridae) are primary successional species on carrion and can lay eggs on a body within minutes of death, thus they are important indicators of post mortem interval (PMI). Traditionally, forensic entomologists have used blow fly developmental stages (sometimes in conjunction with overall body size) to help estimate a PMI. This approach can be reasonably accurate, but some developmental stages span a relatively large range, making PMI estimates broad. Further, error rates for these estimates have not been determined, meaning they do not meet a basic requirement of Daubert.

Extensive laboratory research in the common fruitfly Drosophila melanogaster and other insect species has shown that the complex changes that occur throughout insect development are governed by the regulation of thousands of genes. The primary goal of the research presented today is to use developmental gene expression in blow flies (in this case, the widely distributed green blow fly, Lucilia sericata) to more accurately age them on remains, thus creating a more precise PMI. The utility of this technique is being developed on flies from Michigan, and being confirmed on flies from two other ecoregions. Secondary goals include perfecting laboratory fly rearing techniques so that they best mimic larval growth on a carcass, and developing growth measurement models so that confidence intervals in age estimates can be determined. Towards these goals, more than 2500 individual immature flies (larvae and pupae) from three regions (Michigan, California, and West Virginia) reared under laboratory conditions have been measured, weighed, developmental stage recorded, and been fixed in the RNA preservative, RNAlater. Over 400 Michigan samples at all immature ages have been assayed for gene expression levels. Tested loci included 12 potentially development-specific genes and two housekeeping genes (internal mRNA concentration standards). Three developmental genes were excluded as their time-series profiles yielded little or no useful information. The remaining nine genes were statistically significant predictors of age, and other loci are being tested.

Mathematical models were designed to make predictions of insect age based on the collected length, weight, and stage data, representing those factors typically used by forensic entomologists. The models were able to provide mathematically defined confidence intervals on age predictions, factors important in meeting *Daubert* requirements. A model incorporating stage and length was then compared to an independent data set—larval growth on carcasses (rats) under the same environmental conditions. The models were highly predictive during feeding stages of larval growth, but were not predictive during post-feeding flies (third instar and pupation). Finally, gene expression was added to the equation. While not fully tested, models incorporating gene expression appear much more accurate in predicting age, particularly for the difficult post-feeding stages.

The Use of MiniSTRs as Tools for the Investigation of DNA Degradation and Inhibition

Bruce McCord

In previous work on this project scientists initiated a collaborative study with the National Institute of Standards and Technology to develop a new set of multiplexed PCR reactions for the analysis of degraded DNA. These DNA markers, known as Miniplexes, utilize primers that have shorter amplicons for use in STR analysis of degraded DNA. In the work researchers defined four new STR multiplexes, each of which consists of three to four reduced-size STR loci, each labeled with a different fluorescent dye. Reductions in size of up to 300 base pairs are possible with these new amplicons.

Using the Miniplexes, staff achieved 99.77 percent concordance between these new loci and Profiler Plus. In the 532 samples examined, there were 15 samples that showed discrepancies at one of 12 loci. These occurred predominantly at 2 loci, vWA and D13S317. Developmental validation of these kits has now been completed. Examination of sensitivity, peak balance, PCR conditions, environmental contamination and the resolution of mixtures have been carried out. Quantitative PCR has been used to assess DNA quality and the results show the Miniplexes to perform well at template concentrations above 125 pg. They are also quite useful for low copy number DNA. In the analysis of human skeletal remains, 64 percent of the samples generated full profiles when amplified with the Miniplexes, while only 16 percent of the samples tested generated full profiles with the Powerplex® 16 commercial kit.

In the current work, project staff are examining the application of the Miniplexes to degraded and inhibited DNA samples. It is the goal to create the tools necessary to provide laboratories with better information on how reduced size STR markers respond to PCR inhibitors as well as degraded DNA. They will examine the effect of amplicon size on inhibition processes and work to better understand the mechanisms involved. Investigators will then use this data to provide guidelines on how to best address such samples.

