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Development and Implementation of Forensic Science Research and Training Programs at the University at Albany’s Northeast Regional Forensic Institute (NERFI)

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ABSTRACT

The Northeast Regional Forensic Institute (NERFI)-instructed ABI 3130xl™, ABI RTPCR™, and Leadership Assessment workshops were attended by forensic scientists from across the United States. Course evaluations were completed and indicate a favorable assessment of the workshops content, the instructors, mode of instruction, and NERFI facilities and support staff. Offering travel and per diem for all participants, linking NERFI’s web site with NIJ’s web site, and meeting with various directors and supervisors from the forensic community provided the impetus on educating 104 trainees in DNA Analysis and Leadership Assessment skills.

Near-infrared (NIR) Raman spectroscopy was used to measure spectra of dried human semen samples from multiple donors. The major chemical components that contributed to the Raman spectrum of semen were determined and used to identify the principal spectral components. Advanced statistical analysis of spectra obtained from multiple spots on dry samples showed that dry semen is heterogeneous and its Raman spectra could be presented as a linear combination of a fluorescent background and three spectral components. The relative contribution of each of the three components varies with donor, so no single spectrum could effectively represent an experimental Raman spectrum of dry semen in a quantitative way. The combination of the three spectral components could be considered to be a spectroscopic signature for semen.

The Judicial Awareness of Forensic Science project evaluated the use, impact, and effectiveness of forensic evidence in the courtroom. The preliminary results of this review indicate that forensic evidence definitely played a crucial role in some of the cases and investigations reviewed under this project. In addition, eighty first responders were trained on the proper collection, handling and storage of biological evidence. Furthermore, funding from this award was used to purchase equipment from Porter-Lee and update existing technology to maximize the ability of law enforcement to process crime scenes in the most efficient and effective manner and to permit real-time access to uniform evidence inventory and management files by the District Attorney as well as the police department.

The objective of the ChatMinder project was to conduct a study of the dialogues occurring in the on-line chat rooms. A secure chat-room at the ILS Institute was established and chat data collected from experiments from recruited subjects (SUNY students under an IRB protocol). Over 20 hours of chat involving groups of 3 to 6 people on topics ranging from movies to organic food to state of the economy was collected for this study. VCA technology represents an important advance in automated human-computer communication with potential applications in cross-cultural social modeling, influence operations, advertising, law enforcement, and national security.

Analyzing crime data to make defensible judgments has become increasingly difficult due to the disparate sources of data - each element of which paints only a part of the complete picture. In addition, data can be both structured (log files, spread sheets, and databases) and unstructured (plain text, web pages, word documents etc.). Amalgamating all the evidence into a cohesive stream of evidence requires analysis capability that not
only handles varied data sets but was able to find correlations among the data. Under the Capitol Region Cyber Crime Partnership section of this award, a suite of tools was employed to collect data from open sources, analyze text data, and correlate information from multiple sources including sexual predator behavior and detecting attacks on the network.
EXECUTIVE SUMMARY

As described in the topical headings below, the overall goal of the National Institute of Justice Congressionally-directed award (2008-DD-BX-K301) was to provide the University at Albany’s Northeast Regional Forensic Institute (NERFI) funds for forensic science research, and to develop and implement various professional development training programs for the criminal justice community. Most of the research described in this report was funded at levels only to explore novel concepts in the forensic sciences. The majority of funds from this grant were used to support cost-free instruction and travel in state-of-the-art DNA Analysis Technology and Managerial Assessment workshops. In view of the diverse nature of the individual activities carried out under this project, each activity is characterized herein as a “Module”.

Module: Forensic DNA Training, Leadership Assessment and Research
- **A**: ABI 3130xl™ Training (Appendix 1)
  - Three separate weeks of lecture plus hands-on
    - 6-8 trainees per session
- **B**: ABI RTPCR™ Training (Appendix 7)
  - 2 - 2 ½ day training sessions
  - At least 6 trainees per session
- **C**: Leadership Assessment Workshops for Managers (Appendix 13)
  - 2 - 2 day training sessions
  - 6-10 trainees per session
- **D**: Raman Method Validation Applied to Biological Evidence

Module: Judicial Awareness of Forensic Science
- Determine the impact of new forensic technologies in the court room
- Identify forensic evidence applications in Schenectady County criminal cases
- Train Schenectady law enforcement first responders in the recognition, collection, and preservation of forensic evidence (Appendix 17)
  - Approximately eighty (80) officers were trained in groups of 20
- Set up a system from Porter-Lee to bar-code evidence in the Schenectady, New York DA’s Office and to permit real-time tracking for evidence analysis, inventory control, and disposition purposes in a shared system with the Schenectady PD.

Module: ChatMinder: A Safe Internet Tool for Parents
Graduate level assistance is needed to perform:
- Design and apply new advanced language and information technologies developed at the University at Albany for assisting automated intelligence gathering and analysis to detect and prevent of online crime against children as well as other persons or organizations.

Module: Capitol Region Cyber Crime Partnership
- Design and apply new Cyber Crime tools to identify and prevent sexual offenses against children on the internet.
Module: Forensic DNA Training, Leadership Assessment Workshops, and Research

The forensic science community needs structured training and research in an academic environment. It is important to note, that over 700,000 sworn police officers have available 632 police academies in the United States (Hickman, 2002). The academies are collectively expending over $725,000,000. The Alcohol Tobacco and Firearms (http://www.atf.treas.gov/training/arsonex.htm) funds (except travel and per diem) an eleven month firearms academy and numerous other short courses. The time is now to properly support forensic DNA training programs.

NERFI has a proven record of providing structured learning in an academic environment. NERFI was conceived to address a similar need for structured forensic laboratory training developed with graduate level academic standards delivered in an intensive 16-week program in lieu of inefficient, unstructured mentor-based training programs traditionally afforded newly hired forensic laboratory personnel in state and local crime laboratories. NERFI’s five-year record of accomplishment for delivering graduate level forensic training for DNA casework and databank scientists is unique and well established for expansion. New York State DNA legislation (Article 49-B – NYS Executive Law) was enacted in 1994 and then expanded in 1999, 2004 and 2006 increasing designated offenses from serious felonies to all felonies and selected misdemeanors. This unprecedented expansion led to the signing of a Memorandum of Understanding in 2000 between the University at Albany (UA) and the New York State Police (NYSP) to collaborate on forensic science work force development and research programs. Accordingly, in 2002 the UA and NYSP developed a Graduate Program in Forensic Biology (FB) which now matriculates 6-12 students per year and received the full accreditation from the Forensic Education Program Accreditation Commission (FEPAC) in 2008.

NERFI’s programs provide a long term investment in forensic laboratories’ most valuable assets: forensic scientists. Structured workshops providing forensic scientists professional development is a necessity in the forensic science community. Most forensic laboratories have little or no training funds and view training as a non-reoccurring cost / expenditure instead of an investment in the most significant asset (human resources) in their organizations. Laboratories need training for new and experienced employees in all disciplines and all job titles. In addition, complimentary skills, such as presentation of results, team work, communication (verbal and written), and testimony are also the major focus of in-house training after hiring. Lack of access to efficient training programs and resources can also be seen as a contributor to delays in analyses or backlogs in DNA analyses of criminal cases. Local and federal funding must be utilized to stabilize these education and training programs for all disciplines.

Governmental agencies will need to invest in future criminal justice training to keep abreast of new technology issues related to DNA analyses and CODIS data base needs, and provide the necessary funds to advance basic and applied forensic science research.
In doing so, the following training and research programs were included in award 2008-DD-BX-K301: Module: Forensic DNA Training, Leadership Assessment Workshops, and Research.

A: Capillary Electrophoresis & Data Analysis Using Applied Biosystems’ ABI™ 3130xl Genetic Analyzer & GeneMapper® ID Course (3 one-week laboratory workshops with 6-8 trainees per session):
This 5-day course was designed to provide the theoretical and practical background necessary to perform capillary electrophoresis and data analysis. Today’s advanced technology has led to an exponential number of cases being submitted to the crime lab for DNA testing. For this technique to be successful, it is imperative that the biological evidence be processed and analyzed effectively. A total of twenty-three DNA Analysts attended three different ABI 3130xl™ workshops.

B: ABI™ RT-PCR training Real-Time PCR Using Applied Biosystems’ 7500 Instrument and Chemistries (2 – 2 ½ day with at least 6 trainees per session):
This 2.5 day course was designed to provide the theoretical and practical background necessary to perform quantitative PCR and data analysis. This course also included a multiple-choice exam and issuance of completion certificates. NERFI originally proposed at least six trainees per training session. However, a total of sixty-two DNA Analysts attended the 2-2½ day training sessions on Real-Time PCR Using Applied Biosystems’ 7500 Instrument & Chemistries.

C: Leadership Assessment Workshop for Managers, Supervisors and Directors:
NERFI provided two 2 - day workshops for managers, supervisors, and directors in human resource strategies to help increase efficiencies in selection, retention and promotion of forensic scientists and managers. The first group of forensic managers attending Leadership Assessment: Developing the Next Generation of Leaders in January, 2010, included three lab directors, an assistant director, three managers, and a lab supervisor, two technical managers, and a senior scientist from various forensic sections – crime scene, biology, latent prints, drug analysis, trace, and QA. The second group of forensic managers attending Leadership Assessment: Developing the Next Generation of Leaders in February, 2010, included one director, a forensic science coordinator, three supervisors, a senior scientist, two technical leaders, a quality manager, and a latent print examiner with no management experience. Professionals from the forensic science fields of DNA, drug chemistry, crime scene, firearms and toolmarks, QA and latent prints were represented in this session.

The Leadership Assessment Workshops focused on developing both strategic and behavioral skills for managing employees. These leadership assessment workshops provided participants tools to increase efficiency in selection and retention of new hires and internal promotion decisions, thereby reducing costly turnover. Turnover costs can exceed twice the annual salary plus benefits of the employee (Cascio, 2000).

The leadership assessment workshops also provided an introduction to behavioral observation techniques. This can be used by forensic managers, supervisors and directors
to enhance their skills in the effective assessment of employees. The workshop used managerial exercises with behavioral examples to familiarize participants with the assessment concept. There was an enthusiastic and strong positive response by all participants in these workshops and a high demand for more of these kinds of sessions (Appendix 16).

**D: Raman Method Validation—The use of Raman Technology to Detect Human Biological Fluids:** The identification and characterization of body fluids and stains discovered at a crime scene is a major part of forensic investigation today. The three most common fluids found are blood, semen, and saliva, and there are several methods used currently to distinguish one from another. Blood can be presumptively tested for using different color spot tests, but these tests are destructive to the sample and can also have false positives (Siegel, 2000). If only a small amount of sample is available, careful decisions must be made as to whether the presumptive test is necessary. There are also confirmatory tests for blood that conclusively prove blood is present, and some of these tests can distinguish between species. Semen is similar in that there are destructive presumptive tests as well as confirmatory tests. Saliva, however, has no confirmatory tests. So, an examiner can never be positive about the presence of saliva (Siegel, 2000). Most presumptive tests can be performed in the field, but some sample preparation such as extraction is often necessary. Most confirmatory tests must be done in the laboratory, so forensic experts responding at a crime scene will not know the confirmed identity of a fluid until much later on. The largest problem with these tests is the consumption of the sample. Sometimes a case can be broken with just the smallest amount of biological evidence, so it is crucial that these small quantities are examined as efficiently as possible and nondestructively at the crime scene. Another issue is the ambiguity of the tests. Current simple on-field screening tests cannot confirm the presence of a particular fluid, and saliva can never be confirmed. Finally, mixtures of fluids are frequently found, and this can make identification and subsequent DNA analysis even more difficult. The forensic community is in need of a reliable and ultimately portable method that can exclusively distinguish between the common and uncommon body fluids, as well as not destroy the sample in the process.

Long-term goal is to develop an intelligent and user-friendly methodology with in-the-field capabilities based on Raman spectroscopy for characterizing traces of body fluids at a crime scene. Our hypothesis is based on the fact that every body fluid has a unique composition and should have a unique Raman spectroscopic signature, which can be used for its identification. Recently, Virkler and Lednev reported that Raman spectroscopy can be potentially used to distinguish different body fluids (Virkler and Lednev, 2008) as well as provide non-destructive, confirmatory identification of body fluids at the scene of a crime (Virkler and Lednev, 2009). However, this analysis was carried out on only one sample of each body fluid and did not take into account any variations that might occur between different donors of the same fluid. Since each donor’s sample is heterogeneous within itself due to many different chemical components, we would also like to investigate the effect these chemical components have on the Raman spectral components of a body fluid. The main purpose of this project was to understand the role of heterogeneity within a sample as well as among multiple donors for human semen.
This project was designed to determine the level of spectral heterogeneity of human semen based on principal components and to find out how much variation there is in the spectra from different donors. If there is very little change in the spectrum from one donor to another, then the technique of Raman spectroscopy can be considered to be reproducible in identifying a sample to be semen-based on the application of a calculated spectroscopic signature. This signature, which could be fitted to a semen sample collected from any donor, could be produced based on several spectral components found in semen that are present due to the heterogeneous distribution of the many chemical species in semen. A unique signature can ultimately be developed for other body fluids as well so that an unknown body fluid discovered at a crime scene could potentially be identified in a confirmatory manner.

A spectroscopic signature for human semen was developed based on the heterogeneous chemical composition of semen using NIR Raman Spectroscopy. A set of 50 semen samples were obtained from anonymous donors at an in-vitro fertilization clinic. No information about the donors was available. Statistical analysis found that the spectrum of a dried semen sample contained three major spectral components in addition to a fluorescent background component; a component matching tyrosine, a component containing albumin and choline, and a component matching spermine phosphate hexahydrate. This project also demonstrated qualitatively that there are no significant visual changes in the Raman spectra of dried semen acquired from multiple donors, and we showed that the spectrum of dried semen varies considerably when compared to the spectra of dried blood and saliva. We did not perform any quantitative statistical analysis to compare Raman spectra acquired for dry semen sample collected from different donors. Instead, a novel approach has been developed based on multi-dimensional spectroscopic signature to take into account both sample heterogeneity and possible variations with a donor. The combination of the three principal components can be used as a unique spectroscopic signature to identify the presence of semen and possibly distinguish it from other body fluids and substances of artificial nature found at a crime scene. The term “possibly” was used to indicate that several important steps need to be taken before the final conclusion about the differentiating power of the method could be made. These steps include (1) the development of multidimensional spectroscopic signatures for all body fluids of interest for forensic science, (2) validating the application of the developed method for mixtures of body fluids, (3) validating the application of the method for body fluid traces containing various contaminants, and (4) testing the interference of substrates. The signature’s specificity to semen is additionally reinforced by the determination that two of the three spectral components are dominated by choline and spermine, respectively, and these chemical components are unique to semen and have been used as forensic identification techniques for semen in the past. This spectroscopic signature can be fitted to all of the dried semen samples with high goodness-of-fit statistical results, and this outcome shows how the signature can be applied to any human semen sample to potentially identify it. This proof of concept experiment showed promising results, but many more samples with known demographic information should be investigated.
We envision the potential use of this method for nondestructive detection and confirmatory identification of semen at a crime scene, both in its pure form and even as part of a stain. In addition, the ability to not damage the sample while making these conclusions would be a valuable feature since it would allow the possibility of additional testing on the same sample. More experiments need to be performed involving semen stains on different materials such as clothing, paper, wood, etc., but the technique introduced in this paper shows the potential for the Raman spectroscopic signature of semen to be useful in identifying semen at crime scenes.

Continuing investigation of semen samples and other body fluids is currently taking place in our laboratory. Future work will focus on developing unique spectroscopic signatures for other body fluids to support the assumption that the different fluids can be distinguished from one another using Raman spectroscopy since they are composed of different chemical components. We are also experimenting with a more advanced statistical method which uses principal component analysis (PCA) to mathematically compare multiple spectra of different body fluids as well as spectra from different animal species of the same fluid.

**Module: Forensic Science Impact in the Court Room**

There is very little extant research studying the impact of forensic evidence and technologies in their impact on the outcomes of criminal investigations. Even more alarming is that we have limited documentation of the impact, in general, of the overall forensic science process to outcomes in violent criminal investigations. While we have studied the impact of new technology on forensic scientists (for example, see, Becker & Dale, 2007; Dale & Becker, 2005) the impact on the wider community remains undocumented.

The criminal justice community (prosecutors, detectives, medical examiners) benefits from a team-based structure. The forensic science community has benefited from advances in technology with concomitant increased capacity for evidence processing and analysis. Technological advances raise new questions related to the effectiveness of how agencies respond in concert to violent crime investigations. Most critically this involves how personnel in mission-critical areas function as a single enterprise, as in the coordination and execution during real-time response to a violent crime. These important questions are best addressed using the extant knowledge from social science research in teamwork that will help the forensic community adapt and apply this knowledge quickly.

The application to the criminal justice community is that it must measure and manage the impact of DNA and advanced technology to violent crime investigations. Combined DNA Index System (CODIS), National Integrated Ballistic Identification Network (NIBIN) and Automated Fingerprint Identification System (AFIS) represent technologies that have revolutionized forensic science, providing critical leads to help assure successful criminal investigations. The criminal justice community is a customer of the services of forensic science. However, the criminal justice community lacks explicit measures of the effectiveness of dollars spent on advanced technology. District attorneys and detectives were involved in the construction of the measures in this project. This is
important in that we recognize that those involved in solving high-profile crimes demand quick processing of evidence.

Increased synergy between forensic constituencies will help to stop criminals earlier in their criminal careers, in that offenders identified more quickly as a result of minor criminal activity are not able to advance to more serious crimes. Ninety-four percent of convicted offenders previously committed minor crimes (Haapanen, 1998). Felons whose most serious prior convictions were for forgery or passing bad checks had DNA matches in 12 rape cases, 8 homicides, 1 rape-homicide, an assault, a robbery, and a carjacking (Simon, 1997).

This pilot project analyzed the amount and type of evidence and forensic science technology as used by law enforcement agencies in Schenectady County, New York.

Plea bargains and verdicts were reviewed. The review assessed whether evidence could have been collected that may have improved the outcome of the case. An assessment was also made on whether the prosecutors could have further enhanced the case by seeking other evidence after arrest. Best practices were evaluated with respect to interaction with the lab throughout the criminal justice process.

This project also evaluated the use and effectiveness of forensic technology in the courtroom. Verdicts were examined and some jurors were asked, after trial, to voluntarily take a survey to assist in determining how important the presence or lack of, forensic science was in the verdict. In addition, training for eighty first responders from the Schenectady PD on proper evidence collection was done. Furthermore, funding was provided to purchase equipment from Porter-Lee to bar-code evidence in the Schenectady, New York DA’s Office.

**Module: ChatMinder: A Safe Internet Tool for Parents**

Graduate level assistance was provided with funds from this award to perform literature research and interviews with professionals to identify the need for a new advanced language and information technologies developed at the University at Albany for assisting automated intelligence gathering and analysis to detect and prevent of online crime against children as well as other persons or organizations. Job analysis was performed to identify the necessary fundamental knowledge and skills needed by cyber crime investigators. New Chat Minder tools were then designed using the data from literature research, job analyses and interviews with criminal justice cyber crime professionals.

In recent years, there has been a proliferation in the use of Internet chat rooms – virtual online communities where users from all over the world can interact. Chat rooms attract people of all ages, from all walks of life, although their use is particularly prolific among teenagers. Because encounters are anonymous, the risk of being deceived is high. For example, many chat room sites offer areas for specific age groups, but there is little to prevent someone from creating a profile reflecting a user of that age, and entering the room. There is no guarantee that the users of children’s chat areas are in fact all children.
While the vast numbers of interactions in such chat rooms are innocent, there are an increasing number of documented instances of pedophiles posing as children and ‘grooming’ target children for potential abuse. In response, a number of countries have adopted policies to target online crime against children. In the U.S., the bill is called the Children’s Internet Protection Act (CIPA). In 2003, the Virtual Global Taskforce was created to address online crimes against children (http://www.virtualglobaltaskforce.com/). Despite these efforts, current technological capabilities to detect and prevent on-line crime are extremely limited.