Working Lunch, Tuesday, 12:30 pm – 2:00 pm

Demonstrations and Practitioner Posters

Polymeric Microfluidic DNA Analysis System for Forensic Analysis

Laurie Locascio, Annelise Barron, Michael Gaitan, Jon Geist, John Kakareka, Nicole Morgan, Tom Pohida, Jayna Shah, Paul Smith, Wyatt Vreeland

Microfluidic technology is a promising alternative to capillary-based techniques due to its great potential to miniaturize, simplify, integrate, automate, and mulitplex analyses for higher throughput and speed. This technology has already shown that it is the next revolution in DNA technology, with analysis often complete in less than ten percent of the time required for more traditional capillary technology. Although DNA analysis systems based on microfluidics technology have recently been commercialized, these systems do not meet the specific needs of the forensic community due to poor separation resolution of the relatively long fragments (order 100 to 400 bps) as well as incompatibility with standard test procedures.

A team made up of researchers from several federal and academic institutions have used their expertise in microfluidics, microfabrication, DNA separations, as well as state-of-the-art optics and detection to build a prototype system that can be used as a model to demonstrate the

advantages of applying these latest technologies for forensic identification. The system has the following constituent components: integrated multicolor optical fluorescence detection with multichannel free space laser excitation, fiber optic fluorescence collection, and multichannel/multicolor spectral imaging; automated solid state voltage sequencing with current monitoring and recording; data processing software for spectral deconvolution, electropherogram baselining, and peak calling; a plastic microfluidic device with optimized surface treatment and coating, 2-dimensional injector design, and 8 fluidic separation channels; and a high viscosity polymeric matrix pneumatic loading device. This system is now capable of analyzing forensic DNA samples to establish human identity with approximately a five-fold increase in the speed of analysis over current state-of-the-art instrumentation. The heart of the system is the inexpensive plastic-based microfluidic device. The plastic microfluidic device defines the NIST work as significantly different from other microfluidic platforms that are fabricated in expensive glass substrate materials. The use of plastic materials presented significantly different design challenges compared to glass, particularly with respect to the need for novel surface coatings and chemistries as well as new detection approaches. Project scientists chose to use plastic substrates due to the fact that the raw materials and associated microfabrication technologies are significantly less expensive and may be used to fabricate single-use systems thereby eliminating any doubt about sample cross contamination in forensic cases. The low cost of these devices also makes them more accessible to budget-limited laboratories.

A Miniaturized Forensic DNA Analysis System for SNP-based Detection

Nathaniel C. Cady, Carl A. Batt, Viktor Koltko, Scott J. Stelick

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 by 25cm by 15cm unit, weighing approximately 8 lbs. 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. With 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, we have developed assays that distinguish biological samples as originating from males or females using multiple loci 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. In parallel, a fluorescent bead-based assay is being developed to provide highly multiplexed detection of up to 20 different targets. Finally, the microFLUIDICS DESKTOP system is being modified to include on-board data analysis as well as wireless data transmission for teleforensics applications.

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

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

The project is developing microfabricated capillary array electrophoresis (mCAE) instruments with the ultimate goal of integrating sample amplification, product clean-up and multiplex STR electrophoretic analysis of up to 96 samples on a single-wafer system. The 96-lane mCAE system has been tested at Berkeley using samples conventionally prepared with the PowerPlex® 16 and AmpFlSTR® Profiler Plus® kits. Studies on the speed and resolution as well as analyses of sensitivity, mixture and non-probative samples have been successfully performed (1). A prototype of the mCAE instrument has also been developed, and researchers are in the process of installing this instrument at the Virginia Division of Forensic Science for critical evaluation.

Project scientists are also working on the development of improved reagents and methods for forensic identification. An enhanced multiplex PCR system using energy-transfer (ET) cassette labeled primers (2) for the loci used in the PowerPlex® 16 kit is being constructed. The expected sensitivity improvements range from 2-fold for the FAM-labeled loci to 8-fold for the TAMRA-labeled loci; these improvements should enable amplification from lower copy number samples or with fewer PCR cycles. Using these ET-labels, scientists have also successfully constructed a 4-multiplex sex chromosome typing system consisting of the amelogenin, DYS390, DYS393 and DYS439 loci. The production of small Y-specific DNA fragments combined with the improved sensitivity provided by ET labels will be particularly useful in forensic case work on sexual assault evidence and for male sibling identification.

More advanced analysis systems that include sample cleanup are also in development based on the use of a linear acrylamide gel conjugated with oligos designed to capture specific STR products in an amplified sample (3). This gel-phase capture approach permits the allelic products to be normalized and desalted for effective electrophoretic analysis. Project staff expects this approach to be particularly useful for low copy number amplification and degraded DNA amplification.

A portable genetic analysis microsystem has also been developed for fast on-site forensic DNA identification. Please refer to Peng Liu's research for details of the demonstration.

The National Clearinghouse for Science, Technology, and the Law (NCSTL)

Carol Henderson

The relationship between law, science, and technology is both an essential alliance and a reluctant embrace. One reason for this tension is the lack of a free flow of information between the legal and scientific communities. Worldwide, developments in science and technology are occurring at a rapid rate. Legal challenges are being made to emerging fields like biometrics. Even scientific evidence that has been relied upon for years is facing challenges. This explosion of litigation, coupled with the "CSI Effect," has resulted in the forensic science community being overwhelmed by the amount of information required to meet these challenges.

Until recently there was no one centralized source that allowed one to navigate all the existing case law, journals, reports, and resources necessary to conduct effective investigations and litigation. Supported by a National Institute of Justice grant, NCSTL has developed a

searchable online database accessible at www.ncstl.org. The database includes more than 30 topics ranging from bloodstain pattern analysis to voice analysis. Within each major topic, the following resources are available: court cases, scientific and legal books and periodicals, legislation, conference proceedings and courses, and other relevant information.

In addition to the searchable online resource, NCSTL is building partnerships with universities, agencies and professional associations. Educational opportunities are being developed in the form of a national conference, seminar series, primers, and training modules. NCSTL is also building a reference collection, which is available through interlibrary loan. The Clearinghouse is supported by a grant from the National Institute of Justice (#2003-IJ-CX-K024).

The Forensic Resource Network

Debra A. Figarelli

Debbie Figarelli, with the National Forensic Science and Technology Center (NFSTC) will be available to discuss current NFSTC projects. These will include external DNA audits, grant progress assessment, and the President's DNA Initiative: Analyst Training programs.

Combined Pressure and Electro-Extraction and Concentration of DNA for Microfluidic Forensic DNA Analysis

Joan Bienvenue, Jerome P. Ferrance, James M. Karlinsey, James P. Landers

The effective extraction of DNA for forensic applications requires purification of DNA from a wide variety of sources. Current methods for extraction and purification involve the use of extensive chemical extraction protocols that are time- and reagent-consuming, labor-intensive, and costly. These methods have, in part, contributed to the casework backlog currently facing crime laboratories on local, state, and federal levels. Expedition of these preparatory steps through miniaturization, in concert with multiplex analysis for higher sample throughput, provides an attractive means for addressing this problem. In conjunction with ongoing research to design microchip-based methods for STR analysis of DNA, the proposed research seeks to develop a microchip-based dual pressure-electro extraction and concentration method for the extraction of DNA from forensically-relevant samples. This research demonstrates the use of an electric field during the DNA elution phase of the SPE to enhance recovery and to provide a more concentrated sample for downstream genetic analysis. A glass device designed with dual pressure/electro-elution capabilities is described, with results from preliminary testing detailed. The device allows for continuous, syringe-driven flow to be accomplished while an electric field is applied. Using this device, a typical solid phase extraction (sample load, protein wash, DNA elutions) using pressure-driven flow is accomplished, with the electric field imposed during the final elution step to both trap DNA as it exits the device as well as to enhance DNA recovery. The ability to trap and retain DNA during flow is demonstrated, with further demonstration of precision DNA elution following termination of applied field. Retrieval and concentration of DNA from a silica-based solid phase using the dual purpose design is demonstrated. The reduction in sample volume/increase in sample concentration as it relates to current microchip PCR methods will be discussed.

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

Eric Buel

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. The "faster, more robust and less labor-intensive identification of sperm in the analysis of DNA evidence" can assist the forensic scientist in determining the appropriate use of resources and of crime scene samples. A method that could quickly and accurately screen slides for sperm could decrease the case turn around time for sexual assault cases, more accurately determine which cases would be suitable for autosomal versus Y STR analysis and re-direct staff to assist in other aspects of the biological analysis of the case. Such processing and analysis could give the analyst valuable sample assessment information, saving time and money that could be directed to other analyses.