The aim of this project was to leverage advanced language and information technologies developed for assisting automated intelligence gathering and analysis and apply them to detection and prevention of online crime against children as well as other persons or organizations.

Analysis of the collected data led to construction of preliminary models of social behavior in online discourse. Conversations were annotated for communicative links, dialogue acts, and topic and focus shifts, which created the basis for building computational models of conversational behavior. Some of these models, e.g., how to effectively change the topic of conversation, were subsequently implemented into an automated Virtual Chat Agent (VCA), a Chat Minder prototype. VCA has been demonstrated to perform effectively and convincingly in Internet conversation with human participants.

**Module: Capital Region Cyber Crime Partnership**

For this part of the project, the team continued to work with the district attorneys in the Capital Region, the New York State Division of Criminal Justice Services, and the New York State Computer Crime Unit to expedite the prosecution of computer crime and capital cases involving computer forensic evidence.

It is no longer sufficient to use a single data source or a single analytic technique while analyzing data. In addition, there is a need to analyze unstructured text data since it can provide valuable clues on criminal behavior and intentions. The three methods discussed are mutually complementary and address the needs for law enforcement in fighting crime: 1) open source data collection, 2) natural language processing, 3) identifying correlations between disparate data sources. The data collection robots automate the process of collecting online data making it efficient. Linguistic analysis can be used for behavior analysis and integration tools can be used for tagging and correlating data.

Law enforcement is saddled with a growing backlog of cases of online crime and traditional crime that rely on online evidence. Crime labs around the country have been increasing capacity to handle this growing backlog. In addition to increasing capacity, efficiency of analysis also needs to increase. The crime scene today is often not a physical location but the Internet. Being able to rapidly collect data from online sources will make it feasible for investigators to pursue more crimes. Being able to gather corroborating evidence from chats, instant messaging, and web sites can improve the rate
of conviction. A suite of tools for data analysis will be employed for a host of problems including, 1) psychological profiling of sexual predators and determining precursors to crime 2) identifying hacker motivations for committing crime.

Future studies will involve developing algorithms for data correlations and developing best practices for law enforcement to use. In addition, other linguistic characteristics beyond content analysis while examining text data will be developed and tested. Some of the techniques developed above will be useful for identifying recidivism in certain crimes.
The Northeast Regional Forensic Institute (NERFI) was created in 2003 as a more resourceful, efficient, and cost-saving approach to meet the critical training needs of new DNA personnel hired by public forensic laboratories to perform DNA analysis. Education and training programs are the core of NERFI’s mission to support the forensic science and criminal justice community in staying abreast of the ever-evolving technological field. NERFI also strives to develop novel, state-of-the-art applications of new technologies through on-going research. Past and current interactions between the University and crime laboratories; other federal and state governmental agencies; NIJ’s Office of Science and Technology; and DoD are essential to advancing education, training, and research. These cooperative relationships culminate to improve the speed, accuracy, and delivery of forensic DNA analysis.

Since 2005, NERFI has provided education and training in the form of DNA academies and professional development workshops. For example, the NERFI 16-week intensive DNA academy has successfully trained over 120 newly hired, full-time forensic scientists in the theory and practice of DNA analysis; with most trainees completing all of their training requirements, and beginning casework, in 6-8 months (as opposed to the 12 to 18 months typically required to prepare casework-ready DNA scientists through in-house mentoring programs routinely used by crime laboratories).

Studies have shown that the current population of forensic scientists in the United States is about 10,000 (Becker and Dale, 2004; Dale and Becker, 2003); however, an additional 10,000 new forensic scientists will have to enter the work-force in the next 10 years to alleviate the expanding casework backlog (Fisher, 2003; Long 2001). NERFI’s education and training programs are designed to produce critical thinking, problem solving scientists - not technicians. NERFI’s generic DNA training curricula are designed to embrace all forensic laboratory methods using fundamental scientific theory. The NERFI program strives to provide each trainee with the fundamental forensic biology knowledge and instrumentation necessary to perform the laboratory techniques properly and execute corrective actions when unacceptable results occur.

A major component of this project is to offer to the forensic community a set of workshops that include three separate weeks of AB 3130xI™, two 2.5-day sessions of AB RT-PCR™, and two 2-day training sessions on Leadership Assessment for Managers.

- A: AB 3130xI™ Training
  - Three separate weeks of lecture plus hands-on
    - 6-8 trainees per session
- B: AB RT-PCR™ Training
  - Two 2.5-day training sessions
• At least 6 trainees per session
• C: Leadership Assessment Workshops
  • Two 2-day training sessions
  • 6-10 trainees per session

2. Methods: A-C

Instruction:
A key factor in providing continuing professional development to the forensic community was to employ a team of highly qualified scientists with years of practical experience, combined with a passion for educating and training the next generation of forensic DNA analysts and managers. In addition, NERFI only hires instructors and support staff willing and capable of being a team player. NERFI’s ultimate goal is to serve the needs of the customer by providing cost-effective, high-quality training and education in forensic DNA and leadership assessment. Nearly all of the current NERFI instructors, support staff, and expert contractors have participated in all twelve DNA academies and countless workshops. For Modules A-C, NERFI trainers used various methods of instruction for the AB 3130xl™, AB RT-PCR™, and Leadership Assessment Workshops. For example, the AB 3130xl™ training used a combination of PowerPoint lectures (Appendices 2, 3, and 4) with hands-on instruction, whereas the AB RT-PCR™ employed mostly traditional lectures (Appendices 8 and 9). Both the AB 3130xl™ and AB RT-PCR™ instituted an interactive review session prior to the final examination. Instructors for the Leadership Assessment workshop predominantly used round-table discussions of case studies from journal articles; example-driven discussions on critical topics; and role-playing with modeling combined with traditional PowerPoint presentations (Appendix 14).

Assessment and Evaluation:
NERFI’s professional development curriculum incorporates trainee performance measures and assessments that are substantially more rigorous than those typically found in traditional forensic training programs. For example, trainees in the AB 3130xl™ and AB RT-PCR™ workshops were required to earn a grade of ‘B’ or better on their final written examination (Appendices 5, 10 and 11) in order to receive a NERFI completion certificate. All workshop examinations were developed and reviewed by a team of NERFI instructors and staff, and each examination was graded for content by at least two instructors. NERFI has continuously improved the professional development program from feedback provided by: past trainees, NERFI and UAlbany faculty and staff, and members of the forensic science and criminal justice community.

Dissemination:
NERFI explored several avenues to inform the community about its grant-funded training opportunities. Approximately four thousand brochures were sent to the forensic community in February 2009; John Hicks and Dr. Becker spoke at sessions during the 37th Annual ASCLD Symposium on September 16, 2009; and encouraged word of mouth between members of the forensic community familiar with NERFI’s quality programming.
Unfortunately, there was limited response, with many laboratory managers/supervisors/directors suggesting travel and per diem be included along with workshop attendance. Based on feedback from the forensic community and poor response generated from advertising, NERFI sent NIJ a revised budget for award 2008-DD-BX-K301 of which was approved on July 10, 2009. The revised budget included trainee travel and per diem for all workshops under this award. NERFI also updated their website in early May 2009 with information on Module #1 workshops and linked the newly updated website to NIJ’s website (http://www.ojp.usdoj.gov/nij/training/welcome.htm) - many inquiries from all over the U.S. (and world-wide) were received.

NERFI also held a one-day conference on May 28, 2009, inviting laboratory directors, technical leaders, and supervisors from the northeast to discuss the upcoming AB 3130xl™, AB RT-PCR™, and managerial workshops - as well as other possible future training opportunities. Those in attendance were also asked to complete a brief survey regarding training. From the information gathered, NERFI was able to determine that these technologies (Applied Biosystems’ capillary electrophoresis 3130xl and real-time PCR 7500) were priorities. Next, NERFI set about delivering programs to fulfill the needs expressed by the community. As such, the following dates were scheduled for the above mentioned workshops:

- **A: AB 3130xl™ Training**
  (Three separate weeks of lecture plus hands-on training for 6-8 trainees)
  - January 11-15, 2010, at Westchester County Forensic Lab
  - January 25-29, 2010, at NERFI
  - February 8-12, 2010, at Honolulu Police Dept.

- **B: AB RT-PCR™ Training**
  (Two 2.5-day training sessions for at least 6 trainees per session)
  - June 3-5, 2009, at Massachusetts State Police Crime Laboratory
  - January 5-7, 2010, at Westchester County Forensic Lab

- **C: Leadership Assessment Workshops**
  (Two 2-day training sessions for 6-10 trainees)
  - January 14-15, 2010, at NERFI
  - February 18-19, 2010, at NERFI

3. **Results: A-C**

   **A: AB 3130xl™**
   (Three separate weeks of lecture plus hands-on training for 6-8 trainees)
   *December 7-11, 2009, at NERFI:*
   NERFI had to cancel the first AB 3130xl™ due to lack of attendees.

   *January 11-15, 2010, at Westchester County Forensic Lab:*
   Six trainees from the Westchester County Crime Laboratory (Table 1) were in attendance for this on-site workshop held Jan. 11-15, 2010. Three of the trainees had several years of experience and were acting as casework scientists. The other three were technicians, processing case samples in the lab. All of the trainees were well-versed in the use and maintenance of the 3130 instrument.
The workshop began with theoretical lectures on electrophoresis, the 3130xl instrument, and GeneMapper® ID (Appendices 2, 3, and 4). Because many of the trainees possessed years of hands-on experience with the 3130xl, and the group showed a heightened interest, NERFI staff chose to focus the workshop on the analysis and interpretation of electronic simulated case data, beginning with simple data followed by more challenging and complex.

On the final day of the workshop, NERFI staff held a review session and presented information on the recently released AB GeneMapper® ID-X software. In addition, each trainee completed a final exam (Appendix 5), an evaluation form, and received a completion certificate. Table 1 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

**Table 1: Participants and their Agency—January 11-15, 2010 at Westchester Lab**

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vialotti</td>
<td>Angela</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>King</td>
<td>Nicole</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Leung</td>
<td>Helen</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Gonzalez</td>
<td>Lissette</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Davis</td>
<td>Alexandra</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Bradshaw</td>
<td>Niyrai</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
</tbody>
</table>

_January 25-29, 2010, at NERFI:_
Nine students from across the continental U.S. descended on the University at Albany / Northeast Regional Forensic Institute the week of January 25-29, 2010, for a 5-day workshop on Capillary Electrophoresis & Data Analysis.

There were several trainees attending this workshop (Table 2) that were currently responsible for instrumentation maintenance in their labs, as well as one individual who was slated to be the first analysts in a new DNA section. In addition, another trainee was working as a fingerprint examiner with no prior DNA bench experience, with the remaining students somewhere in between.

NERFI staff started the workshop with theoretical lectures on electrophoresis, the 3130xl instrumentation, and GeneMapper® ID v3.2.1 (Appendices 2, 3, and 4). Afterwards, each trainee did analysis and interpretation using GeneMapper® ID v3.2.1 from electronic simulated case data. All trainees were required to replace the 16-capillary array, perform both routine polymer delivery system maintenances, and both the spatial and spectral calibrations on the 3130xl. The workshop concluded with a presentation on GeneMapper® ID-X software and a review session. Each trainee completed the required exam and course evaluation.
Table 2 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

**Table 2: Participants and their Agency—January 25-29, 2010 at NERFI**

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hou</td>
<td>Geoge</td>
<td>Los Angeles County Sheriff’s Office</td>
<td>X</td>
</tr>
<tr>
<td>Couch</td>
<td>Amy</td>
<td>AZ DPS</td>
<td>X</td>
</tr>
<tr>
<td>Fejes</td>
<td>Ildiko</td>
<td>AZ DPS</td>
<td>X</td>
</tr>
<tr>
<td>Oliver</td>
<td>Dianne</td>
<td>TX Dept. of Public Safety</td>
<td>X</td>
</tr>
<tr>
<td>Bryan</td>
<td>Lauren</td>
<td>North LA Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Punte</td>
<td>Dana</td>
<td>North LA Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Ho</td>
<td>Ranee</td>
<td>St. Louis Metro PD</td>
<td>X</td>
</tr>
<tr>
<td>Smith</td>
<td>Juline</td>
<td>Trinity DNA Solutions</td>
<td>X</td>
</tr>
<tr>
<td>Lockhart</td>
<td>Brigid</td>
<td>Oakland Co. Sheriff’s Office</td>
<td>X</td>
</tr>
</tbody>
</table>

**February 8-12, 2010, at Honolulu, HI:**

Eight scientists, including the DNA Technical Leader, from the Scientific Investigation Section of the Honolulu Police Department (Table 3), hosted and participated in the Capillary Electrophoresis & Data Analysis workshop on-site. The workshop consisted of several theoretical lectures (Appendices 2-4) and introductory 3130xl instrumentation hands-on work as well. Because many of the trainees already had years of hands-on experience with the 3130xl, NERFI staff spent the majority of the workshop on the analysis and interpretation of electronic simulated data sets and providing a lecture on real-time PCR.

On the final day of the workshop, the trainees participated a review session covering the instrumentation and GeneMapper® ID v3.2.1 software. Afterwards, each trainee completed the required exam (Appendix 5) and workshop evaluation form. Table 3 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

**Table 3: Participants and their Agency—February 8-12, 2010 at Honolulu, HI**

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuoka</td>
<td>Cathy</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Chua-Chiaco</td>
<td>Barrie</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Kashimoto</td>
<td>Samantha</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Tsang</td>
<td>Elizabeth</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Crabbe</td>
<td>Sean</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Fuller</td>
<td>Kim</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Esaki</td>
<td>David</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
<tr>
<td>Young</td>
<td>Michael</td>
<td>Honolulu Police Dept.</td>
<td>X</td>
</tr>
</tbody>
</table>

**B: Two 2.5-day training sessions on “Real-Time PCR Using Applied Biosystems’ 7500 Instrument & Chemistries”**
June 3-5, 2009, at Massachusetts State Police Crime Laboratory:
Thirty-six scientists from four New England Forensic Laboratories attended from June 3-5, at Massachusetts State Police Crime Lab (Table 4), the Advanced Lecture with Bruce McCord Ph.D., the Introductory Lecture with NERFI staff, or both. This 2.5-day workshop was divided into one day of Advanced Lecture (Appendix 9) followed by one and a half days of Introductory Lecture (Appendix 8).

The Advanced Lecture (Appendix 9), presented by Bruce McCord from Florida International University, consisted of five separate lectures covering a range of topics related to and affecting quantitation (via real-time PCR) of human DNA in forensic casework. Topics included: 1) DNA Quantitation by Real-Time PCR: Advanced Issues, 2) Investigation of the Effects of Sample Degradation and Inhibition in Forensic DNA Typing with Reference to qPCR, 3) A Comparison Between Plexor and Quantifiler Duo, 4) qPCR and Low Copy Template, and 5) Y STRs and qPCR. Dr. McCord lectured throughout the day and answered, as well as posed, insightful questions as he went. The attendees were engaged and focused.

The Introductory Lecture (Appendix 8) with NERFI staff began with an in-depth look at: why there is a need to quantitate, possible outcomes of not quantitating, history of quantitation, Polymerase Chain Reaction, Real-Time PCR, Fluorescent Detection (Stokes Shift & Emission Spectra), Filters, Multicomponent Analysis, AB Human Quantifiler kit, TaqMan Probe Technology, Definitions associated with Real-Time Analysis, Standard Curves and three criteria (slope, y-intercept, correlation coefficient), Troubleshooting the Standard Curve, Instrument Calibrations, and a Software Demo. The trainees were then divided into groups of two and issued laptops with SDS software and sample data. The trainee pairs were encouraged to explore the many functions of the software as the instructors worked with each pair individually.

The following day the students reviewed with NERFI staff all of the material covered in the past two days in a game of RT-PCR Jeopardy followed by a final examination (Appendices 10 and 11) and an evaluation of the workshop. The NERFI staff found this method of review to be very successful; the students were very engaged and interactive. Table 4 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

Table 4: Participants and their Agency—June 3-5, 2009 at Massachusetts Lab

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ross</td>
<td>Joseph</td>
<td>City of Boston Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Lynch</td>
<td>Julie</td>
<td>City of Boston Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Webster</td>
<td>Rebecca</td>
<td>City of Boston Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Pilla</td>
<td>Angela</td>
<td>City of Boston Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Muniec</td>
<td>David</td>
<td>Maine State Police Lab</td>
<td>X</td>
</tr>
<tr>
<td>Sabean</td>
<td>Jennifer</td>
<td>Maine State Police Lab</td>
<td>X</td>
</tr>
<tr>
<td>Name</td>
<td>First Name</td>
<td>Affiliation</td>
<td>Attendance</td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
<td>------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Waterhouse</td>
<td>Christine</td>
<td>Maine State Police Lb</td>
<td>X</td>
</tr>
<tr>
<td>LaFountain</td>
<td>Marcia</td>
<td>Vermont Forensic Laboratory</td>
<td>X</td>
</tr>
<tr>
<td>Herrick</td>
<td>Rebekah</td>
<td>Vermont Forensic Laboratory</td>
<td>X</td>
</tr>
<tr>
<td>Drugan</td>
<td>Cailin</td>
<td>Massachusetts State Police</td>
<td>X</td>
</tr>
<tr>
<td>Farnam</td>
<td>Leanna</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Harrington</td>
<td>Kim</td>
<td>Massachusetts State Police</td>
<td>X</td>
</tr>
<tr>
<td>Walsh</td>
<td>Sharon</td>
<td>Massachusetts State Police</td>
<td>X</td>
</tr>
<tr>
<td>Sgueglia</td>
<td>Joanne</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Haddad</td>
<td>Sandra</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Ruiz</td>
<td>Elisse</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Tremblay</td>
<td>Kara</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<td>Jennings</td>
<td>Laurie</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Ordyna</td>
<td>Chrissy</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<td>Simson</td>
<td>Crystal</td>
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<td>X</td>
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<td>Sullivan</td>
<td>Kristen</td>
<td>Massachusetts State Police</td>
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<tr>
<td>Collins</td>
<td>Sidney</td>
<td>Massachusetts State Police</td>
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<tr>
<td>Gould</td>
<td>Kathleen</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Barber</td>
<td>Amy</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Scott</td>
<td>Abbey</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<td>Brachold</td>
<td>Jaime</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Lemire</td>
<td>Christine</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Frederick</td>
<td>Alanna</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Marengo</td>
<td>Denise</td>
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<td>Lindauer</td>
<td>Kim</td>
<td>Massachusetts State Police</td>
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<tr>
<td>Wilcox</td>
<td>Kenton</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>Dindinger</td>
<td>Matt</td>
<td>Massachusetts State Police</td>
<td>X</td>
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<tr>
<td>O'Connor</td>
<td>Jessica</td>
<td>Massachusetts State Police</td>
<td>X</td>
</tr>
<tr>
<td>Schneeweis</td>
<td>Lynn</td>
<td>Massachusetts State Police</td>
<td>did not attend full</td>
</tr>
</tbody>
</table>
January 5-7, 2010, at Westchester County Forensic Lab:
Twenty-six scientists from three New York Forensic Laboratories attended the Advanced Lecture with Bruce McCord (Jan. 8, 2010), the Introductory Lecture with NERFI (Jan. 6-7, 2010), or both. The Advanced Lecture, presented by Dr. Bruce McCord covered the same topics and material presented during June 3-5, 2009. The Introductory Lecture with NERFI staff covered the same topics as described above during the June 3-5, 2009 RT-PCR. On the last day of the workshop, a review session was held using the Jeopardy-type format. Once again the NERFI staff found this method of review to be extremely successful. At the conclusion, each attendee completed the assessment examination (Appendices 10 and 11) as well as an evaluation form. Table 5 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramprashad</td>
<td>Alanna</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Davis</td>
<td>Alexandra</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Vialotti</td>
<td>Angela</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>D'Amato</td>
<td>Chris</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>San Pietro</td>
<td>David</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Schwartz</td>
<td>Elayne</td>
<td>Westchester Co. Crime Lab</td>
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<tr>
<td>Leung</td>
<td>Helen</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
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<tr>
<td>O'Connor</td>
<td>Holly</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
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<tr>
<td>Hoey</td>
<td>Jaime</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Reilly</td>
<td>Jennifer</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
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<tr>
<td>Chernjawski</td>
<td>Joselyn</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
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<tr>
<td>MacLaren</td>
<td>Kevin</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
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<tr>
<td>Gonzalez</td>
<td>Lissette</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Stout</td>
<td>Lynn</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Tsocanos</td>
<td>Maria</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>Eustace</td>
<td>Mary</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
<tr>
<td>King</td>
<td>Nicole</td>
<td>Westchester Co. Crime Lab</td>
<td>X</td>
</tr>
</tbody>
</table>
C: Leadership Assessment Workshops
(Two 2-day training sessions for 6-10 trainees)

January 14-15, 2010, at NERFI

The first group of forensic managers attending Leadership Assessment: Developing the Next Generation of Leaders in January, 2010 included three lab directors, an assistant director, three managers, and a lab supervisor, two lab managers, and a senior scientist from various forensic sections – crime scene, biology, latent prints, drug analysis, trace, and QA (Table 6). All of the nine participants had some management experience, from as little as 3 to as much as 21 years. As shown in Table 6, experience in forensic science ranged from 7 to 30 years.