Two vendors have each supplied a system that consists of a microscope with motorized focus; a computer-driven stage that accepts multiple slides; a video system to import images into a computer; and software to drive the microscope stage, focus the scope, and interpret images. The software utilized for this project has been employed for other cell discrimination purposes and is being modified to locate spermatozoa. At the present, both systems have implemented spermatozoa identification algorithms designed to be used on "Christmas-Tree" stained smears and can search a slide for the presence of spermatozoa. However, the software is still in the developmental stage and is being refined to optimize performance. One system currently takes about 15 -20 minutes to completely scan a slide at 400 X. However, scanning time is typically much shorter on sperm positive slides. The system automatically saves images of candidate spermatozoa found and the identified cells can be easily reviewed on the computer monitor for user confirmation. In addition a "return to" capability is being added, to enable optional review of candidate sperm by a user directly through the eyepieces of the microscope. A key area of continuing work involves sparse cellular preparations which occasionally cause problems for the currently implemented automated focusing algorithm, because there are too few objects on which to reliably focus. Automated focus performance in such instances has improved by utilizing focal references derived from an inscribed circular sample boundary printed on the surface of the slides. These references enable a default plane of focus to be computed that may then be automatically "walked" during scanning. In addition, to providing more reliable focus, this modification is expected to further speed slide scanning. The system is presently useful, as multiple slides can be loaded and assessed in a single run, and cell locations on the slides are noted automatically. Additional algorithm refinement and research activities are underway to reduce particular types of false positives, to further examine the limitations, and to extend the practical utility of the searching program. Of interest is the question: Can the instrument be trusted to call a slide "negative for sperm?"

DNA.gov – Assistance, Information and Training

Lee Mockensturm

DNA.gov is an evolving site. We are working on some new training courses and kicking around different ideas but would love to know what you would like to see included. Examples of what we're working on include training courses for DNA analysts, and prosecutors and an update to current officer training; State-specific profiles including data and reports; and adding greater technical resources for forensic scientists. New features already included are home-pages for various audiences, lists of NIJ-funded research articles, training for officers of the court, and presentation of case studies that show the range of uses for DNA evidence.

NIST: On-going Projects to Aid the Human Identity Testing Community

John M. Butler, 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 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. The staff conducts 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 this work are made available on the STRBase website: http://www.cstl.nist.gov/ biotech/strbase/ NISTpub.htm. This presentation will inform meeting participants regarding latest work in DNA quantitation, miniSTR marker development, new Y-STR loci, and mtDNA analysis.

Plenary Session, Tuesday, 2:00 pm – 4:45 pm

Resources and Assistance for the Forensic DNA Community

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. The 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.

NIJ's Grant Progress Assessment Program

Mark Nelson

The Grant Progress Assessment (GPA) Program was created by the National Institute of Justice (NIJ) in order to improve grant oversight and program management. The assessments are funded by the NIJ through its cooperative agreement with the National Forensic Science Technology Center (NFSTC) and conducted in conjunction with the Free DNA Audit Program delivered by the NFSTC. This presentation will provide an overview of the GPA program, its goals and objectives, and NIJ plans to expand the GPA beyond DNA grants to include the Paul Coverdell Forensic Science Improvement Program and the Solving Cold Cases with DNA program grants.

Update on the Grant Progress Assessment Program

Jeff Hickey

The Grant Progress Assessment (GPA) program is a 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 the National Forensic Science Technology Center (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, and as well as future plans and deliverables. In addition, information will also be provided on the personnel resources NFSTC has to staff these assessments, how to prepare for a GPA or DNA program assessment (or both), and resource information available to the community on the NFSTC website.

The National Institute of Justice Expert Systems Testbed Project: Analysis of Single Source Samples and Casework Samples

Rhonda Roby, John Paul Jones

Phase I, the evaluation of expert systems for single source samples, is complete. The National Institute of Justice Expert Systems Testbed (NEST) Project Team has evaluated three (3) commercially available expert systems, conducted five (5) demonstration sessions, produced one (1) workshop, and written a handbook. The handbook, entitled "Forensic DNA Databasing: Expert Systems for High-Throughput Processing of Single Source Samples," is now available.

In summary, the NEST Project Team conducted a thorough evaluation of the commercially available software programs/systems that met the definition and scope of an expert system for the purposes of this project. These expert systems can be validated and adopted into the workflow of the laboratory to improve efficiencies in the data review of single source samples. Each laboratory should consider its specific needs when selecting an expert system. Once an expert system is purchased, the laboratory should validate the system according to Appendix B in the NDIS DNA Data Acceptance Standards Operational Procedures and request approval from the NDIS Custodian prior to use for upload of convicted offender samples into NDIS.