Table 6: Participant Position and Experience—January 14-15, 2010 at NERFI

<table>
<thead>
<tr>
<th>Participant Title</th>
<th>Years of Forensic experience</th>
<th>Years in Management</th>
<th>Years in Current Job</th>
</tr>
</thead>
<tbody>
<tr>
<td>Director</td>
<td>30</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Assistant Director</td>
<td>25.5</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Director</td>
<td>22</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Quality Manager</td>
<td>21</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Lab Manager</td>
<td>18</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Section Supervisor</td>
<td>17</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Director</td>
<td>15</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Lab Manager</td>
<td>14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Senior Scientist</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

The first day of the two-day workshop began with a look at the leadership challenges facing lab managers today, including forensic scientist turnover. This was followed with an overview of the need for responsive leadership in forensic labs as implicated in the NRC’s Strengthening Forensic Science in the United State: A Path Forward (NAS Report, 2009) and of the importance of leadership training in such a technology-intensive field. After acknowledging the paucity of forensic lab management studies, Dr. Becker summarized the lessons learned from the few management studies that have been conducted in forensic lab situations. Dr. Pavur compared the roles of the forensic scientist and the supervising scientists, which centered on the nature of intellectual capital. Both instructors also discussed methods of assessing and managing intellectual capital by
focusing on various models of organizational behavior – creating Forensic Advisory Boards for stakeholder input, “people make the place,” the Attraction-Selection-Socialization-Attrition (ASSA) framework, and STAR interviewing practices (Situation or Task, Action, Result).

The second day began with a tour of the New York State Police Forensic Investigation Center followed by instruction and discussion of management leadership theory, concentrating on the theories of Mary Parker Follett and the relationship between power, leadership and conflict. Dr. Becker introduced the concepts of empowerment and collaborative learning (Dr. Gary Yukl’s articles) and offered specific strategies for effective leadership. Both instructors ended the workshop by leading a role play activity involving experimentation with eleven influencing behaviors.

Over the two days, the NERFI Leadership Assessment workshop engaged in co-instructional techniques of case studies from journal articles, example-driven discussions on critical topics, Power Point presentations, and role play with modeling. Table 7 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

Table 7: Participants and their Agency—January 14-15, 2010 at NERFI

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adamo</td>
<td>Robert</td>
<td>Westchester Co. Crime Laboratory</td>
<td>X</td>
</tr>
<tr>
<td>Baral</td>
<td>Sanghamitra</td>
<td>Prince Geoge's Co. PD</td>
<td>X</td>
</tr>
<tr>
<td>Crenshaw</td>
<td>Karin</td>
<td>Palm Beach Co. Sheriff's Office</td>
<td>X</td>
</tr>
<tr>
<td>Grady</td>
<td>David</td>
<td>Worcester PD</td>
<td>X</td>
</tr>
<tr>
<td>Kamb</td>
<td>Valerie</td>
<td>Johnson Co. Crime Laboratory</td>
<td>X</td>
</tr>
<tr>
<td>Lakhkar</td>
<td>Bharat</td>
<td>Westchester Co. Crime Laboratory</td>
<td>X</td>
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<tr>
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<td>Prince Geoge's Co. PD</td>
<td>X</td>
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<td>Murga</td>
<td>Kim</td>
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<tr>
<td>Eastman</td>
<td>Dr. Allison</td>
<td>Forensic Identity &amp; Profiling</td>
<td>X</td>
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</tbody>
</table>

February 18-19, 2010
The second group of forensic managers attending Leadership Assessment: Developing the Next Generation of Leaders in February, 2010, included one director, a forensic science coordinator, three supervisors, a senior scientist, two technical leaders, a quality manager, and a latent print examiner with no management experience. Professionals from the forensic science fields of DNA, drug chemistry, crime scene, firearms and toolmark, QA and latent prints were represented in this session (Table 8). Nine of the ten participants had some management experience, from as little as 6 months to as much as 9 ½ years. Experience in forensic science ranged from 3 to 24 years. Table 8 below summarizes the position and experiences of the ten participants.
The workshop content presented, and activities conducted, were nearly identical to the first workshop.

Table 9 below describes each attendee, their associated agency, and whether or not they received a completion certificate.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Agency</th>
<th>Certificate</th>
</tr>
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<tr>
<td>Werry</td>
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<td>Ohio State Highway Patrol</td>
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<td>Saul</td>
<td>Doug</td>
<td>DuPage Co. Sheriff's Crime Lab</td>
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</tr>
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<td>Levine</td>
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4. Conclusions and Implications: A-C  
A: AB 3130xl™  
(Three separate weeks of lecture plus hands-on training for 6-8 trainees)  
A total of twenty-three DNA Analysts attended three different AB 3130xl™ workshops.  
A fourth workshop was originally scheduled for the week of December 7, 2009, at NERFI—unfortunately, the workshop was cancelled due to a lack of interest. Feedback from the forensic community indicated that the lack of interest was due to the workshop’s
scheduling too close to the holiday season, and that most DNA analysts had already satisfied their required 8 hours of continuing education for 2009.

Each workshop varied in content due to the level of expertise—however, the basic theory of capillary electrophoresis and GMID software, plus the data analysis and interpretation using simulated data was included for all three workshops. In addition, trainees also had hands-on training with the AB 3130xl™. All three workshops reviewed the material prior to taking the final examination. Attendees who were present for the entire workshop and who passed the assessment exam (letter grade of ‘B’ or better) were issued a completion certificate (Tables 1-5, 7, and 9). All attendees participating in the NERFI workshops passed with a ‘B’ or better. NERFI goes to great lengths to present the information in various formats: presentation, handouts, animation, and discussion - by more than one instructor, with interactive review sessions, to ensure the students in the group have the best possible chance of coming away with the intended knowledge. In addition, one of the necessary qualifications of a NERFI instructor is that they are knowledgeable, approachable and have years of forensic science training and education experience. These traits have proven to be invaluable when trying to engage students and encouraging them to ask questions when they are unsure. NERFI instructors are also well versed in recognizing students that may be apprehensive and approaching them after lectures, starting conversations, and enticing questions from these students that may not otherwise ask.

A program evaluation for all three AB 3130xl™ workshops provided feedback (Poor 1 to Excellent 5) on five areas: Program Overall, Speakers, Quality of Audio-Visual, Quality of Handouts and Facilities (Appendix 6). Attendees rated the program very high, especially with regard to the Course Overall and Instructors, with comments indicating the topics covered were very informative and thorough. The instructors themselves were described as clear and well-spoken and sufficiently answered all of the questions. Participants enjoyed the small group interaction and commented that they would like to see similar offerings in the future.

B: Two 2.5-day training sessions on “Real-Time PCR Using Applied Biosystems’ 7500 Instrument & Chemistries”
NERFI originally proposed at least six trainees per training session. However, a total of sixty-two DNA Analysts attended the two 2.5-day training sessions on Real-Time PCR Using Applied Biosystems’ 7500 Instrument & Chemistries. The interest level for the two RT-PCR workshops was exceptional for two reasons. First, having two separate host-sites in the northeast allowed more trainees to attend per session. Second, many of the trainees appreciated the advanced lectures on real-time PCR presented by Dr. Bruce McCord from Florida International University. However, high marks from trainee feedback, for both workshops, were given to NERFI staff for their Introductory RT-PCR presentations (Appendix 12).

C: Leadership Assessment Workshops
(Two 2-day training sessions for 6-10 trainees
January 14-15, 2010, at NERFI
An evaluation requested scored feedback (*Poor* 1 to *Excellent* 5) on five areas: Program Overall, Speakers, Quality of Audio-Visual, Quality of Handouts and Facilities (Appendix 16). Participants scored all aspects of the program favorably, with the highest marks given to the speakers themselves as professional, knowledgeable, highly specialized, and effective. Participants enjoyed the small group interaction and commented that they were leaving with management tools they could use in their current positions.

*February 18-19, 2010, at NERFI*

A program evaluation requested scored feedback (*Poor* 1 to *Excellent* 5) on five areas: Program Overall, Speakers, Quality of Audio-Visual, Quality of Handouts and Facilities. Participants scored all aspects of the program favorably, with the highest marks given to the Program Overall and a comment indicating the topics covered were useful (Appendix 16). The instructors themselves were described as very knowledgeable and praised for prompting very thought-provoking discussions. Participants enjoyed sharing experiences with other forensic scientists. Small group interaction and commented that they would like to see similar offerings in the future.

The most interesting /useful topics included influencing tactics, the relationship between morale and retention, and the psychology of being a leader.

The instructors for the Leadership Assessment workshop revised the content and schedule for the second session based on the comments from the first session participants, the experience level of the second group, and questions from the participants on the first day. The January group indicated that the influencing tactics were quite useful. Therefore, the instructors moved that activity to the first day to provide more time to practice influencing behaviors. In addition, there was discussion on morale, engagement, and loyalty, based on questions from the January session. The February group (Table 8) had fewer long-tenured senior managers or directors than the January group (Table 6), so the second day included more team relationship and customer aspects, and less material on external liaisons for upper management. As the instructors make adjustments to meet the needs of the participants, some of the PowerPoint presentations are modified and do not follow the pre-printed handouts.

*Lessons learned, future thoughts for improved workshops:*

Over the years NERFI has relied heavily on feedback from the forensic community for all of its education and training programs. For example, after each training session NERFI instructors and staff review evaluations and revise the curricula accordingly. NERFI has future plans for all potential attendees to complete a brief questionnaire before attending a workshop. Some of the topics covered in the newly developed pre-workshop questionnaire will include: level of experience, current job title, and training and education needs. Armed with this information, NERFI plans to offer two workshops for each topic in the future, one introductory and one advanced. This approached was used for the real-time PCR workshop and the NERFI instructors found this method of instruction to be very successful. It was noted that this information would have been very useful for the 3130xI™ training workshop.
After reviewing the Leadership Assessment evaluation forms it was determined that the attendees would like to extend the workshop to include one more day. One experienced manager explained it quite well when she commented that she felt like she had the background after two days and was ready to use it if she had just had one more day. Part of the issue is that the sessions are mixed with new managers, people in line of succession to be managers, and very experienced managers. Because of this, the instructors covered all -- theory, research -- and yet still offered training in specific skill sets. New managers acquired a huge amount of what they needed but experienced managers, while appreciating the theory and research, are ready for the more seasoned business management approach which there was just not time for in these sessions.

**Implications for the field of criminal justice and the study of forensic science**
Because of the recent economic downturn and budgetary constraints, training budgets are the first items to be eliminated in most forensic science labs. However, the FBI Quality Assurance Standards require that all DNA Analysts receive at least 8 hours of continuous education each fiscal year. In Modules A-C, over 100 trainees from across the nation received their annual mandatory professional development by successfully completing the NERFI 3130XL, RT-PCR or Leadership Assessment for Managers workshops. More importantly, all trainees received this specialized and required training free-of-charge. All of the trainees that took the written examinations scored at least an “80” or better (no failures) with an overall average of 90.38.

**Dissemination**
NERFI is in the process of producing a peer-reviewed report on the various methods of training currently being done throughout the forensic science community. Information obtained from the 3130XL, RT-PCR or Leadership Assessment for Managers workshops will be included in this paper, and presented to scientists, policy makers, and practitioners at a criminal justice and scientific meetings.

**D—Raman Method Validation--The use of Raman Technology to Detect Human Biological Fluids**
(Author: Dr. Igor Lednev)

1. **Introduction: D**
In recent years, forensic analysis has become one of the most growing areas of bioanalytical chemistry (Brettell et al, 2007). The ability to identify traces of body fluids discovered at crime scenes is a very important aspect of forensic investigations (Li, 2008; Shaler, 2002; Jones, 2005). With DNA analysis being one of the most popular and informative forensic techniques, it is imperative that any potential body fluid sample is not destroyed during the initial identification process. Fluids such as blood, semen, saliva, and vaginal fluid can be very useful in identifying a victim or suspect (Best, 2007), and they can also help answer questions regarding the events of a crime. An analytical technique that could identify a particular body fluid rapidly, simply, and non-destructively at the scene of a crime would be a valuable tool for forensic investigators.
Virkler and Lednev have recently reported that Raman spectroscopy can be potentially used to distinguish different body fluids (Virkler and Lednev, 2008) as well as provide non-destructive, confirmatory identification of body fluids at the scene of a crime (Virkler and Lednev, 2009). However, this analysis was carried out on only one sample of each body fluid and did not take into account any variations that might occur between different donors of the same fluid. Since each donor’s sample is heterogeneous within itself due to many different chemical components, we would also like to investigate the effect these chemical components have on the Raman spectral components of a body fluid. This paper investigates the role of heterogeneity within a sample as well as among multiple donors for human semen.

In addition to blood, semen is one of the most prevalent body fluids found during criminal investigations, especially in cases involving sexual assault (Shaler, 2002). There are currently several tests, both presumptive and confirmatory, that can be used to identify an unknown fluid found at a crime scene to be semen (Virkler and Lednev, 2009). Some of the popular presumptive tests include searching for stains using an alternate light source (ALS) and looking for the presence of seminal acid phosphatase (SAP) using chemical tests (Li, 2008; Greenfield and Sloan, 2003). Some commercial ALS instruments have been developed such as the Wood’s Lamp (Santucci et al, 1999), Bluemaxx™ BM500 (Nelson and Santucci, 2002), Polilight® (Vandenberg and vanOorshot, 2006), and the Lumatec Superlight 400 (Fielder et al, 2008), but these are not exclusive to semen identification and can only be used as a screening technique. The tests for SAP (Li, 2008; Watson, 2004) are much more reliable, but they are destructive to the sample and there is still some potential for false positive results. The most popular methods for confirmatory identification of semen include the microscopic visualization of sperm cells using specific stains and immunological tests for prostate specific antigen (PSA) (Li, 2008; Greenfield and Sloan, 2003). The staining method will of course not be helpful if the donor is azoospermic, so this technique has limited applications including having to perform the test in a laboratory. Several commercial PSA test kits which can be used at a crime scene have been developed including Biosign® PSA (Maher et al, 2002), OneStep ABACard® (Hochmeister et al, 1999), Chembio, Medpro, Onco-screen (Healy et al, 2007), PSA-check-1, Seratec® PSA Semiquant (Hochmeister et al, 1999), and SMITEST (Sato, etal 2002; Yokota et al, 2001). Like with the presumptive tests, these PSA test kits do show false positive results and are destructive to the sample.

The destructive nature of both the presumptive and confirmatory tests is the largest concern that needs to be addressed. Sometimes a very small amount of semen evidence can solve a case if examined properly, so it is crucial that the available evidence is processed efficiently and non-destructively so that further analysis, including DNA typing, can be performed (Budowle and vanDaal, 2009). Another issue is the potential of false positive results (Denison et al, 2004). The current easy-to-use test kits do not absolutely confirm the presence of semen either in pure form or as part of a stain. The forensic community is in great need of a reproducible, non-destructive, and portable method that can exclusively identify the presence of semen at a crime scene and distinguish it from other body fluids.
Raman spectroscopy is a forensic technique that has increased in popularity over the last several years (Macleod and Matousek, 2008; Bartick, 2002), and it can be paired with infrared (IR) absorption spectroscopy to gain information about the structure and properties of materials based on their vibrational transitions (Nafie, 2001). Some applications being used today involve the identification of fibers (Thomas et al, 2005), drugs (Hodges and Akhavan, 1990), and lipsticks (Rodger and Broughton, 1998), as well as ink (Mazzella and Buzzini, 2005), paint (Suzuki and Carrabba, 2001), and condom lubricant (Coyle and Anwar, 2008) analysis. The theory behind Raman spectroscopy involves the inelastic scattering of a low-intensity, monochromatic, and nondestructive laser light by a solid, liquid or gas sample. There is little to no sample preparation, and no reagents are needed for analysis. Most importantly, the required amount of sample needed for Raman analysis can be as low as several picograms or femtoliters, and the sample will not be destroyed so that further analysis can still be performed. A typical Raman spectrum reveals a specific vibrational signature of the sample being measured based on the energy of the scattered light, and this feature is very useful in identifying an unknown substance. Raman spectroscopy is also very appropriate for the analysis of disordered and heterogeneous samples (Colomban and Gouadec, 2009) which are common properties of body fluids. Finally, Raman spectroscopy shows very little interference from water (Grasselli, 1981) which makes it a great technique for analyzing body fluids and their traces. Portable Raman spectrometers are available now (Eckenrode et al, 2001; Yan and Vo-Dinh, 2007), and these designs along with advanced software could be applied to the identification of semen at a crime scene.

Surprisingly, there have been no publications of any experiments involving the identification of semen using Raman spectroscopy. The objective of this study is to determine the heterogeneity of a dried semen sample from one donor as well as analyze the qualitative variation among samples from different donors using NIR Raman spectroscopy. Determining the specific principal components that contribute to the overall spectrum of semen is an important task since it is unlikely that a single library spectrum of semen will match an unknown semen sample. If careful statistical analysis is not performed, there could possibly be a false positive result for another body fluid that is similar in composition. The goal is to use automatic mapping to develop a spectroscopic signature specific to dried semen which will be generated by combining multiple spectra based on the different components that make up a basis sample due to heterogeneity. Our hypothesis was that advanced statistical analysis could be used to separate the basis semen spectrum into individual components, and that these components could potentially be characterized based on the known chemical composition of semen. The characterized principal components could then be used in a “multi-dimensional analysis” of dried semen as opposed to a “single-dimensional analysis” which only involves the comparison of a single average library spectrum. The principal components found from the basis semen sample will be fitted to the average dried semen spectrum obtained from multiple donors to illustrate the capability of a unique spectroscopic signature to be applied to all semen samples. herein this study, the NIR Raman dried semen component spectra found by analyzing a single semen sample as well as the spectra obtained from analyzing dried semen samples from many donors. Preliminary assignments of major Raman peaks and possible identities of the semen components were made based on literature data.
2. Methods: D

2.1 Samples
A set of 50 semen samples were obtained from anonymous donors at an in-vitro fertilization clinic. A small 10 µL drop of each sample was placed on a circular glass slide designed for use with an automatic mapping stage and allowed to dry completely. The samples were analyzed using automatic mapping that scanned a sample area of 75x75 µm and measured Raman spectra from 10 random points within the area with 6 ten-second accumulations at each point.

A single basis semen sample was prepared in the same way as the others, and automatic mapping was again used scanning 36 random points with 6 ten-second accumulations at each point. The spectra obtained from this sample were used to determine the number and identities of the principal components of semen and to develop the spectroscopic signature.

2.2 Raman Microscope
A Renishaw inVia confocal Raman spectrometer equipped with a research-grade Leica microscope, 20x long-range objective (numerical aperture of 0.35), and WiRE 2.0 software were used. For the automatic mapping, the lower plate of a Nanonics AFM MultiView 1000 system was set up under the microscope, and measurements were taken using Quartz II and QuartzSpec software. A 785-nm laser light was utilized for excitation. The laser power on the dried samples was about 115 mW.

2.2 Data Treatment
All of the spectra obtained from the automatic mapping of the dried semen samples were first treated using GRAMS/AI 7.01 software to remove any cosmic ray interference. The spectra were then imported into MATLAB 7.4.0 for statistical analysis and normalized to adjust for the varying amount of background interference in each spectrum. The number of principal components in the basis sample was determined using significant factor analysis (SFA), and the individual component spectra were extracted using the alternate least squares (ALS) function. The components found in the original basis sample were used to create a spectroscopic signature, and this signature was fitted to each average spectrum found from the remaining semen samples. The Curve Fitting Toolbox in MATLAB was used to perform residual analysis on the difference between the fitted and experimental spectra, and “goodness-of-fit” statistics were calculated based on how well the signature matched the experimental spectrum.

3. Results: D

3.1 Main approach
The main goals of this study were to determine the level of spectral heterogeneity of human semen based on principal components and to find out how much variation there is in the spectra from different donors. If there is very little change in the spectrum from one donor to another, then the technique of Raman spectroscopy can be considered to be reproducible in identifying a sample to be semen based on the application of a calculated spectroscopic signature. This signature, which could be fitted to a semen sample collected from any donor, could be produced based on several spectral components found...
in semen that are present due to the heterogeneous distribution of the many chemical species in semen. A unique signature can ultimately be developed for other body fluids as well so that an unknown body fluid discovered at a crime scene could potentially be identified in a confirmatory manner.

3.2 Single Sample Heterogeneity
A single basis semen sample was used to develop the spectroscopic signature that would be applied to all samples. The basis sample spectra were imported into MATLAB, and SFA analysis was performed to determine the number of principal components that were present. The results of this analysis (data not shown) indicated 6 principal components. The ALS function was applied to extract the spectra of each of these components, and further examination of the results revealed that there were actually only 3 unique components that were spectral representations of the chemical species in the semen sample. The remaining components consisted of background fluorescence, a virtual duplicate of one of the 3 real components, and a nonsense component that appeared to just be noise. Figure 1 shows the average spectrum of the basis sample along with the 3 principal components.

![Fig. 1. The average Raman spectrum of the basis semen sample (a), and the Raman spectra of semen spectral components 1 (b), 2 (c), and 3 (d) with major peaks labeled.](image)
The wave number range of 670-1750 cm\(^{-1}\) is shown in the figure and will be the used to create the spectroscopic signature since this is the region that contains most of the important characteristic peaks. The major Raman peaks that define each component are labeled and are listed in Table 1.

Table 1 – Raman peak assignments of dried semen

<table>
<thead>
<tr>
<th>Raman shift (cm(^{-1}))</th>
<th>Spectral component</th>
<th>Vibrational mode</th>
<th>Chemical component</th>
</tr>
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<tbody>
<tr>
<td>641</td>
<td>1</td>
<td>Ring deformation (^{38})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>715</td>
<td>2</td>
<td>CN stretching (^{36})</td>
<td>Choline</td>
</tr>
<tr>
<td>759</td>
<td>2</td>
<td>Ring vibrations (Trp) (^{39})</td>
<td>Albumin</td>
</tr>
<tr>
<td>798</td>
<td>1</td>
<td>CH(_2) deformations in ring (^{38})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>829</td>
<td>1</td>
<td>Ring breathing (^{38},^{38})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>848</td>
<td>1</td>
<td>Ring bending (^{38},^{60})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>888</td>
<td>3</td>
<td>Phosphate mode (^{45})</td>
<td>SPH</td>
</tr>
<tr>
<td>958</td>
<td>3</td>
<td>PO(_4)^{3-} sym. stretching (^{39},^{61})</td>
<td>SPH</td>
</tr>
<tr>
<td>983</td>
<td>1</td>
<td>CH(_2) wagging (^{38})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>1003</td>
<td>2</td>
<td>Aromatic ring breathing (Phe) (^{43})</td>
<td>Albumin</td>
</tr>
<tr>
<td>1011</td>
<td>3</td>
<td>CC stretching (^{36},^{38})</td>
<td>SPH</td>
</tr>
<tr>
<td>1055</td>
<td>3</td>
<td>CN sym. stretching (^{36})</td>
<td>SPH</td>
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<td>CH(_2)/NH(_3) rocking (^{38})</td>
<td>Tyrosine</td>
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<td>1200</td>
<td>1</td>
<td>CC stretching (^{61})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>1213</td>
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<td>CH(_2) twist and rock (^{38})</td>
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<tr>
<td>1240</td>
<td>2</td>
<td>Amid III (^{44})</td>
<td>Albumin</td>
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<tr>
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<td>Sym. ring deformation (^{62})</td>
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<td>CH(_2), CH(_3) bend (Trp) (^{32})</td>
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<td>CH(_2) bending (^{32})</td>
<td>SPH</td>
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<tr>
<td>1494</td>
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<td>NH(_3) sym. bending (^{49})</td>
<td>SPH</td>
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<td>1</td>
<td>CC stretching (^{60})</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>1668</td>
<td>2</td>
<td>Amid I (^{41})</td>
<td>Albumin</td>
</tr>
</tbody>
</table>

Possible assignments and vibrational modes for each peak are also listed, and these assignments were made based on the known composition of semen and literature data.

The spectral components of semen are complex, and some have contribution from multiple chemical species. According to several literature sources (Altman, 1961; Owen and Katz, 2005; Mann, 1975), some of the chemical components of semen that are present in the highest concentrations are fructose, choline, spermine, citric acid, acid phosphatase, and albumin. Other chemical components of lower abundance are glucose, inositol, lactic acid, and urea. Despite this list of dominant chemical species, component 1 shown in Figure 1 appears to be a match to the amino acid tyrosine. The peaks at 641, 798, 829, 848, 983, 1179, 1200, 1213, 1265, 1327, and 1616 cm\(^{-1}\) are almost exactly the same as peaks depicted in literature sources on the Raman spectrum of tyrosine (De...
Gelder et al, 2007; Johnson et al, 1986). There are also residual peaks from other chemical species, but these are more dominant in the other two components. It is surprising that a single amino acid would be found to be one of the major principal spectral components of semen instead of a more complex chemical compound, but there are free amino acids known to be in semen (Altman, 1961), and it is possible that tyrosine is abundant in albumin and acid phosphatase which are both large contributors to the composition of semen. Phosphorylated tyrosine residues have been reported to have a protecting effect on the membranes of sperm cells and help stabilize lipids (Sancho et al, 2006), so an abundance of free tyrosine in semen is practical. In addition, it has been found that tyrosine phosphorylation occurs when sperm undergo capacitation which is necessary before fertilization (Liu et al, 2006).

It is obvious at first glance that component 2 is dominated by a protein due to the presence of amid I and amid III (Carter and Edwards, 2001) peaks at $1668 \text{ cm}^{-1}$ and $1240 \text{ cm}^{-1}$, respectively. It has been reported that the protein albumin makes up about one third of the protein content of semen (Owen and Katz, 2005), therefore this is a logical assignment as a contributor to component 2. Comparison of literature data on the Raman spectrum of albumin supports this conclusion (Liang et al, 2008; Ivanov et al, 1994), with matching peaks occurring around $759$, $1003$, $1336$, and $1448 \text{ cm}^{-1}$ in addition to the amid I and III peaks already mentioned. It is also possible that the enzyme acid phosphatase is contributing to this component due to its protein qualities and large abundance in semen, but there is not a lot of literature data available to compare with, so albumin will be considered the major contributor of component 2 for now. Finally, choline also appears to be present in component 2. The large peak at $715 \text{ cm}^{-1}$ is very likely due to the C-N symmetric stretch found in choline which has been previously reported (Edsall, 1943; Koyama et al, 1977; Spiker and Levin, 1975). It is also likely that the CH$_2$ scissoring in choline is contributing to the peak at $1448 \text{ cm}^{-1}$ (Koyama et al, 1977). This peak is very large in component 2 so it is probable that more than one chemical species is contributing to it. As with component 1, there are other peaks present that are much stronger in the other two components, so they are not considered to be dominating in component 2. Component 3 appears to oppose component 1 when comparing spectra from one donor collected from different spots. When the peaks present in component 1 are strong, the peaks for component 3 are weak and vice versa (Figure 2).
Fig. 2. A dry trace of semen is strongly heterogeneous. Raman spectra acquired from different spots in the same dried semen sample dominated by component 1 (a) and component 3 (b).

The chemical contributor to component 3 was fairly simple like with the case of component 1. The spectrum for component 3 is a match to spermine phosphate hexahydrate (SPH) that has previously been reported (Bertoluzza et al, 1983; Eapen and Joe, 1997). The peaks found in component 3 at 888, 958, 1011, 1055, 1065, 1125, 1317, 1461, and 1494 cm\(^{-1}\) all appear in the known spectrum of SPH and are listed in Table 1 with vibrational assignments. As previously mentioned, spermine is present in large concentrations in human semen, and it has even been the basis of forensic semen identification in the past (Gonmori et al, 1994; Tsutsumi, 1987; Suzuki et al, 1980; Sato et al, 1996). The basic nature of spermine causes it to interact with the phosphoric acid groups of nucleic acids and form strong bonds (Bertoluzza et al, 1983; Eapen and Joe, 1997). This binding leads to a precipitation of SPH in semen (Iitaka and Huse, 1965). These crystals were first observed by Leeuwenhoek in 1678 (van Leeuwenhoek, 1678), and they have since been observed in semen as the fluid begins to dry (Rosenheim, 1924). These properties regarding the presence of SPH in semen make it an understandable assignment of component 3. As with the other two principal components of semen, component 3 contains residual peaks from chemical species which are more dominant in components 1 and 2.

3.4 Multiple Donors
The second objective of this study was to determine the amount of spectral variation from one semen donor to another. The first step was qualitative in nature and involved the
visual comparison of the average spectrum from all of the different donors. All of the spectra appeared to be very similar and contained all of the same major peaks. There were changes in intensity of some peaks for different donors, but this is expected since the relative contribution of the chemical species in semen will likely change with each donor and can even change within the same donor (Mann, 1975). Figure 3 shows the average spectra of five semen samples (black lines) from different donors as an example of their similarities. To demonstrate the spectral differences between body fluids, the spectra of blood and saliva that we have previously reported (Virkler and Lednev, 2008) are also included in this figure. Unlike the slight intensity changes within the semen samples, blood and saliva have major peak differences which make them distinguishable. The other features of Figure 3 will be discussed in more detail in the following paragraphs.

After achieving visual confirmation that there is consistency among the spectra of semen from different donors, a more quantitative approach was developed. A spectroscopic signature was created that consisted of the 3 principal components found in the basis semen sample along with a horizontal line and a line with a slope equal to that of the fluorescence background. These five basis spectra were linearly fitted to the average basis semen spectrum, and the two spectra overlapped very well. The spectroscopic signature was also applied to the spectra from each of the remaining 49 semen samples to determine if it could universally be fitted to a sample from any donor. Figure 3 shows the fitting to only five representative semen samples, but all of the samples had very similar fits. The bottom of Figure 3 contains the results of fitting the spectroscopic signature to the spectra of blood and saliva, and it is visually obvious that they are very poor matches.
Fig. 3. The average Raman spectra of five semen samples (black) with the fitted spectroscopic signature (a-e), and the Raman spectra of blood (f) and saliva (g) with the fitted spectroscopic signature.

These results qualitatively show the specificity of this signature to semen and its potential ability to be used as an identification technique for forensic purposes.

A quantitative statistical analysis was performed to determine how well the spectroscopic signature fit the experimental spectra. Using the Curve Fitting Toolbox in MATLAB, the intensity values for the basis experimental spectrum and fitted spectrum were plotted on an axis as the x- and y- coordinates, respectively. All of the spectra were normalized to a maximum value of 1, so that is the highest value for both the x- and y- axis. Two identical spectra would yield a scatter plot matching a line with the equation of y=x, so this line was used for comparison and was fit to the plotted data points of the basis sample evaluation to determine how close of a match the experimental spectrum and signature were. The statistical result was three quantitative goodness-of-fit values which statistically confirmed the qualitative match of the experimental and fitted spectra (Table 2).

Table 2 - Goodness-of-fit statistical results for semen signature fitting

<table>
<thead>
<tr>
<th>Sample</th>
<th>SSE</th>
<th>R-square</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen basis</td>
<td>0.0556</td>
<td>0.998</td>
<td>0.00630</td>
</tr>
<tr>
<td>1</td>
<td>0.299</td>
<td>0.990</td>
<td>0.0146</td>
</tr>
<tr>
<td>2</td>
<td>0.379</td>
<td>0.982</td>
<td>0.0164</td>
</tr>
<tr>
<td>3</td>
<td>0.450</td>
<td>0.979</td>
<td>0.0179</td>
</tr>
<tr>
<td>4</td>
<td>0.311</td>
<td>0.981</td>
<td>0.0149</td>
</tr>
<tr>
<td>5</td>
<td>0.319</td>
<td>0.979</td>
<td>0.0151</td>
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<td>0.200</td>
<td>0.992</td>
<td>0.0119</td>
</tr>
<tr>
<td>7</td>
<td>0.324</td>
<td>0.987</td>
<td>0.0152</td>
</tr>
<tr>
<td>8</td>
<td>0.164</td>
<td>0.996</td>
<td>0.0108</td>
</tr>
<tr>
<td>9</td>
<td>0.161</td>
<td>0.992</td>
<td>0.0107</td>
</tr>
<tr>
<td>10</td>
<td>0.189</td>
<td>0.989</td>
<td>0.0116</td>
</tr>
<tr>
<td>11</td>
<td>0.107</td>
<td>0.996</td>
<td>0.00875</td>
</tr>
<tr>
<td>12</td>
<td>0.111</td>
<td>0.995</td>
<td>0.00888</td>
</tr>
<tr>
<td>13</td>
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<td>0.995</td>
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<td>0.107</td>
<td>0.996</td>
<td>0.00875</td>
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<td>15</td>
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<td>0.0142</td>
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<td>16</td>
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<td>0.0117</td>
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<td>17</td>
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<td>19</td>
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<td>0.00725</td>
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<td>0.993</td>
<td>0.0112</td>
</tr>
<tr>
<td>25</td>
<td>0.177</td>
<td>0.993</td>
<td>0.0112</td>
</tr>
<tr>
<td>Blood</td>
<td>1.38</td>
<td>0.967</td>
<td>0.0313</td>
</tr>
<tr>
<td>Saliva</td>
<td>2.25</td>
<td>0.822</td>
<td>0.0401</td>
</tr>
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</table>
These values are the sum of squares due to error (SSE), R-square, and root mean squared error (RMSE). The SSE value measures the total deviation of the data points from the y=x line, and a value closer to 0 means there are fewer random errors (Wakefield, 2008). The R-square value indicates how well the y=x best-fit line explains variation in the data, and a value closer to 1 indicates that a higher proportion of the variance is accounted for by the line (Wakefield, 2008). A value closer to 1 also means that the fitted signature and experimental spectrum are a better match. Finally, the RMSE value estimates the standard deviation of the random data components. Again, a value closer to 0 indicates that the y=x line is a better fit (Wakefield, 2008), and that the signature better fits the experimental spectrum.

This same fitting procedure was performed for the remaining 49 samples. The graphical result of a typical fit is shown for sample 22 in Figure 4A as an example. The top half is a graph showing the fit of the line y=x to the comparison of the signature and sample, and the bottom half is a plot of the residuals which are found by subtracting the best fit line from the scatter plot. A residual plot with random points around zero that do not form a pattern indicates a good fit (Wakefield, 2008), as is the case here. The results for SSE, R-square, and RMSE for 25 of the 50 samples are shown in Table 2. As expected, the basis sample results indicated the best match since that sample was the template for the spectroscopic signature determination, but the statistical values for all of the samples are very close fall within a certain range that suggests a good fit. To put in perspective how well the signature fits the semen samples, it was also applied to the spectra of human blood and saliva which we have already reported (Virkler and Lednev, 2008). The goodness-of-fit statistics for those fits are listed at the bottom of Table 2, and it is easy to see how poorly the semen signature matches the spectra of the other two body fluids when all three statistics are taken into account. The R-square value for the semen signature fit to blood is not too much different than the average fit to semen, but the SSE and RMSE values definitely indicate a much worse fit. The visual results of the signature fit to the saliva sample are also shown in Figure 4B.
Fig. 4. Quantitative evaluation of the fitting quality. Comparison of the line $y=x$ with the semen signature fit for a semen sample (4A, top) along with the residual plot (4A, bottom). The semen signature fit for a saliva sample (4B, top) along with the residual plot (4B, bottom).

There is a large amount of disagreement between the best fit line and scatter plot in the top graph, and there is an obvious pattern of digression away from zero in the residual plot.
As revealed in Figure 3, Figure 4, and Table 2, the semen signature closely matches the experimental semen spectra and clearly does not fit the blood or saliva spectra. This result shows that a spectroscopic signature created from one basis semen sample can be fitted to multiple other semen samples from different donors, and this technique can potentially be used to identify an unknown sample to be semen without yielding a false positive match with other body fluids. However, at this stage of the project, the identification could be made only for individual body fluid traces (not mixtures of body fluids) without any contamination or substrate interference as indicated above (steps for further research).

4. Conclusions and Implications: D
A spectroscopic signature for human semen was developed based on the heterogeneous chemical composition of semen using NIR Raman Spectroscopy. Statistical analysis found that the spectrum of a dried semen sample contained three major spectral components in addition to a fluorescent background component; a component matching tyrosine, a component containing albumin and choline, and a component matching spermine phosphate hexahydrate. The results demonstrated qualitatively that there are no significant visual changes in the Raman spectra of dried semen acquired from multiple donors, and that the spectrum of dried semen varies considerably when compared to the spectra of dried blood and saliva. The combination of the three principal components can be used as a unique spectroscopic signature to identify the presence of semen and possibly distinguish it from other body fluids and substances of artificial nature found at a crime scene. The signature’s specificity to semen is additionally reinforced by the determination that two of the three spectral components are dominated by choline and spermine, respectively, and these chemical components are unique to semen and have been used as forensic identification techniques for semen in the past. This spectroscopic signature can be fitted to all of the dried semen samples with high goodness-of-fit statistical results, and this outcome shows how the signature can be applied to any human semen sample to potentially identify it. This proof of concept experiment showed promising results, but many more samples with known demographic information should be investigated.

We envision the use of this method for nondestructive detection and confirmatory identification of semen at a crime scene, both in its pure form and even as part of a stain. A forensic investigator would be able to determine the true identity of a suspected semen sample and whether it was pure and not contaminated. The ability to make these identifications and conclusions, especially at the scene of a crime, would be major progress in the area of forensic semen analysis. In addition, the ability to not damage the sample while making these conclusions would be a valuable feature since it would allow the possibility of additional testing on the same sample. More experiments need to be performed involving semen stains on different materials such as clothing, paper, wood, etc., but the technique introduced in this paper shows the potential for the Raman spectroscopic signature of semen to be useful in identifying semen at crime scenes.