Phase II of the NEST Project is the evaluation of commercially available expert systems for forensic casework samples including inhibited DNA, degraded DNA, and mixture DNA data. The NEST Project Team will study the ability of different software systems to evaluate challenged samples and controlled mixture data for major and minor profiles and apply this information to casework analyses. Currently there are no guidelines for an expert system involving the review of mixed DNA results. The Project Team is currently developing a set of evaluation criteria for the assessment of mixture data for Phase II. These evaluation criteria, the design of the mixture experiments and challenged samples, and a summary evaluation of Phase I will be presented.

Selecting an Expert System: Making the Right Decision

Sindey Schueler

The National Institute of Justice's (NIJ) Convicted Offender DNA Backlog Reduction Program has made tremendous progress in addressing the offender samples waiting to be profiled for CODIS. However, the success of profiling the offender backlog has created a bottleneck in performing the required 100 percent technical review of data for many forensic laboratories. The technical review must be completed before entry into CODIS, and many forensic laboratories are faced with the challenge of performing the technical review of massive amounts of data in a timely manner with limited staff. One option available to eliminate this bottleneck is to implement an expert system for data review. Expert systems will enable the forensic community to meet the challenge of performing timely technical review of data before CODIS entry. Several potential expert systems have been tested through NIJ Expert Systems Test Bed Project. Since several options are available, forensic laboratories are faced with making a decision in selecting the "right" potential expert system. This presentation explores some of the factors taken into consideration by a small state laboratory in the decision-making process of selecting a potential expert system.

Expert Systems at the California Department of Justice: Lessons Learned

Scott Nagy

On November 2, 2004, California voters passed Proposition 69, establishing an all-felon DNA Data Bank that also includes adults arrested for specific felony offenses and sex and arson registrants. Since that time, the number of samples submitted to the CAL-DNA Data Bank has increased from approximately 3,000 blood samples to over 20,000 buccal samples per month. To reduce the resulting backlog, we are assessing the value of expert systems to augment or significantly reduce manual data analysis and technical review of data bank STR profiles. During this evaluation, we identified a number of issues critical to the success of implementing an expert system. Although the present protocol for buccal sample analysis provides a high

success rate, much of the data requires manual review and confirmation due to low peak height or the presence of artifacts that aren't resolved or identified by some expert systems. Because advanced STR typing software now requires less effort to manually review high quality data, an expert system would not significantly reduce our analysis time unless the concentration and yield of DNA recovered from these buccal collections could be increased. To accomplish this, existing extraction protocols have been changed and an alternative validated, along with increasing buccal collection training. Since NDIS procedures require a separate validation for each analytical method generating data to be analyzed using an expert system, having a mature and firmly established analysis method is essential prior to undertaking the validation of an expert system.

Validation of the TrueAllele® Automated Data Review System 2.0

Barry Duceman, Tom Leach, Peter Wistort, Jill Roberts J, Carole Schweigert, Nicole Carter

The Convicted Offender DNA Databank at the New York State Police Forensic Investigation Center in Albany has completed developmental validation of TrueAllele® System 2.0, which is an expert software system, designed by Cybergenetics Inc., to automatically perform quality analysis of DNA profiles from single source samples. Like other state convicted offender laboratories, the New York DNA Databank has recently been confronted with a significant increase in the number of offender submissions. To meet this challenge, the forensic community continues to advance in the application of high-throughput DNA extraction and analytical technology. As a result, downstream data review has become a significant bottleneck. The results of the present study indicate that the TrueAllele® technology offers a viable approach to solving this problem by significantly reducing the time required for data review. The developmental validation of TrueAllele® System 2.0 which the project has completed was designed and performed to be in accordance with the NDIS Appendix B guidelines and the Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories. The validation is specific for samples amplified with AmpFlSTR Cofiler® and Profiler Plus® kits and subsequently analyzed with the ABI Prism® 3100 Genetic Analyzer. The validation consisted of a reiterative optimization (or "calibration" phase) that is required to establish the rules and custom thresholds for analyzing STR DNA data. After calibration, an extensive concordance study was performed to ensure agreement with the results from manual review of data generated in Genotyper®. In total, 2,303 unique samples were processed for the validation study.

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

Forensic DNA Research and Development: Ongoing Projects II

DNA NanoChips: Pushing Technology Limits to Nanoscale and Single DNA Molecule Analysis

Laura Mehrmanesh

Presenters report on progress made in the development of a broadly enabling NanoChip technology platform aimed at advancing the base technology of DNA Forensics to a fundamentally new level, pushing technology limits to the nano-scale and enabling applications for single DNA molecule analysis, proteinomics, and mutation detections. A multi-disciplinary team of researchers, consisting of materials scientists, chemists, biomedical engineers, electrical engineers, and physicists, has been assembled at Brown for this ambitious undertaking. Two fundamentally new and complementary nanotechnologies for DNA identification are under development by the team. One is based on the molecular sieving function of a highly ordered carbon nanotube array with a 100nm periodicity and a 40nm gap. DNA strands are separated by length at a rate that is theoretically projected to be an order of magnitude faster and over a distance that is many times shorter than conventional electrophoresis. The second approach is complementary, bringing the detection limit down to single DNA strands and enabling a direct on-chip electronic readout detection method. This approach focuses on the change in the ionic current over time as STR-tagged DNA passes through the nanopore. The STR-specific double-stranded DNA current is different from the untagged single-stranded DNA current, therefore allowing real-time DNA profiling.

Significant progress has already been made in this initial (1.5 year) phase of the ambitious long-term development of a completely new base technology for DNA forensics. Scientists have designed, fabricated, and experimented with several types of nanotube arrays as well as silicon nanoposts, gold nanorods, and polymeric nanoposts for DNA separation by molecular sieving. Researchers have also succeeded in developing a fabrication process for and creating several single-nanopore structures that would otherwise have taken several years by more conventional (and more costly) approaches. We have designed and fabricated microfluidic channels with NanoChip molecular sieves incorporated into them, and tested the fundamental aspects of DNA transport and separation efficiency. The project has also developed and scaled up nanofabrication methods and nanofabrication facilities to enable the undertaking.

A Dog mtDNA Database

Marc Allard

Project staff is in the process of building a dog mtDNA database for the control region sequences for 500 dogs, and a second database of the complete mtDNA genome sequence for 100 dogs. The second database will support the first in providing additional sites that will break up the most common haplotypes observed. Tissues and blood have been collected for a wide diversity of dog samples. This includes both registered animals as well as unregistered mixed bred dogs. DNA has been extracted on over 200 specimens. Sample preparation, PCR, and sequencing have been optimized for dog DNA with 10 primers covering the control region and 86 primers for the complete mtDNA genome. Sequencing has been conducted largely on an ABI

capillary sequencer. Sequences are read in both directions to insure fidelity to the sample and those contiguous are assembled using the sequencer software. The hypervariable repeat region is excluded from the alignment and from further analyses. Consensus sequences are compiled into the Nexus format where they are phylogenetically analyzed using software. Additional data editing is conducted for analysis using population-based software. Preliminary evidence shows considerable variation in both regions, with over 100 variable SNPs observed for the complete mtDNA genome and approximately 40 SNPs in the control region. Together these discriminate most dogs though there are still some animals that share common haplotypes. Our projections are that the control region sequencing will be completed in October and the complete mtDNA genome sequencing with be completed by the end of the year.

Microarray Based Resequencing of MitoDNA: Promises and Pitfalls for Forensic Applications

Linda D. Strausbaugh, Michael Adamowicz, Dione Kampa Bailey, Richard Chiles, Giulia Kennedy, Carl Ladd, Henry C. Lee, Lu Li, Heather Nelson, Igor Ovtchinnikov, Dan Renstrom, John Schienman, Josh Suhl, Sean Walsh

Microarray-based resequencing of the mitochondrial genome presents several attractive features for forensic applications (especially for reference/known samples), including built-in redundancy for sequence confirmation and the capacity for very rapid acquisition of the entire genome sequence. This project has assayed the accuracy of resequencing by challenging arrays with DNA from ten individuals who represent different maternal lineages from the Connecticut Geographical and Genetic History Collection. Scientists find that quality results can be obtained with small amounts of freshly prepared or stored total genomic DNA isolated from buccal swabs. The call rate is very high and all SNPs identified (compared to the RCRS) were identical in replicate samples. When "n" calls are made, they often occur at or adjacent to the poly-C runs or in dense areas of SNPs. In seven of the ten samples, there is complete concordance of sequences in HV1 and HV2 between microarrays and standard capillary sequencers. One of the DNA samples (Jamaican lineage) generates numerous "n" calls in HV1 and HV2 upon resequencing due to multiple SNPs in short intervals. The remaining two samples (Russian lineage and Chinese lineage) have the same base miscalled at the beginning of a polyC run. Indels in the hypervariable regions may also be problematic for resequencing. The performance of microarray-based sequencing outside of the hypervariable regions is excellent with respect to both call rate and accuracy. Immediate and long range strategies for improving microarray resequencing will be presented. Strengths and weaknesses of this approach for forensic applications will be discussed.