Continuing investigation of semen samples and other body fluids is currently taking place in our laboratory. Future work will focus on developing unique spectroscopic signatures
for other body fluids to support the assumption that the different fluids can be distinguished from one another using Raman spectroscopy since they are composed of different chemical components. In addition, a more advanced statistical method which uses principal component analysis (PCA) to mathematically compare multiple spectra of different body fluids as well as spectra from different animal species of the same fluid will be tested in future work.

Implications for the field of criminal justice and the study of forensic science:
The needs and current status of various methods for detection and identification of body fluids for forensic purposes are briefly outlined above. In much greater details, we have disseminated this information in our recent review article “Analysis of Body Fluids for Forensic Purposes: From Laboratory Testing to Non-Destructive Rapid Confirmatory Identification at a Crime Scene” (Forensic Sci. Int. 2009, 188, 1-17). Great progress has been made in developing multi-dimensional Raman spectroscopic signatures for dry traces of various body fluids. In addition to the future research steps outlined above, practical application of our new method will require the understanding (i) how aging effect the spectral response from body fluid traces. In addition, our preliminary results clearly indicate that Raman spectroscopy combined with advanced statistical analysis is capable to (ii) differentiate species based on the blood traces. Another area of potential expansion of the method capabilities is (iii) genetic profiling, which is of great importance for forensic science. The overall project includes two major phases: Phase 1 targets the development and evaluation of the proposed novel methodology under controlled (laboratory) conditions. Phase 2 will involve building and certifying a portable easy-to-use automatic instrument based on the requirements identified in Phase 1.
1. Introduction

This study will examine the extent to which forensic DNA technology is exploited in the range of criminal investigations within Schenectady County and to assess the impact of its use in the outcomes of the individual investigations and subsequent proceedings in court. Published studies suggest benefits to the expanded use of DNA technology (Cascio, 2000; Weedn and Hicks, 1998). Under this project, training will be developed and instructed by NERFI staff for first responding officers on the identification and collection of potential DNA evidence at “routine” crime scenes – a “routine” scene being one in which the department’s crime scene/evidence collection unit would not typically be deployed.

To further facilitate the use of forensic services – especially DNA testing – an integration system initiative through Porter Lee will be set up to link the evidence management and tracking systems of the Schenectady DAs office with the Schenectady PD and with the NY State Police Forensic Investigations Center. When fully implemented, the Schenectady County initiative will serve as the pilot for a wider proposal (subject to available funding) linking ten Counties in the Capital Region as described herein.

Schenectady County is one of sixty-two counties in New York State, located in an area known as upstate New York or the Capital District area and one of ten Counties within the NY State Police Troop G territory. Schenectady County is 206 square miles in size with a population of more than one hundred and fifty one thousand. The District Attorney, Robert Carney, holds the highest office in the county judicial structure. Seven organized law enforcement agencies operate within the County, the largest of which is the Schenectady City Police Department. DA Carney’s office is staffed with approximately thirty-seven employees. The Schenectady Police Department patrols the City and is the sixth largest law enforcement agency within the State of New York. All law enforcement forensic needs of the county are performed by the New York State Police Forensic Investigation Center located just a few miles away in Albany.

The New York State Division of Criminal Justice Services (DCJS) annually provides the following data in the “Crime Index Crime Summary 2008” (New York State Division of Criminal Justice Services, 2008) Schenectady County years 2007 vs. 2008 statistics indicate an increase in all categories except motor vehicle theft as illustrated:

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Violent</th>
<th>Forcible</th>
<th>Aggravated</th>
<th>Property</th>
<th>Burglary</th>
<th>Larceny</th>
<th>MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>5,355</td>
<td>689</td>
<td>5</td>
<td>36</td>
<td>288</td>
<td>360</td>
<td>4,666</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>5,634</td>
<td>740</td>
<td>9</td>
<td>43</td>
<td>311</td>
<td>377</td>
<td>4,894</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td>5.2%</td>
<td>7.4%</td>
<td>80.0%</td>
<td>19.4%</td>
<td>8.0%</td>
<td>4.7%</td>
<td>4.9%</td>
<td>9.6%</td>
</tr>
</tbody>
</table>

Law enforcement’s success in solving crime depends on its ability to identify, collect and preserve physical evidence of probative value and then to present that evidence to a
forensic laboratory for analysis. Based on studies cited above, the recovery of DNA evidence in any criminal investigation is expected to improve the “solvability factor” significantly through the exclusion or inclusion of suspects or particular acts and has been demonstrated to significantly affect the course of the investigation and subsequent judicial process (Becker and Dale, 2007; Dale and Becker, 2005).

To determine the scope and extent of the use of forensic DNA evidence within Schenectady County, the primary service provider, the New York State Police Forensic Investigation Center was asked to provide statistics on cases presented to them from all law enforcement agencies within the County. The following graph displays a summary of the data provided:

![Graph](image)

Figure 1. Comparing the results of the Index Crime Summary to the Laboratory’s DNA statistics on the same four (4) categories for period 2007 vs. 2008 provided the following results.

**Larceny**
The Index Crime Summary 2007 vs. 2008 revealed an increase of 6.1% on reported Larceny investigations whereas the laboratory reports an increase of 55.6% in Larceny cases submitted with DNA evidence.

**Burglary**
The Crime Index 2007 vs. 2008 revealed an increase of 9.6% on reported Burglaries investigations whereas the laboratory reports an increase of 14.3% in Burglary cases submitted with DNA evidence.
Robbery
The Index Crime Summary 2007 vs. 2008 revealed an increase of 8% on reported Robbery investigations whereas the laboratory reports a decrease of 17.2% in Robbery cases submitted with DNA evidence.

Homicide
The Index Crime Summary 2007 vs. 2008 revealed an increase of 80% on reported Homicide investigations whereas the laboratory reports a decrease of 38.4% in Homicide cases submitted with DNA evidence.

During the period covered by these data, it is significant to note the dramatic increase in physical evidence submissions from law enforcement agencies within the County requesting DNA analysis – especially for “non-traditional” DNA crimes.

2009 New York State Index Crime statistics are not yet available but will prove to be extremely interesting as DNA submissions 2008 vs. 2009 increased for the reported categories by 23.8%.

2. Methods

For purposes of this project, DA Carney authorized a part-time employee (a representative of the Northeast Regional Forensic Institute) to be placed within his office to serve the following functions:

- Liaison between the police agencies, the District Attorney’s Office, and the NYSP Forensic Investigation Center
- Facilitate training for first responding (patrol) officers of the Schenectady PD in the recognition, detection, and collection of forensic evidence - a total of eighty officers (Appendix 17).
- Coordinate the acquisition and installation within the District Attorney’s Office of a fully automated evidence inventory and disposition tracking system (purchased from Porter-Lee, Schaumberg, Illinois) that is fully compatible with the system in use by the Schenectady PD.
- Identify and measure evidence collection data and, to the extent possible within the period of this study, to monitor case outcomes:
  - Types of crime reported
  - Amount and types of evidence collected
  - Identify opportunities to apply new forensic technologies
  - Determine impact of evidence and new forensic technologies on case outcomes

Plea bargains and verdicts were reviewed. If evidence could have been collected that may have improved the outcome of the case, police work was also reviewed. If the
prosecutors could have further enhanced the case by seeking other evidence after arrest, this was explored.

This project also attempted to evaluate the use and effectiveness of forensic technology in the courtroom. Verdicts were to be examined and jurors asked after trial to voluntarily take a survey to assist in determining how important the presence or lack of, forensic science was in the verdict; however, as noted below, timely access to the jurors and to individual cases before the courts was precluded by legal and policy considerations.

3. Results and Conclusions and Implications
Attempting to identify case outcomes became very problematic as the gathering of case information critical to this study was challenging. Through a cooperative arrangement with the State Police Forensic Investigation Center, a spread sheet was created from case information gleaned from the laboratory DNA case submission information for years 2006 through 2008 but significant problems were encountered which precluded the populating of this record as conceived in the original project proposal. Historical case records are all paper boxed, labeled and stored at an off-site location to the limited office space. The existing District Attorney’s office electronic records did not contain detailed information on the nature of specific evidence items collected and forensic analysis outcomes. In addition, the length of time required for a criminal case to go from arrest and charging action to court disposition proved counterproductive to the strategies originally conceived for this study. A significant amount of time was spent in the Special Victim Unit which practices vertical prosecution, trying cases of domestic and sexual assault. During the grant period, some time was spent actually observing trial testimony of witnesses including testimony of forensic laboratory analysts in cases in which DNA evidence was introduced.

One noteworthy case resulted in a sentence of 75 yrs. to life. DNA evidence played a crucial role in the investigation of a sexual assault as the assailant was unknown and unidentifiable to the victim who was rendered unconscious due to strangulation and very severely beaten.

A review was conducted of sentences imposed in ten historical sexual assault cases. Five of the cases resulted in plea bargaining and five resulted in trial. In the cases in which plea bargains were negotiated, the sentences imposed averaged twelve years. The cases that went to trial resulted in an average sentence of forty-two years.

An anonymous and voluntary survey form was designed to be completed by jurors when available; however, upon review by the District Attorney and Counsel to the DA, it was determined that such a survey would prove problematic based on a recent court decision weighing post-trial interview and survey of jurors.

As an ancillary development in this project, the DA’s office requested a review of two 1995 cold case homicides with DNA evidence that remain unsolved. After reviewing the investigations and analyzing the evidence secured, a spread sheet was created organizing and coordinating the evidence recovered at the scene. Several new items that potentially
contain DNA evidence were identified. Several meetings were held with the District Attorney’s office, Schenectady Police Department and the New York State Police Forensic Investigation Center resulting in the submission of new evidence for testing and resubmission of older DNA evidence.

“Crime Scene Analysis Evidence Collection and Packaging” training was conducted for eighty one (81) officers (first responders) attending four 4-hour training sessions at the Schenectady Police Department on forensic evidence recognition and collection (Appendices 17 and 18) by NERFI staff covered under this grant. In addition, all eighty-one officers received four hours of further training by Schenectady Police Department staff (not covered by this grant) which included a redirection on policy and procedure with regard to “routine” crime scenes. The Schenectady Police Department is implementing a new policy where first response officers collect appropriate forensic evidence from misdemeanor offenses, primarily automobile break-ins, for submission to Forensic Investigation Center. The program included an overview of the New York DNA Database program and qualifying offenders who are required to provide DNA specimens to the Database (Appendix 18). Other lectures were given on the capabilities of a forensic laboratory, the potential for effective use of DNA evidence, and general contamination and collection issues. A Schenectady Police Department detective and supervisor of the Crime Scene Unit provided a four hour agency specific collection and processing training session to the officers, alternating with the forensic laboratory training lectures.

The training provided officers with an overview describing the benefit of forensic evidence to a criminal investigation, this included discussion of cases where forensics was critical to identifying and convicting the perpetrator of the crime.

This grant provided the ability to purchase an evidence management system (“The Beast” by Porter-Lee corp.) for the Schenectady County District Attorney’s Office. A system from the Porter-Lee Corporation was purchased due to its compatibility with the Laboratory Information Management System (LIMS) developed by the same corporation currently in use by the Schenectady PD evidence management unit and the New York State Police Forensic Laboratory. Not only will this evidence management system be used to record and track the evidence in the District Attorney’s office it will also be used to track the volumes of boxed case material needed for prosecution. These boxes are stored throughout the District Attorney’s office until they are moved offsite. Additionally the Schenectady Police Department is a subscriber to the Porter-Lee system and the largest contributor to case load for prosecution by the District Attorney’s office. The system has the potential of allowing the District Attorney’s office to remotely view the evidence and case records of the Schenectady Police Department. This alone would benefit a prosecutor in case preparation and evidence transfer for trial along with assisting the two agencies in decisions needed for evidence retention and destruction. The installation of this system would have made this study much more successful. Unfortunately, full installation and operability could not be completed within the project cycle.
The case management system will be delivered and installed in the District Attorney’s office by Porter-Lee during the month of March 2010. Though this grant will have expired, the project manager retained under the grant, Ron Stevens, has agreed to assist in the implementation and training to insure the successful implementation of this system.

When implemented, the Schenectady PD/Schenectady DA/State Police Forensic Investigation Center integration project will serve as a pilot for a wider, ten County, Capital Area Initiative linking these agencies with the Forensic Investigation Center. The concept and basis for the expanded initiative is described further below.

Bipartisan legislation now pending in Congress would require the federal government to collect data on the number of untested rape kits nationwide, and to prioritize the testing of this evidence. A pending House bill also would provide incentives to state and local law enforcement to eliminate the backlog of untested kits. The U.S. Department of Justice has declared its support for initiatives that help assure rape kit evidence is processed on a timely basis. When implemented, the integration project will help assure that criminal justice agencies can readily and effectively identify criminal cases in which physical evidence has been recovered and track the progress of laboratory analyses.

Other federal legislation has been proposed to establish DNA evidence retention requirements. The legislation is in response to expressed concerns that such evidence may be subjected to testing using technological advances unavailable at the time of the original crime and ensuing investigation that may lead to new information bearing upon the guilt or innocence of suspects or persons convicted of those crimes.

Criminal justice agencies face significant challenges in maintaining the integrity and control of the wide variety of physical evidence recovered during the course of criminal investigations. Automated systems are available from several sources that are designed to aid in inventory control, in documenting the chain of custody as required when introduced in subsequent court proceedings, and in tracking the progress of forensic processing of the evidence.

The NYSP Forensic Investigations Center uses a Laboratory Information Management System (LIMS) to track all cases in which physical evidence is submitted for laboratory analysis. The system employs bar-coding of physical evidence specimens and is a product developed by the Porter-Lee Corporation, Schaumburg, IL. The same system is used by many of the public crime laboratories operating in New York State. Porter-Lee also produces an evidence management system (EMS) which is used by many police departments across the state.

Implications for the field of criminal justice and the study of forensic science:
This project will prove helpful in identifying and addressing the technical specifications and operating rules necessary to (1) effectively track and document the movement of physical evidence from the point of collection to . . . (2) storage at the police agency to . . . (3) transfer to the laboratory for forensic analysis to . . . (4) return to the police agency to . . . (5) transfer to the District Attorney for introduction in a court proceeding and . . . (6) ultimate return for retention at the police evidence storage and control. Throughout this movement of the physical evidence, interested agencies (the PD, DA and Lab) will be able to independently inquire
electronically in real time on the location of the evidence, the status of pending forensic tests, and obtain reports of the results of forensic testing on the evidence. The documentation involved in the transfer of evidence will be accomplished without redundancy in data input as the two-dimensional bar-codes employed will automatically track and capture the specimen identification number with descriptive information, the relevant case information, and the submitting and receiving agencies identifying information and points of contact.

The acquisition of computer equipment and software for other District Attorneys offices in the capital region and for selected law enforcement agencies within those counties will proceed as resources are available. The police departments at Albany and Troy, the Rensselaer County Sheriff’s department, and Albany District Attorneys office already use the Porter-Lee evidence management system. In discussions held with State Police administrators and IT managers as well as FIC managers, there is strong interest in expanding this system to include the major users of forensic testing services available through the FIC.

With the improved capacity to manage forensic evidence, training will be provided to departments with an emphasis on the effective use of DNA technology to resolve a wider variety of crimes. Traditionally, DNA technology has been applied primarily in the investigation of violent crimes. Crime data show that there are nearly twice as many burglaries in New York State as compared to the number of violent crimes — with most burglaries going unsolved. Recent advances in the technology, coupled with the expanded processing capacity of public forensic DNA laboratories (largely accomplished with federal funding assistance), provide opportunities for its effective use in property crimes. According to recent studies, DNA is more likely to be recovered from a crime scene than a fingerprint, and DNA Databank “hits” linking offenders to evidence from a violent crime showed that in 75% of the cases the basis for collecting the offender’s DNA specimen was a conviction for a “lesser” crime such as a burglary or drug offense. The Capital Area Integration Initiative will assure the first responding officers to a burglary scene will be equipped and trained to recognize potential DNA evidence and properly collect it in a manner that meets the requirements of the forensic laboratories and, ultimately, the courts. In order to insure the quality and reliability of DNA analysis services provided to the law enforcement agencies in the capital region and to assure analysts are prepared with the latest and most efficient analytical techniques, highly specialized training will be provided to forensic DNA laboratory personnel in workshops and seminars.

Finally, the immediate success of Forensic Science Impact in the Courtroom can not be judged solely on the statistics and information provided from the results of this study. Instead, future studies may be warranted to determined the overall success from the advise, instruction and equipment provided under Module—Forensic Science Impact in the Court Room.
MAIN BODY: Module - ChatMinder—A Safe Internet Tool for Parents

Author: Dr. Tomek Strzalkowski

1. Introduction
The objective of ChatMinder project is to conduct and deliver a study of dialogues occurring in the on-line chat rooms. A secure chat-room at the ILS Institute will be used to collect chat data from experiments with recruited subjects (SUNY students under an IRB protocol). Approximately 20 hours of chat involving groups of 3 to 6 people on topics ranging from movies to organic food to state of the economy will be collected and analyzed for this study.

One hypothesis pursued is that an automatic agent could be developed to operate in a live chat room, monitoring conversational behavior. When this automatic agent detects behavior that is unsuitable, it can try to intervene. There is a range of actions such an agent could take, including reporting malicious behavior to some administrator. However, a more subtle intervention could be an effort at changing the topic of conversation, presumably away from potentially dangerous areas, to safer topics.

2. Methods
Methods used in this research include data collection and annotation, conversational modeling, and software development and testing.

1 Data
Chat data was collected through controlled exercises with participants in the secure chat-room located at ILS labs. While large volumes of data may be obtained from public chat-rooms, it was of limited value for the type of modeling tasks that were of interest in part because of the high-level of noise, lack of focus, and rapidly shifting, chaotic nature, which makes any longitudinal studies virtually impossible. Public chat-rooms may be excellent sources of data for studies involving on-line language usage (e.g., novel uses of vocabulary, syntax), general conversational etiquette, and related issues. However, for deriving more complex models of conversational behavior, this project required the interaction to be reasonably focused on a task and/or social objectives within a group. There are a number of available resources that demonstrate that data collected under monitored conditions is still effective for modeling language (cf. ICSI-MRDA corpus (Shriberg et al., 2004), Switchboard corpus (Jurafsky et al., 1998) and Map Task corpus (Anderson et al, 1991)). The purpose of conversational data analysis was two-fold: (1) understanding how certain social behaviors are reflected in language use, and (2) building an automated chat agent that could effectively use appropriate linguistic forms to achieve certain social objectives in the chat-room.

One specific social behavior that was focused on in this project included Topic Change, which was an attempt by a participant to change the flow of discussion from one topic to another. Modeling this behavior was of interest because it was directly applicable to a future Chat Minder prototype. To ensure that this social behavior was present in our chat
data, a multi-tiered collection process was devised in which the subjects started from simple, free-flowing conversations and progressed towards more complex and structured interactions. Approximately 20 hours of chat dialogue spread out over 14 sessions of 90 minutes each were collected during this study, amounting to a total of 7317 individual utterances.

2 Data Annotation

The data was annotated for presence of linguistic elements that correlate with social behavior on three different levels, as detailed in Sections 2.1 through 2.3 below. In Section 2.4, we describe the annotation tool used to facilitate annotation. Section 2.5 describes the annotated set and provides pertinent statistics.

2.1 Communication Links

Communication links capture associations between utterances. Utterance includes the sequence of words that are entered by a participant in a single turn in chat. In multi-party chat the relationships between utterances are often ambiguous; it is not readily apparent who is speaking to whom, particularly when there is no addressing information in the utterance. However, it is an integral part of understanding how social behavior is manifested in language, as we need to determine between which participants the conversation flows. These are situations where one participant’s utterance responds or relates to a previous utterance by another participant. This also includes situations where one participant is addressing another participant or a group of participants who may subsequently respond to him or her.

At this level, we were interested in annotating utterances that were addressed to the entire group or to some specific participant, uttered in response to a specific previous utterance, or are continuations of a previous utterance by the same participant. Accordingly, there are 3 possible communication links, one of which was assigned to each utterance in our annotated data: Addressed-to (a specific participant), Response-to (a specific prior utterance by a different participant), and Continuation-of (a specific prior utterance by the same participant).

2.2 Dialogue Acts

The functional or dialogic aspect of an utterance has to do with its role or purpose in conversation. Statements, questions, answers, offers, acceptances and rejections, as well as expressions of thanks are all examples of such functions in a dialogue, which we call Dialogue Acts (DA). At this level our objective was to capture how an utterance functions in dialogue, which may or may not be directly related to its form. For example, the utterance “Can you close the window?” can function as a question or as a directive, depending upon the context in which it is used. It was therefore important to consider the context of the utterance to make a decision of which dialogue act label to assign. We created a hierarchy of 21 dialogue act labels, which we grouped into three top-level categories, namely – Statements and Responses, Questions and Directives and Conversational Norms.
By marking utterances at this level, we wanted to identify the pragmatic composition of an utterance. This is especially useful when trying to discover certain social behaviors, such as the change of conversation topic. The mechanism of changing the topic may vary by participant, the kind of topic change, and by the time at which it is attempted relative to the sequence of conversation. A participant may introduce a new topic by asking a question about that topic, thereby obligating the other participants to respond to them; or they may make a provocative statement and thus attempt to steer the conversation towards another topic. We wanted to determine whether there was any correlation between utterances marked as topic change utterances (explain in subsection 3.3 below) and the dialogue act assigned to them.

2.3 Topic and Focus of Conversation
A topic is the subject matter under discussion in some part of the dialogue. Focus is another semantic property of an utterance and it pertains to an entity or event that is the most salient in the utterance. The difference between topic and focus is often only the matter of degree: topics are larger subjects (such as technology, movies, or politics), while foci are more narrow subthemes within a topic (e.g., cell phones, or Tom Hanks, or Eiffel Tower). However, it is often impossible to define topics and focus a priori (e.g., Tom Hanks may be a focus of a discussion about movies; but it would be a topic if the discussion is about his career, while a specific movie he starred in may be the focus.)

In this exercise we primarily concentrated on topic and focus changes. In other words, we wanted to be able to tell where a topic/focus starts and when it ends in a dialogue. We postulate that annotating topic change and focus shifts will help us determine behavior, which induces a modification of the flow of conversation. We asked annotators to determine the topic and the focus of each utterance using labels of their own choosing (based on what is being said). When the subject of the dialogue changed in any significant way, we asked the annotators to change the topic or the focus label. That utterance would then denote the beginning of a new topic. Note that in chat room conversations, due to the asynchronous nature of dialogue and multiple participants, there may be different topics being discussed within a short period of time, often being interleaved with one another. The same may be the case for focus as well.

2.4 Chat Annotation Tool
An annotation tool (developed under another project) was used to facilitate the annotation process. This tool was implemented in Java and deployed as a web applet, so that annotators were able to work from remote locations. All annotators were trained on a sample set of data. Since our annotation paradigm is multi-tiered, we asked the annotators to annotate each level in separate passes through the data to reduce cognitive effort. This also had the effect that annotators become more familiar with the data with each pass. Figure 1 shows a screenshot of the Chat Annotation Tool.
Figure 1. Screenshot of Chat Annotation Tool

In the screenshot above, note that every utterance by the participants was represented as a row and all participants were separated into columns. Annotators mark the communicative links and dialogue acts which show in the utterance cell – e.g. turn 102 by participant Lance was marked as “addressed-to-Kerri” communicative link and a “Positive-Answer” dialogue act. Each different color represents a separate topic, so in this short snippet of conversation in the screenshot there were three, albeit interleaved, topics that were annotated.

2.5 Annotated Data Set

Of the 14 sessions we collected, we selected 10 for annotation, with at least 3 annotators for each session. In Table 2 some of the overall statistics computed from this set are shown. We computed inter-annotator agreement on all three levels of our annotation, i.e. Communication Links, Dialogue Acts and Topic/Focus Shifts. Topic and Focus shifts had the highest inter-annotator agreement scores on different measures such as Krippendorf’s Alpha and Fliess’ Kappa. In Figure 2, we show inter-annotator agreement measures on Topic/Focus shift annotation. With such high degree of agreement, we can reliably derive models of topic shift behavior from our annotated data.
Figure 2. Inter-annotator agreement measures for Topic/Focus shifts

Table 2. Selected statistics from annotated data set

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Sessions Annotated</td>
<td>10</td>
</tr>
<tr>
<td>Number of annotators per file</td>
<td>3</td>
</tr>
<tr>
<td>Total Utterances Annotated</td>
<td>4640</td>
</tr>
<tr>
<td>Average number of utterances per session</td>
<td>~520</td>
</tr>
<tr>
<td>Total topics identified per session</td>
<td>174</td>
</tr>
<tr>
<td>Total topic shifts identified per session</td>
<td>344</td>
</tr>
</tbody>
</table>

3. Results

Analysis of the collected data led to construction of preliminary models of social behavior in online discourse. Conversations were annotated for communicative links, dialogue acts, and topic and focus shifts, which created the basis for building computational models of conversational behavior. Some of these models, e.g., how to effectively change the topic of conversation, were subsequently implemented into an automated Virtual Chat Agent (VCA), a Chat Minder prototype. VCA has been demonstrated to perform effectively and convincingly in Internet conversation with human participants.

3.1 Conversational Modeling for VCA design

A virtual chat agent is an automated program with the ability to respond to utterances in chat. Our VCA was distinctive in its ability to participate in multi-party chat and manage to steer the flow of conversation to a new topic. We exploit the dialogue mechanism underlying HITIQA (Small et al. 2009) to drive the dialogue in VCA.

The topic as defined by the information contained in the participant’s utterance was used to mine outside data sources (e.g., the web) in order to locate additional information about that topic. The objective was to identify some of the salient concepts that appear
associated with the topic, but are not directly mentioned in any recent utterances. Such associations may be postulated because additional concepts are repeatedly found in many web pages near the concepts that are mentioned in chat.

Based on our annotated corpus, we also determined that a common method that participants employ to achieve a topic change in conversation is to introduce a new concept (or aspect) that is shared between the current topic and the new topic. This was schematically illustrated in Figure 2 below, where the current conversation topic (technology) was changed to a new topic T2 (music bands). Participant K introduces the topic of music bands by finding a common concept that forms a bridge between these two topics, which is “Lars Ulrich”. By introducing Lars Ulrich K opens the window for the conversation to shift to music bands, which indeed happens as speaker N picks up on the association, as intended. Subsequently, K clinches the transition by adding another utterance on the new topic.

![Figure 2. Schematic illustration of a topic change.]

Figure 3. Example of topic change by participant K.

The effect of topic change was apparent when a subsequent utterance by another participant is about the same topic. This is a successful attempt at changing the topic. Below is an example of topic shift annotated in our chat data collection.

**Example 1. A topic change in dialogue**

Note that in this example, the second part of utterance by participant KA – “what kind of music do you guys listen to?” is deployed to shift the topic from “music band, dc for cutie” to “music”. This, in turn allows participant KN to shift the topic to You Tube.
Another example is participant KA’s utterance in the transcript below inducing participant KI to change her topic from navigation to texting:

**KI:** I actually went on a 3 hr drive yesterday, i just used a map instead of his GPS  
(TOPIC: navigation technology/GPS)

**JR:** hahaha its still possible to exist without all the technology but google and mapquest have def made my life more livable-  
(TOPIC: navigation technology/google, mapquest)

**KA:** what about text messaging  
(TOPIC: technology, BRIDGE: text messaging)

**KI:** I hate texting. I'd rather just talk on the phone  
(TOPIC: text messaging)

**Example 2. Another topic change in dialogue**

We found this model of topic change fairly consistently exhibited, where the participants would ask an open question, to get other participants to respond to them, thereby changing the course of conversation. We collected all utterances marked topic shifts and created a set of templates from them. These templates served as a model for the VCA to utilize when creating a response.

Another model of behavior that we found as a consequence of topic change was topic sustain. This is an instance where the utterance was marked to be on the same topic as the one currently being discussed, for example, JR’s utterance in the Example 2 above. Topic-sustain utterances typically offer a new in-topic aspect, but provide no bridge to another topic as noted previously. Typical linguistic forms used were offers of support, an agreement with a previous utterance, or a question about any known in-topic aspect.

**3.2 VCA Software Architecture**

The chart in Figure 4 shows an overall architecture of the VCA.

![VCA Architecture and Components](image)

**Figure 4. VCA Architecture and Components.**
Chat Analyzer
Every utterance in chat was considered to be a candidate for response by the VCA. It was first analyzed by the Chat Analyzer component. This process removes stop words, emoticons and punctuation, as well as any participant nicknames from the utterance. We postulate that the remaining content bearing words in the utterance represent the topic of that utterance. We call this analyzed utterance our chat “query” which was sent in parallel to the Document Retrieval and NL Processing component.

Document Retrieval
The document retrieval process retrieves documents from either the web or a test document corpus. We use Google AJAX api for our web retrieval process and InQuery (Callan et al., 1992) retrieval engine for our offline mode of operation. The test document corpus was collected by mining the web for all utterances in our data collection, thus creating a stable document set for experimental purposes. In addition, this test corpus ensured that we had a collection of documents pertinent to the realistic topics that were discussed by the participants in chat sessions. Currently, the document corpus contains about 1Gb of text data. We retrieve, on average, 20 documents per chat query, for both online and offline modes of operation.

Clustering
Paragraphs in documents were retrieved using clustering method in Hardy et al. (Hardy et al. 2009). This process groups the paragraphs containing salient entities into sets of closely associated concepts. From each cluster, we choose the most representative paragraph, usually called the “seed” paragraph for further NL processing. Each seed paragraph and the chat query undergo the same further NL processing sequence.

NL Processing
We process each chat query by performing stemming, part-of-speech tagging and named-entity recognition on it. Each seed paragraph is also run through same three natural language processing tasks. We are using Stanford POS tagger for our part-of-speech tagging. For named entity recognition, we used BBN’s IdentiFinder.

Framing
Frames were from the entities and attributes found in both the chat query and the paragraphs. A frame is a structured template that provides salient information about the underlying text. This work extends the concept of framing developed for HITIQA (Small et al, 2009) and COLLANE (Strzalkowski, 2009). The frame built from chat query was the Chat Frame and those frame built from paragraphs are Paragraph Frames. Framing provides an informative handle on text, which can be exploited to compare the underlying textual representations, as we explain in the next section.

Scoring and Frame Matching
Using the information in the frames built in the previous step, we compared the chat query frame to the frames created from the paragraphs. We assign a score for each paragraph frame based on how many attributes and their corresponding values match. Note that for a paragraph frame to be eligible for scoring it should contain all the attributes found in the chat query frame. Of all the paragraph frames we select the highest scoring frames and select the attribute-value pairs that are not part of the chat query frame. For example, as show in Figure 5 below, the chat utterance “Aruba might be nice!” created the following chat query frame.
Correspondingly, we will select all PLACE type entities from the highest-ranking paragraph frames. These are shown in Figure 6 as Aruba Entity list. The entities “NASCAR”, “Women Seeking Men” and “Mateo” are not of entity type – PLACE, we assign them a score of 0. Only the entities that match the type of entity in the chat query frame get a positive score. This score is the frequency of occurrence of that entity in the paragraph; in this example it is found to be 1. Assigning scores by frequency of occurrence ensures that the most commonly occurring concept around the one that is being discussed in the chat query utterance will be used to respond with.

**Template Selection**

Once we have chosen the entity to respond with, we select a template from the set of templates for that entity. These were templates that were created based on the models created from topic change utterances annotated in our data set. For a select group of entities, which were quite frequently encountered in our data collection such as PLACE, PERSON, ORGANIZATION etc., we have a set of templates specific to that entity type. We also have several generic templates that may be used if the entity type does not match the ones that we have selected. For example, a PLACE specific template is “Have you ever been to ___?” and a PERSON specific template is “You heard about ___?” Not all templates are formulated as questions. An example of a generic template is “___ rules!”.
3.3 VCA Experiments
Figures 7 and 8 represent examples of the VCA in action in a simulated environment. In both examples the VCA was the participant “renee”. Figure 7 shows an example of a topic sustain behavior while Figure 8 shows an example of a topic change behavior.

Figure 7. Example of VCA in action: topic change

Figure 8. Another example of VCA in action: topic sustainment
3.4 Preliminary Evaluation

We have developed a two-stage evaluation protocol in order to test the effectiveness of the VCA prototype in a realistic setting. In the first stage, we tested the performance of the VCA in the laboratory chat room with human participants. At some point during the dialogue, one of the participants was tasked to withdraw from the chat and (silently) pass the control to this VCA for a period of time. This participant remains on-line but all conversation was now conducted by the VCA. After N minutes, where N is an experimental parameter, the control of conversation returns to the human user. In this test, it was important that the other participants were not aware ahead of time when the switch was supposed to occur.

The initial metric of VCA effectiveness was calculated as a proportion of utterances generated by the VCA during the number of utterances made by all participants during this period. These utterances were subsequently judged for appropriateness using the metric developed for the Companions Project (Webb, 2009). In general, utterances that were not on topic or out of place were judged as not appropriate. Such utterances may give off the VCA to other members of the group.

Since a VCA was tasked to perform a specific function in the chat room – i.e., to accomplish a topic change – the effectiveness with which this task was accomplished was also measured. One simple metric that was developed included an index of subsequent mentions of the new topic by others in the discourse. Initially, annotators track the topics manually over the log of the chat (we noted previously that there is a high level of inter-annotator agreement in this category). For the purpose of automating this metric, the topic may be equated with the named entity introduced by the VCA into the conversation for the first time, e.g., “How about Dallas?” introduces Dallas as a topic of conversation if it wasn’t a current topic, and has not been one before (or at least not recently). We count the number of utterances by other participants in the dialogue that contain references to the new topic (e.g., Dallas) in the immediately following dialogue. The topic change was considered successful if at least one subsequent mention of the topic occurs in the dialogue. The topic change was considered lasting if there are at least two subsequent mentions by two different participants. The topic change was considered permanent, if the dialogue does not return to the topic before the change occurred.

Evaluation of topic sustain action was performed in a similar manner. In this case the VCA picks up an already introduced topic and generates an utterance meant to induce a response from other participants that would require a mention of the topic, e.g., “What one can do in Dallas?” Again we measure the number of subsequent mentions of this topic, which may be direct or via a pronoun. We also count the number of turns in between these to measure the extent of dialogue over which the topic continues and to what degree it engages the participants.

4. Conclusions and Implications

In the ChatMinder project, a study of language uses had been conducted from dialogues occurring in the on-line chat rooms. At least 20 hours of chat involving groups of 3 to 6 people on topics ranging from movies to organic food to state of the economy had been analyzed for this project. We subsequently performed initial studies with a prototype autonomous chat agent (VCA) that can effectively change the topic of conversation in a
chat room with human participants. VCA technology represents an important advance in automated human-computer communication with potential applications in cross-cultural social modeling, influence operations, advertising, law enforcement, and national security.

It was our continuing hypothesis that an automated agent such as this can be used to monitor live chat rooms where at risk constituents (such as children) can be participating in online conversation. There were a number of flags and filters that can be used to spot potentially troubling conversation. One method of dealing with these was to report transgressors to chat room administration. Another, more preventative measure could be to allow automated agents to change the topic of conversation when it believes the current topic was dangerous or inappropriate. This method could also be used in situations where people were posting inflammatory statements about some issue, and rather than terminate the discussion an automated agent could attempt to divert the course of the conversation, rather than allow it to continue. Of course, these are potential future applications of this technology, which require more extensive evaluation of the current prototype.

For a more realistic future evaluation, a live Internet chat room experiments need to be conducted, where the VCA is entered either autonomously or with a human “handler”. Future work needs to focus on transferring VCA technology to the chat-rooms used by children. This may be accomplished in collaboration with researchers at UA School of Social Welfare, the Families Together of Albany County, and Prevent Child Abuse NY, and would include additional studies of suspicious on-line behavior when a change of topic intervention by a VCA may be warranted.

Implications for the field of criminal justice and the study of forensic science:
There are mechanisms for interaction specific to groups we may wish to target. For example, the language used, or social conventions that any automated agent some observe if communication is likely to be successful (e.g., the ECO project currently underway at ILS). The strength of our model is that it doesn’t rely on any domain model or prior knowledge, which is costly to include and brittle to maintain. A weakness is that there may have to be adaptation to individual user groups, or some sort of maintained ‘hot list’ of topics that should be monitored. These will need to be acquired, either from monitoring the groups themselves, or more likely, by law enforcement or monitoring agencies, who are likely aware of the issues facing particular groups or communities.

Future research may thus focus on (a) adapting the findings of this study to other chat-room user populations, specifically to children; and (b) building and evaluating of an autonomous chat agent. The deliverable from this project is the information included in this report.
1. INTRODUCTION

Crime data analysis necessitates combining pieces of information from disparate sources to make meaningful deductions. The number of sources continues to increase and the data is complex – making it difficult for forensic analysts to collect and process data manually. Consequently, the process of crime analysis is often slow and labor-intensive. While, it is not possible to replicate human deductive ability tools can be used to process data and reduce the cognitive overload on the analysts. The objective of this investigation is to develop techniques that improving the efficiency and effectiveness of crime data analysis, including, collection and analysis. Crime data comes from several sources, such as: computer logs, past crime records, databanks, and reports. The data can be both unstructured such as reports, articles or structured such as databases and spreadsheets. The goal of this research is to develop crime analysis techniques using both structured and unstructured data. There are three components of this work. Our goal was to develop a set up an infrastructure that will assist in data collection and analysis. This includes:

1) Determination of data sources
2) Creation of data collection robots from online sources
3) Development of natural language processing capability from text data
4) Creation of techniques for tagging and correlating data

Our initial goal was to use the infrastructure to investigate the problem of recidivism in crime (especially sexual crime) based on crime records available as well as information from public sources. However, obtaining criminal data, especially DNA data, proved to be very difficult and we focused our efforts on public data sources and creating the infrastructure to efficiently analyze them.

The focus of this work is to build the infrastructure that will allow for efficient data collection, and correlation and analysis of data to draw reliable conclusions. Infrastructure building involved in house code development as well as acquisition of third party tools. Law enforcement already has capabilities of searching through databases efficiently. They now also have standard forensic procedures for electronic data and new procedures are evolving as technology evolves. There are however missing elements of the infrastructure which could add to the repertoire of law enforcement agencies, including: 1) online data collection (e.g. robots), 2) text data analysis, and 3) correlations across disparate data sets.

2. DATA COLLECTION

Data collection for crime analysis has traditionally come from law enforcement agencies and other government sources. Increasingly, data for crime analysis is coming from public sources. Clues to motivations and behaviors of criminals can be buried in their online activities which can provide precursors to future criminal activity or help in
corroborating evidence from other sources. The data set can contain both text and numerical data which needs to be analyzed in conjunction.

The focus of this work has been on Internet Crimes against Children (ICAC) and we have identified several public and non-public sources to compare and analyze. The non-public sources were collected as a part of a previous investigation by the Capital Region Cyber Crime Partnership. The DCJS data includes level 1-3 sex offender data with arrest dates, address information, and charges of those individuals who used a computer to commit their offense prior to 2007. Site Key, Avalanche, Justin, Underscore, and Falcon are sting operations conducted by the New York State Police where data was collected from websites offering child pornography subscriptions which included address, IP, and purchase dates. A database schema including attributes is included below.