Plenary Session, Wednesday, 10:10 am – 11:50 am

DNA Training Programs

NIJ's DNA training for the Criminal Justice Community under the President's DNA Initiative: Products for the Community

Charles Heurich

NIJ is the research, development, and evaluation agency of the U.S. Department of Justice and is dedicated to researching crime control and justice issues. NIJ provides objective, evidence-based knowledge and tools to meet the challenges of crime and justice, particularly at the State and local levels.

To increase the use of DNA technology in the criminal justice system on March 11, 2003, President Bush announced a five-year, more than $1 billion initiative: "Advancing Justice Through DNA Technology." One of the goals of this initiative is to maximize the use of DNA technology by developing training and providing assistance on the collection and use of DNA evidence to the wide variety of professionals involved in the criminal justice system including police officers, prosecutors, defense attorneys, judges, forensic scientists, medical personnel, victim service providers, corrections officers, and probation and parole officers. This presentation gives an overview of the progress and products that some of the projects funded under the President's DNA Initiative have produced to this point, as well as some thoughts for the future.

DNA Training Efforts of the Forensic Resource Network

William J. Tilstone

The main training tools developed by NFSTC in the last 12 months, funded under the President's DNA Initiative, include the release of an interactive CD "DNA for Officers of the Court", with an accompanying workshop program; the completion of a pre-release version of the DNA Academy training for new DNA analysts using interactive multi-media instruction for the knowledge base, converted from a face-to-face to distance delivery format, and accompanied by a practical manual and competency record; delivery and capture of a series of workshops on DNA topics, including: Population Genetics; Method Validation; Mitochondrial DNA Screening; Quantitative PCR; and Implementation of GeneMapper ID software (for release as DVD / computer-based instruction packages; and the development of a curriculum for pre-DNA screening of biological samples for face to face delivery monthly in July through December 2006.

NIJ Forensic Resource Network

Terry Fenger

Marshall University Forensic Science Center (MUFSC) is a member of the NIJ Forensic Resource Network and has a mission to provide training to the forensic science community. Though the President's DNA Initiative, courses in advanced DNA technologies are being offered to the forensic science community. To date, 14 state agencies have participated in week-long training workshops that include sessions on automation, high throughput instrumentation for

DNA quantitation and analysis, and the use of expert systems to analyze DNA data. The focus of current and future trainings will be presented and feedback on the direction of future training agenda will be sought.

A Virtual Practicum to Train Sexual Assault Forensic/Nurse Examiners in the National Protocol

Joseph Henderson

The Interactive Media Laboratory at Dartmouth Medical School is developing an advanced distance learning program for health care practitioners who perform, or who may perform, sexual assault medical forensic examinations. The program applies IML's Virtual Practicum training model and methodologies to disseminate the concepts and procedures contained in "A National Protocol for Sexual Assault Medical Forensic Examinations" (National Protocol). This protocol was developed by the Office on Violence Against Women, U. S. Department of Justice, under the President's DNA Initiative. The Virtual Practicum incorporates mentor/apprentice learning strategies, patient-based learning via rich-media virtual patients, lectures, computer-based activities, interviews with patients and practitioners, and role-modeling by experts, all in a graphically integrated learning environment. Examples of the Virtual Practicum and other programs can be run from IML's website: iml.dartmouth.edu. A broadband connection is required to run the programs. There are also papers describing the Virtual Practicum model and its theoretical basis.

DNA: A Prosecutor's Practice Notebook

Paula H. Wulff

The American Prosecutors Research Institute, through NIJ grant funding, has developed a 5.5 hour on-line training program designed to provide state and local prosecutors with a basic understanding of DNA and its application in the courtroom. The material is organized into lessons which walk the user through investigating, preparing, and presenting cases involving DNA-related evidence. Throughout the lessons the user is provided with focused "practice points" to supplement the topics presented as well as "knowledge checks" which enable the user to assess their understanding of the lessons. The lessons are interactive and rely upon a variety of scenario-based means to convey the material and its application to the law. *The Notebook* will be available online and in CD-ROM format upon request and will become a pre-requisite for all students attending APRI's live DNA training courses. APRI is grateful to NIJ for its support and to NFSTC for its guidance in developing this project.