We have identified several sources of data including publically available sex offender registries, which can be used to solve important questions related to criminal justice. We are in the process of looking at Perverted Justice chat logs, Wikisposure, pedophile forums (including some which are defunct), PeeJ forums, Absolute Zero and AntiPaedo blogs, Corporate Sex Offender. In addition, business and school information is also being collected.

We hope to be able to develop techniques for identification of online sex offenders through analysis of these sources. Some cyber crime analysis methods can be used for this purpose. Website domain histories can be reviewed as well as IP addresses correlated to geographic locations for associating with known information about an individual.
Identification can also be done through text-analysis and authorship determination (linguistics and psychology).

3. TOOLS AND METHODS

Data Collection Robots
As a part of this work, we have developed data collection robots and models to collect data from publicly available sources using Kapow\textsuperscript{1}. This software tool allows scanning of thousands of sites to collect data and insert it into structured databases. As an initial pilot, the robot was configured to collect information on sex offenders listed in the NYS Department of Criminal Justice Sex Offender Registry. This robot can collect information on personal information, location, crime, and conviction of the sex offenders. Robots were also developed to collect information from hacker forums to observe patterns of behavior.

Linguistic Analysis and Identification
We have also developed software that can do content analysis on unstructured data such as chat logs, web pages, online postings, and emails. Unstructured text can be used to profile criminals, identify their motives, and predict their inclination to coming crimes. Natural language processing allows us to analytically identify such markers in text. In addition, tools were developed for parsing unstructured text and identifying keywords. Frequency of usage of keywords in blogs, chat logs, and postings of internet users can be used to understand the psychological behavior of a user. Using data from known offenders and clean data from other blogs can be used to create a classifier. Keyword markers exist for several different attributes, such as anger, fear, sexual behavior, etc. We have programmed these keywords in the code so that we can conduct behavior/motive analysis based on the content. Beyond use of keywords, we also plan to investigate linguistic features in the text that can provide us clues to their behavior.

Correlating information obtained from the web and attempting to associate these with the existing data on sexual offenders can help in detection of potentially dangerous situations where chat room text analysis can expose precursors to sexual crime. Based on preliminary analysis we can identify keywords that predators may use to ask about clothing sizes, genitalia descriptions, and parental presence. Users using such language may be more likely to be sexual predators.

Correlating Disparate Sources of Data
If these predators can be linked to past crime history the likelihood of their committing another crime increases manifold. Another attribute is the frequency of such behavior, i.e. similar behavior with multiple potential victims. We have deployed the Palantir Government platform, which allows us to tag data from disparate sources and correlate the information creating a comprehensive data view for analysts who can then use their expert judgment while analyzing capabilities. Palantir Government is increasingly being used by intelligence and law enforcement agencies to assist analysts. By working with

\textsuperscript{1}http://www.kapowtech.com
Palantir, we hope our work will be able to help generate or refine an ontology which can benefit law enforcement and justice entities.

Finally, capability has been developed where information from multiple sources can be exposed as XML (semi-structured) and correlations can be made across different sets of information. SOLR search engine has been deployed for data analysis; work is also underway for installing the Palantir Government analytic engine for more sophisticated data correlations. This infrastructure has multiple tools for data collection and analysis and will be leveraged for a variety of research projects mainly focused on cyber crime. Initial investigations planned using this infrastructure are: 1) to analyze behavior of sexual predators and develop psychological profiles that can be linked to their online behavior expressed through online postings; 2) develop identifiers from online chatter that suggest the onset of a cyber attack.

4. RESULTS

One of the most pernicious dangers of the Internet is its potential use for online sexual predation since it allows predator access to children in a relatively anonymous environment. Researchers believe that increased social activity online through chats and social networking sites (i.e. MySpace, Facebook) have increased the chances of contact between potential predators and their victims. A majority of research has focused on the behavior of offenders using data once a crime has been committed. The objective of this research is to identify the potential for a criminal to commit such activities (either for the first or subsequent times) by evaluation of their virtual identities.

Some of the initial analysis has revealed use of some of the techniques discussed can also benefit parole officers. For example, in just a preliminary analysis of the sex offender registry, it was found that some of the listed residences are hotels or motels (temporary housing). By correlating business data (in the hotel/motel category) we can flag residences which are questionable and should be checked out. Also, sometimes addresses listed as places of work refer to large plazas which can include many different places such as restaurants, grocery stores, and child care facilities in close proximity. Other times, places of business are just residential homes. Sometimes, these residential homes come up for sale on real estate sites and alerts relating to this activity are important. These are actual instances that we discovered on live sex offender registry members.

There are psychological markers that are exhibited in sexual predators that may be detected from their online behavior. Similarly there are psychological traits of potential victims that make them more vulnerable to advances of sexual predators. Evaluating either of the two in isolation may not accurately predict the risk of a potential sexual crime. Four components that contribute, in differing degrees and forms, to the development of a sexual predators behavior are 1) arousal, 2) emotional congruence, 3) blockage, and 4) disinhibition (Finkelhor, 1984). Emotional congruence relates to the pedophile’s emotional need to relate to children. It is usually expressed in terms of pedophiles having difficulty relating to other adults. Sometimes children meet pedophile emotional needs, which other adults cannot. A feeling of lacking dominance can be
attempted to be fulfilled with children who are inherently less dominant. Similarly, psychological and social immaturity, low self-esteem, sexual abuse in childhood, narcissism, self-centeredness, and social inadequacy can lead to similar manifestations of emotional need. This can result in blockage – where bad experiences with age appropriate adults, sexual dysfunction, limited social skills, marital disturbance, or social and religious pressures limit age-appropriate sexual opportunities. These experiences can include: difficulty in relating to adults of the opposite sex; deficient social skills; anxiety over sexual matters; unresolved oedipal dynamics; disturbances in adult sexual relationships; and repressive norms about sexual behavior. Disinhibition reflects the abusers lack of control through impulse control deficits, psychosis, alcohol, drugs, stress, or nonexistent family rules-coupled with sexual arousal conditioning. This disinhibition can be caused by impulsive disorders senility, mental retardation, alcohol or drug abuse, situation stress, and tolerance of incest within the culture. Lastly, arousal of an adult by child has frequently been cultural or familial conditioning to sexual activity with children: such as corporal punishment and/or sexual child abuse. Approximately 50% of all sex offenders were victims of sexual assault (Smith and Israel, 1987; Johnson, 1989; Gladwell, 2007), 70% of child sex offenders have between 1 and 9 victims; at least 20% have 10 to 40 victims, and serial child sex offenders may have as many as 400 victims in a lifetime (Elliot et al. 1995; Elliot, 2009). With today’s electronic world, even with cultural intolerance, pedophiles can find support in large virtual communities validating their lifestyles. The objective of this research is to identify markers for these attributes in the writings and evaluate the potential for sexual crime. Data from sources such as chat logs from existing sexual offenders, train a classifier, and then use it to classify logs from current online activity in social networks.

4. CONCLUSIONS AND IMPLICATIONS

Discussion of findings: It is no longer sufficient to use a single data source or a single analytic technique while analyzing data. In addition, there is a need to analyze unstructured text data since it can provide valuable clues on criminal behavior and intentions. The three methods discussed are mutually complementary and address the needs for law enforcement in fighting crime: 1) open source data collection, 2) natural language processing, 3) identifying correlations between disparate data sources. The data collection robots automate the process of collecting online data making it efficient. Linguistic analysis can be used for behavior analysis and integration tools can be used for tagging and correlating data.

Implications for policy and practice: Law enforcement is saddled with a growing backlog of cases of online crime and traditional crime that rely on online evidence. Crime labs around the country have been increasing capacity to handle this growing backlog. In addition to increasing capacity, efficiency of analysis also needs to increase. The crime scene today is often not a physical location but the Internet. Being able to rapidly collect data from online sources will make it feasible for investigators to pursue more crimes. Being able to gather corroborating evidence from chats, instant messaging, and web sites can improve the rate of conviction. A suite of tools for data analysis will be employed for
a host of problems including, 1) psychological profiling of sexual predators and determining precursors to crime 2) identifying hacker motivations for committing crime.

*Implications for further research:* The current work builds the basic infrastructure for analyzing crime using multiple data sources including unstructured text data. This infrastructure can be used for facilitating other problems, i.e. sexual predators, hackers, and white collar criminals. Each of these problems requires an understanding of psychological behavior markers as well as data collection and analysis. Further research will involve developing algorithms for data correlations and developing best practices for law enforcement to use. We also need to add other linguistic characteristics beyond content analysis while examining text data. Some of the techniques developed above will be useful for identifying potential for recidivism in certain crimes.

*Dissemination:* The work will be disseminated through publications that will be written on specific problems that are addressed using this infrastructure. The New York State Police will work with UAlbany on specific analysis using these tools, including, sexual predator analysis, and white collar crime.
REFERENCES


New York State Division of Criminal Justice Services.


Strzalkowski, T 2009. ECO: Effective Communication through Internet Social Media. Internal report. ILS.


APPENDICES

Appendix 1: 3130 XL and GeneMapper ID General Information

NORTHEAST REGIONAL FORENSIC INSTITUTE
UNIVERSITY AT ALBANY
1400 WASHINGTON AVENUE
ALBANY, NEW YORK 12222
WWW.ALBANY.EDU/NERFI

CAPILLARY ELECTROPHORESIS & DATA ANALYSIS
USING APPLIED BIOSYSTEMS’
3130XL GENETIC ANALYZER & GENEMAPPER® ID

Instructors: Lucy A. Davis & Jamie L. Bolrose

COURSE DESCRIPTION
This five-day course is designed to provide the theoretical and practical background necessary to perform capillary electrophoresis and data analysis. Today’s advanced technology has led to an exponential number of cases being submitted to the crime lab for DNA testing. For this technique to be successful, it is imperative that the biological evidence is processed and analyzed effectively.

The course enrollment is limited to six (6) individuals per session – due to the hands-on element.

There are four sessions scheduled:
- December 7 – 11, 2009
- January 11 – 15, 2010
- January 25 – 29, 2010
- February 8 – 12, 2010

Sessions will be held from 8:00am till 4:00pm (with a one-hour lunch) each day at the NERFI labs. We are also interested in travel to host labs and providing this workshop. If your lab is interested in this option, please contact us for additional information. These workshops are being held via NJI funding and you may attend free-of-charge. The cost to attend the course is covered, as well as travel and per diem (CSEA: Albany, NY rate $160/day).

This course will cover a variety of topics (lecture & hands-on) including:

I. Electrophoretic Theory: Ohm’s Law, Cathode, Anode and Molecular Separation

II. Genetic Analyzer:
   A. Sample Preparation with Formamide
   B. Electrophoretic Injection
   C. Sample Stacking
   D. The Capillaries used in Forensic STR Analysis
   E. Electric Osmotic Flow
   F. Performance Optimized Polymer
   G. Constant Charge-to-Mass-Ratio
   H. Stokes Shift
   I. Optics & Fluorescent Detection
   J. Charged-Couple Device Camera
   K. Spectral Calibration, How & Why
   L. Spatial Calibration, How & Why
   M. Instrument Components & Function
   N. Data Collection Software: instrument protocols, results groups, plate records, plate map editor, running the instrument, preparing samples, and maintenance calibrations (daily, weekly, monthly, annually).
III. Data Analysis
   A. GeneMapper Theory:
      1. Internal Lane Standard
      2. Local Southern Method
      3. Analytic Parameters
      4. Baseline Smoothing
      5. Baseline Window
      6. Polynomial Degree
      7. Peak Window Size and Slope Threshold
      8. Advanced / Classic Algorithms
      9. Marker Specific Shutter Ratio
      10. Defining and Analysis Range
   B. Use of GeneMapper
      1. Creating a New Project
      2. Navigating Through the Software
      3. Analyzing / Evaluating Data: Size Match Editor, Raw Data Review, 250bp peak Migration, Ladders (Panels & Bins), Negative Controls, Positive Controls, Unknown Samples, Artifacts and OL Calculations.
   C. Demonstration of GMD-IDX

The course will conclude with a multiple-choice exam and issuance of course completion certificates.

For more information, please contact:

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Appendix 2: Electrophoresis Presentation

Electrophoresis and Fluorescent DNA Detection

Presented by: Jamie L. Belrose
Created by: NERFI staff
Capillary Electrophoresis & Data Analysis

Introductions...

Educational Background
(Degrees and Major):

Lucy A. Davis

Professional Background
(previous employment, current position):

Hobbies (What do you like to do in your spare time):

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Educational Background

AS: Adirondack Community College – Math/Science

BS: University of New Haven – Forensic Science
~ Internship: New York State Police

MS: University at Albany – Forensic Molecular Bio
~ Internship: Federal Bureau of Investigation

Professional Background

Teconic Biotechnology: Assay Design Specialist

OCME NYC: Criminalist II

NERFI: Forensic Training Lecturer & Manager of Daily Operations
Interesting Fact about me

I collect magnets...

I was named after Wonder Woman

My name in French translates to:
Jaime Belle Rose
I love the beautiful rose.”

What is Electrophoresis?

- A method of separating large molecules (such as DNA fragments or Proteins) from a mixture of similar molecules.
- An electric current is passed through a medium containing the sample, and each molecule travels through the medium at a different rate, depending on its electrical charge and size.
- Agerose and acrylamide gels are the media commonly used for electrophoresis of proteins and nucleic acids.
Gel Electrophoresis

- The pH and other buffer conditions are arranged so that the molecules being separated carry a negative charge. This allows them to move within an electric field.
- As they move through the gel, the larger molecules will be held up as they try to pass through the pores of the gel, while the smaller molecules will be impeded less and move faster.
- This results in a separation by size, with the larger molecules closer to the starting point and the smaller molecules further away.

Electrophoresis of DNA

- The Phosphate groups on the backbone of the DNA molecule readily give up their H⁺ ions, therefore nucleic acids are negatively charged in most buffered systems.
- DNA molecules will migrate away from the negative electrode (cathode), and migrate towards the positive electrode (anode).
- The higher the voltage, the greater the force felt by the DNA molecule, and thus the faster they will migrate in an electric field.

Electrophoresis of DNA

- Constant Charge to Mass Ratio:
  - During electrophoresis the DNA fragments are separated based solely on size. This is because each of the molecules, regardless of size, experiences the same amount of pull from the electric current.
  - The structure of DNA is comprised of nucleotide units. Each unit contains: 1 phosphate, 1 sugar, and 1 nitrogenous base. For every unit there is one negative charge from the phosphate.
  - As the DNA molecule grows there are more units added, each with a negative charge.
  - This means the charge is proportional for each DNA molecule. More units = More negative charges!

Topics to be Discussed

- Agarose Gel Electrophoresis
- Acrylamide Gel Electrophoresis
  - Native conditions
  - Denaturing conditions
- Capillary Electrophoresis
  - 3130xl

Gel Matrices Used for Electrophoresis of DNA

- Agarose Gels have fairly large pore sizes and are used for separating larger DNA molecules (e.g. RFLP fragments).
- Polyacrylamide Gels are used to obtain high resolution separations for smaller DNA molecules (STR and DNA sequence analysis).
**Agarose Gel Supplies**

- An electrophoresis chamber and power supply
- Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic.
- Sample combs, around which molten agarose is poured to form sample wells in the gel.
- Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).

**Electrophoresis Buffer**

- Several different buffers have been recommended for electrophoresis of DNA.
- The most commonly used for duplex DNA are TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA). DNA fragments will migrate at somewhat different rates in these two buffers due to differences in ionic strength. Buffers establish a pH and provide ions to support conductivity.
  - TAE has buffering capacity and dsDNA runs faster in TAE vs. TBE

**Agarose Gel Electrophoresis System**

- Loading buffer (for loading samples) contain something dense (i.e., glycerol) to allow the sample to sink to the bottom of the sample wells, and one or two tracking dyes, which migrate in the gel (ahead of the DNA) and allow monitoring or how far the electrophoresis has proceeded.
- A fluorescent dye used for staining nucleic acids, such as Ethidium bromide or Sybr Green.
- Transilluminator or Fluorescent Gel Scanner for photo-documentation

**Agarose Concentration**

- By using gels with different concentrations of agarose, one can resolve different sizes of DNA fragments. Higher concentrations of agarose facilitate separation of small DNAs, while low agarose concentrations allow resolution of larger DNAs.
  - High concentration = small pores = good for small fragments.
  - Low concentration = large pores = good for large fragments.
Ethidium Bromide

- This compound contains a planar group that intercalates between the stacked bases of double-stranded DNA.
- UV radiation at 254 nm is absorbed by the DNA and transmitted to the bound dye.
- The energy is then emitted at 590 nm in the red-orange region of the spectrum.

Agarose Gels – Ethidium Bromide

- Ethidium Bromide intercalates between the stacked bases of the DNA strands.
- Ethidium Bromide can be co-polymerized with the agarose or stained post-electrophoresis by incubating the gel in an Ethidium bromide solution.

After completion of the run, visualize the DNA under UV light.

Acrylamide Gel Electrophoresis

Polyacrylamide Gel Electrophoresis

- Polyacrylamide gels are poured between two glass plates held apart by spacers and sealed with tape.
- The length of the gel can vary between 10 cm and 100 cm depending on the separation required. Longer gels = more separation (sequencing)
- They are always run vertically with TBE as the buffer.

Polyacrylamide Gel Electrophoresis

- Polyacrylamide gels have enough resolving power to separate fragments differing by one base pair in size, but their range is ~ 5 to 1000 bp.
- They are much more difficult to handle than agarose gels, fragile.
Non-denaturing gels (Native)

- Run at low voltages - 6V/cm - and 1X TBE to prevent denaturation of small fragments of DNA by the heat generated in the gel during electrophoresis.
- The rate of migration is approximately inversely proportional to log of their size. However, the base sequence composition can alter the electrophoretic mobility of DNAs such that two DNAs of the same size may show up to a 10% difference in electrophoretic mobility.

Denaturing Gels

- These gels are polymerized with a denaturant that suppresses base pairing between nitrogenous bases - this is usually Urea but can be Formamide.
- Denatured DNA migrates through the gel at a rate which is almost completely independent of its composition or sequence.

Capillary Electrophoresis

- Performing electrophoresis in a capillary allows the heat to be effectively dissipated through the capillary walls which reduces any convection related peak broadening.
- This improved heat dissipation means that higher operating voltages can be used in CE which can produce significantly faster analysis times.

Heat Dissipation

- In conventional slab gel electrophoresis the heat associated with the generation of electric current during separation can cause problems of peak / band broadening.
- Heat causes the formation of convection currents within the gel.
- Heat generation restricts the operating voltages that can be used in slab gel electrophoresis which produces longer analysis times.

Capillary Electrophoresis

- Performing electrophoretic separations in capillaries was shown to offer the possibility of automated analytical equipment and fast analysis times.
- The capillary was inserted through the optical center of a detector which allowed real time capillary detection. You can view the data as it is detected by the instrument.
Capillary Electrophoresis

- Operation of a CE system involves application of a high voltage (typically 10-30 kV) across a narrow bore (25-100 μm) capillary.
- The capillary is filled with electrolytic solution which conducts current through the inside of the capillary.
- The ends of the capillary are dipped into reservoirs filled with electrolyte.

Capillary Electrophoresis

- Electrodes made of an inert material, such as Platinum, are also inserted into the electrolyte reservoirs to complete the electrical circuit.
- A small volume of sample is injected into one end of the capillary.
- The capillary passes through a detector at the opposite end.

Capillary Electrophoresis

- Application of a voltage causes movement of sample ions towards their appropriate electrode passing through the detector.
- The plot of detector response (RFU) versus time (data point) is generated, which is termed an electrophorogram.

Chemistry Involved

- Injection
  - electrokinetic injection process
  - importance of sample preparation (formamide)
- Separation
  - capillary
  - PEP-4 polymer
  - buffer
- Detection
  - fluorescent dyes with excitation and emission traits
  - virtual filters (hardware/software issues)
Injection

- Electrokinetic Injection:
  - Definition: A mechanism that forces a small amount of sample into the capillary as a result of an applied voltage.
  - Process: A small amount of sample is forced into the capillary when the capillary and the electrode are submerged into the sample vial and voltage is applied. Since the sample is ionized, ions from the sample migrate into the capillary.
  - Increasing the amount of time that the capillary and electrode are in contact with the sample should increase the amount of sample (DNA) drawn into the capillary.

The Importance of Formamide

- Formamide and single-stranded DNA
  - Samples to be analyzed are first diluted in a large amount (approximately 1-2X) of high-quality denatured formamide (80°C).
  - Formamide serves two purposes:
    - 1. By dissolving the DNA product in a large volume of solution, the chemical properties of the solution are altered, allowing for the formation of a single-stranded DNA.
    - 2. The formamide forms hydrogen bonds with the nucleic acid bases, preventing complementary nucleotide hybridization, thus promoting the formation of single-stranded DNA. As single-stranded DNA is not stable at high temperatures, it is less able to interact with the capillary, resulting in separation of closely sized molecules and thus increased resolution.

- In solution, secondary structure is eliminated.

Injection

- The efficiency of electrokinetic injection is dependent on the amount of ions present in the sample.
- Sample stacking describes the preferential sample injection between the DNA molecules and the ions.
- As the concentration of a sample increases, the sample conductivity also increases (ionic strength increases). The ions then compete with the DNA in the sample to be injected into the capillary. Thus, fewer DNA molecules are injected.

Sample Stacking:
- When DNA is injected into a solution that is lower in ionic strength, the buffer inside the capillary, sample stacking occurs.
- When the injection voltage is applied, the DNA within the sample solution moves into the capillary. As the DNA enters the capillary, it encounters a buffer that is much higher in ionic strength, bringing the ionic DNA molecules to the top. Regardless of the length of the DNA molecule, the DNA molecules stack on top of one another, forming a sharp band.
- The large DNA molecules will be starting from the same point as the small. This "evens the race through the capillary."
- The sample is focused.
Injections

A few nanoliters of DNA enters the capillary:
- The capillaries used in STR analysis are made of fused silica (glass). They are 47cm in total length and 350μm from the inlet to the detection window. The inner diameter is 50μm.
- Some available lengths of capillary are 47cm and 65cm. The longer the capillary, the longer the separation time, the better the resolution. The longer capillaries are traditionally used in sequencing where high separation is required.

Injection

- The outside of the capillary is coated with a layer of plastic polyamide. This adds strength to the otherwise frail capillary. This coating however interferes with the fluorescent detection and must be removed. A small window is burned away to allow the laser to penetrate the capillary.
- The inside of capillary is uncoated from the manufacturer. It does become coated however, after it has been filled with POP-4.

POP 4

Performance Optimized Polymer – 4
- The “4” refers to the 4% concentration of linear uncross-linked poly(dimethylacrylamide) or PDMA. The PDMA molecules coat the inner wall of capillary masking the charge of the silica. Allowing for non-retarded migration of the DNA.
- POP-4 also contains 8M UREA and 5% 2-pyridilidone to maintain the single-stranded formation of DNA during electrophoresis.
- The buffer that helps to maintain pH is 100mM N-[(hydroxymethyl)methyl]-N-methyl-2-amino propane-sulfonic acid (TAPS). This buffer also aids in sample stacking.

Separation

- Size-based separation due to interaction of DNA molecules with entangled polymer strands.
- Polymers are not cross-linked as in the gel.
- “Digest” is not attached to the capillary wall.
- “Pumpable” – can be replaced after each run.
- Polymer length and concentration determine the separation characteristics.

Separation Issues

- Run temperature – 60 °C helps reduce secondary structure on DNA and improve precision.
- Electrophoresis buffer – Urea in Pop 4 helps keep DNA strands denatured.
- Capillary wall coating – dynamic (constantly changing) coating with polymer.
- Polymer solution – POP-4.

Detection

- Fluorescent dyes
  - spectral emission overlap
  - relative level on primers used to label PCR products
  - dye “blobs” (free dye)
- Virtual filters
  - hardware (CCD camera)
  - software (color matrix)
Do you remember the electromagnetic spectrum?
- Energy travels in waves.
- Shorter waves have more energy.
- A photon is the elementary particle of electromagnetic radiation for a given wavelength (light).

Detection
- Molecules (fluophores) exist at a "ground state" energy level
- Energy (photons) is emitted from a light source (laser) to excite the electrons of a molecule.
- Electrons must absorb or release energy when changing levels:
  - Absorb (increase energy)
  - Release (decrease energy)
- Electrons absorb energy and are elevated to a higher energy level. This excited state is only temporary: quanta 1-10 nanoseconds.
- Interactions and conformational changes within the molecule cause electrons to transition to a lower energy level, causing the release of energy in the form of a photon (light).
- The energy of the released photon is different than the energy that was absorbed from the laser: the difference between these two states is Stokes' shift.

Detection
- Energy absorption and fluorescent emission properties are specific to individual dyes. Therefore one dye can be differentiated from another.

Detection
- Utilizing dyes with different emission fluorescence allows for multiple DNA fragments to be run simultaneously.

Methods:
- Intercalating fluorescent dyes: binds to the DNA, only allows for analysis in a single color
- Adding labeled deoxyribonucleotides (dNTPs) into the PCR product: impacts mobility of the DNA fragments in an electric field; the ionic charge on the dye also changes the charge-to-mass ratio of the DNA-Dye conjugate

Detection
- Dye attached to the 5' end of the amplification primer: Only labels one strand of the amplified product for ease in analysis (second strand is not detected). Multiple amplicons can be labeled simultaneously.
- AB AmpFISTR kits: it is the forward primers that are labeled.
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### Primer - 5' end Dye Attachment

![Diagram of primer - 5' end dye attachment](image)

### Fluorescent Dyes

- Each manufacturer of fluorescent DNA kits/STR Genotyping kits employs a combination of dyes optimized for their multiplex system, the modes of detection, and the laser used.
- The manufacturer also optimizes the concentrations of the dyes to promote balanced electronic signals across each loci in a multiplex system.

### Detection

- Using a multiplex kit requires multi-component analysis to compensate for the spectral overlap.
- Multi-component analysis is mathematically performed by subtracting out the fluorescent contribution of overlapping dyes leaving only the dye of interest.

### Detection Identifier

![Graph of detection identifier](image)

### Fluorescent Emission Spectra

![Graph of fluorescent emission spectra](image)

### Detection

A matrix, or spectral, is used to create a “virtual filter” to permit only the specific dye in a wavelength range to be visualized.

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Detection

- Creating a matrix file involves running dye standards and capturing the fluorescence for each individual dye – no evidence STR data associated.

- The fluorescence is then tabulated and the amount of overlap between the dyes is calculated.

- This calculation is then applied to all subsequent runs of evidentiary samples.

Detection

- The Matrix is defined as a Spectral on the 3100 series instruments.

- The spectral is applied to the data when it is collected by the camera, and is NOT a separate analysis. Thus, there is no true raw data associated with the 3100 series instruments.

Detection

- Since the fluorescent dye is affected by its environment, varying conditions can influence the effectiveness of the matrix. Therefore matrix files should be generated as often as needed when run/detections conditions are altered.

- When are new matrix files needed?
  - When observable artifacts are seen during analysis, aka pull-up.
Appendix 3: 3130XL Presentation:

Applied Biosystems’ 3130xl Genetic Analyzers: The Theory

Northeast Regional Forensic Institute
Capillary Electrophoresis & Data Analysis
Presented by: Jamie L. Belrose
Created by: NERFI Staff

Sample Prep with Formamide

- Samples are first diluted in a large amount (proportionally 1:10) of High Purity Deionized Formamide (HPLC).

- Formamide serves two roles:
  1. Diluting the PCR product in a large volume of solution also dilutes the salts associated with PCR (this is important in the sample injection process in that it minimizes the ionic strength of the sample solution).
  2. Keeps DNA single-stranded.
     - Prevents hydrogen bonds with the nucleotide bases preventing complementary denaturation or reannealing.
     - Why do we want single-stranded DNA?
       - Better resolution (DNA in linear form)
       - Early-strand DNA has increased flexibility allowing for easier movement through the PFG, resulting in better separation of closely related molecules and thus increased resolution.
       - In addition, secondary structure is eliminated.

Electrokinetic Injection

- The single-stranded (negatively charged) DNA is drawn into the capillary via the application of an electric voltage (10kV for 5s, based on validation).
- By using electricity, only a sample's charged species enter the capillary.
- Electrokinetic injection is highly dependent on the ionic strength of the sample solution and the buffer within the capillary.

Electrokinetic Injection

- Since the introduction of DNA into the capillary is based on the application of an electric voltage, the salt ions present in a sample will also be affected. If the ionic strength of a sample is high, the salt ions will be competing with DNA molecules for injection into the capillary.
- The ionic strength of the sample and the amount of DNA injected are inversely proportional to one another.
  - Low ionic strength of sample - Large of DNA on the capillary
  - Iontic strength is also used to "level the playing field”.

Sample Stacking

- When DNA is injected from a sample solution that is drawn in ionic strength, upon the TAE buffer inside the capillary, sample stacking occurs.
- When the injection voltage is applied, the DNA within the sample solution travels into the capillary. As the DNA enters the capillary it encounters a buffer that is much higher in ionic strength, bringing the native DNA molecules to a halt. Regardless of size, the DNA molecules stick on top of one another forming a highly focused band.
- This creates the capillary. The large DNA molecules will be starting from the same point at the small.
- The sample is focused.

Capillary

- Above a pH of 5, silica (glass) is negatively charged.
- If the inside of the capillary were to remain uncoated, the inside wall of the capillary would become covered in a layer of negative charges. This TAE buffer within the capillary would create a layer of positive charges on top of this, creating what is known as the “double-layer”.
- The negatively charged DNA molecules would be attracted to these positive charges.
- When the separation voltage is applied, the native cations (positive) would migrate towards the anode (negative) and pull the DNA molecules into the same direction, back in line.
- The dynamic internal coating of POP-4 is necessary for efficient migration. It also insures no cross-contamination between samples.
**Electro-Osmotic Flow (EOF)**

- In other words, the positive charges (cations from the buffer) that form on top of the negative charges (anions from the glass) will migrate through the capillary and push the DNA back to where it started from.
- This phenomenon is known as electro-osmotic flow (EOF).
- However, the EOF can significantly reduce the binding of the capillary to prevent EOF.

**Separation based on size**

- When the electric field is applied, the DNA begins to migrate toward the positive pole. As it passes through the buffer, it interacts with the DNA in a manner similar to a sieving mechanism.
- The shorter DNA fragments can more readily migrate through the sieving medium and reach the detection window sooner.
- The longer the fragment, the longer it takes to migrate through the length of the capillary.

**Constant Charge-to-Mass Ratio**

- During electrophoresis, the DNA fragments are separated based on size, which is because each of the molecules, regardless of size, experiences the same amount of force from the electric field.
- The structure of DNA is comprised of nucleotide units. Every unit contains 1 phosphate, 1 sugar, and 1 nitrogenous base. For every unit there is one negative charge from the phosphate.
- As the DNA molecule grows, there are more units added, each with a negative charge.
- This means the charge is proportional to molecule's size for each DNA fragment.
- More nucleotide units = More negative charge
  - More negative charges = More mass to pull...

**Fluorescence and Excitation**

- When the DNA fragments reach the detection window, they encounter a laser. The laser is located at the 1 end of the capillary and excites the DNA molecules.
- The excitation energy from the laser causes an electron of the fluorescent molecule to absorb energy and transition to a higher energy level. The electron then undergoes a deexcitation process and returns to the original ground state.
- As it's doing so, it emits a photon of light.

**Stokes Shift**

- The excitation wavelength from the laser is of a shorter wavelength and higher energy.
- The emission wavelength from the molecule is a longer wavelength and lower energy. This enables the detection system to differentiate between the two wavelengths and only detect the fluorescence.
- Each of the 5 dye used has a different emission wavelength, allowing the instrument to determine which dye is present.
- This phenomenon allows us to simultaneously use 5 types.

**Optics and Detection System**

- The laser is the source of excitation energy needed to detect the fluorescent dye emissions.
- 3D CCI has dual laser illumination to ensure all of the capillary is illuminated simultaneously.
Charge-Coupled Device (CCD) Camera

- Uses a small rectangular piece of silicon rather than a piece of film to receive incoming light.
- This piece of silicon is divided into many light sensitive cells, each cell represents one pixel (picture element) of the entire picture. The surface of the camera is 256 pixels wide by 330 pixels long.

Charged-Coupled Device

How does a Charged-Coupled Device work?

- CCD can receive charge via the photoelectric effect and electronic images can be created.
- The photoelectric effect is synonymous with Stokes Shift.
- Each one of the pixels on the surface of the camera accumulates (builds up) an electric charge (from the optical photons) proportional to the light intensity at that spot in time.
- All at once (every 30s), this stored electrical energy is sent as an electronic signal to the data collection software.
- This information is then converted (using mathematical algorithms) into the peaks that you see in an electropherogram.

Optical Calibration

Spatial (3130):

- A spatial calibration maps the position of each of the 16 capillaries on the surface of the CCD camera.
- A spatial is performed when a new array is installed, the detection window is opened, and routinely to ensure proper instrument function.
- The spatial calibration uses the Raman water signal from the polymer within the array to determine the precise location of each array.

Spatial Calibration

Good ✅

False Positives

Bad ❌

Final Results
Spectral Calibrations

Spectral (3120):
- Equivalent to a Matrix on the 310.
- A spectral calibration creates a mathematical algorithm to correct for the overlapping of fluorescence emission spectra of the dyes.
- Spectral calibrations are run if pull-up is seen often, after service calls involving the optics, and approximately once a year to ensure accurate results.

Optical Calibrations

The Spectral:
- Generated by a separate run/analysis of a dye set.
- Contains information about how much of the collected light falling on a virtual filter is due to the intended light (color) emission, and how much is from the overlapping other colors.
- Using a mathematical algorithm (we don’t need to know); the overlapping colors are subtracted out, leaving only the peak color of interest.

Dye Set F (Profiler Plus, COFiler)

Fluorescent Emission Spectra for AB Dyes
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4-dye Matrix
(you can actually view this file on the 310)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>B</th>
<th>G</th>
<th>Y</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1.0000</td>
<td>0.7622</td>
<td>0.0563</td>
<td>0.0027</td>
</tr>
<tr>
<td>G</td>
<td>0.5999</td>
<td>1.0000</td>
<td>0.5624</td>
<td>0.0096</td>
</tr>
<tr>
<td>Y</td>
<td>0.4036</td>
<td>0.7005</td>
<td>1.0000</td>
<td>0.1963</td>
</tr>
<tr>
<td>R</td>
<td>0.1877</td>
<td>0.3812</td>
<td>0.5470</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Raw data (only on the 310)

No color subtraction

Analyzed data
(after the spectral is applied)

This is different for the 3130

- The matrix file is known as a Spectral Calibration.
- For the 3100 series instruments the spectral file is applied to the data as it is collected, thus no true raw data.
- How is the Spectral applied simultaneously as the data is collected, you ask?!

This is Complicated...Listen Closely!!

Excellent Question!
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---

**All Overlaid On Top of Each Other!!!**

---

**Tah-dah!!!**

---

**One intersection point**

- But remember (from slide 18) that the surface of the CCD is 256 X 550 pixels = 140,000.
- ...and each one of the activated pixels on the previous slide is really 3 X 14 = 42 pixels.
- So, 42 * 4 = 168/pipetary
- 168 * 16 = 2688 pixels activated.

---

**Nuts and Bolts of 3130**

---

**The Guts**

---

92
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Creating a Plate Record

GeneMapper Plate Editor

GeneMapper Plate Editor

How to perform multiple injections of the same samples?

- Use the Add Sample Run tool under the Edit column
- Additional Results Group and Instrument Protocol columns are added.
- This is useful if you would like to inject a sample (from the same well) twice with different injection times.

Running the Instrument

- Prepare Samples and Plate Assemblies
- Place Plate onto Autosampler
- Schedule a Run
- Link the Plate
- Run the Instrument

Plate Assembly

- Use correct plate base
- Make sure reagent caps are firmly seated and flat
- Make sure holes in the plate remain aligned with the holes in the sample
The Autosampler
- Places plates correctly for sample injection
- Notes 95 and 304 well plates
- Electric sensors detect presence/angle of plate
- Heats cathode buffer, waste, and waste rinse kits
- Capillaries engaged in electrophoresis
- Autosampler calibration only as needed.

Linking the Plate
- Under the Run scheduler, select the desired plate record
- Click the plate position (A or B)
- Plate position indicator changes from yellow to green when linked
- The green run button becomes active

Running the Instrument
- Very Easy

Run Procedure
1. Capillary Fill
   - Capillary moves from the buffer reservoir to waste reservoir
   - Capillary is filled with sample by the force applied by the syringe
2. Pre-electrophoresis
3. Water wash of the capillary
   - The autosampler moves the sample tray so that the sample plate is below the capillary and recording position
   - The autosampler then raises the tray up to dip the capillaries into the samples
   - 15 kV for 5 seconds to disperse the DNA up into the capillary

Run Procedure (cont.)
5. Water wash of the capillary
   - Capillary moves to buffer reservoir
   - Capillary moves from the buffer reservoir to waste reservoir
7. Electrophoresis
   - Additional voltage is applied to allow the DNA to traverse the capillary
   - DNA begins to separate through the polymer
   - Approximately 45 minutes
5. Detection
   - Data collection begins
   - Mathematical algorithms established by the spectrophotometer are applied simultaneously
   - Can see the data in real-time

Things to be mindful of...
- LED lights located on the front of the instrument
  - Steady green = ready
  - Blinking green = running
  - Steady green and blinking amber = end of run
  - Blinking amber = paused state, door open, self test
  - Steady red = failure (check alarm)
- Cathode
  - Made of platinum (ZnS)
  - Check for crystals before each run
  - Very easy to bend! Wipe gently with Kimwipes and distilled water
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**Things to be mindful of…**

- Pump Blocks
  - Polymer is changed every 7 days
  - Or sooner if you notice a decrease in resolution (broader peaks)
  - Be sure the polymer blocks are clean and dry

**Things to be mindful of…**

- Capillary
  - Change it when you notice poor resolution
  - Do not let the ends of the capillary be exposed to air for more than 30 min
  - Be sure the capillary window is over the laser detector window, no fingerprints!
  - The capillary is tough, yet fragile!
    - Don’t be afraid to handle it; but avoid applying undue stress, such as twisting.
    - Capillary window is fragile

**3130x/ Maintenance Tasks**

The following should be performed BEFORE EACH BATCH OF RUNS:

- Reservoir caps are firmly seated and fast
- Holes in the plate do not align with the holes in the caps
- Plate assembly sits snugly on the plate deck
- Clean instrument surfaces, checking for dried polymer
- Check for leaks around array, knobs and interconnections

**3130x/ Maintenance Tasks**

The following should be performed DAILY or BEFORE EACH BATCH OF RUNS:

- Clean and replenish water and 1X GAB (Genetic Analysis Buffer) reservoirs.
- Remove bubbles in the polymer system (via Wizard)
- Check the loading-end header to ensure the capillary tips are not crushed, damaged, or have visible crystals.
- Check the layer of polymer to ensure that there is sufficient volume for runs intended.
  - Apx. 0.05-0.1 ul per set of 10 injections

**The Wonders of Wizards**

- **Change Polymer Wizard**
  - To change different polymer types, e.g., POP4 to POP6
- **Instrument Preparation Wizard**
  - To prepare instrument for being idle more than one week
  - Repeat -polymer pattern
- **Dispense Array Wizard**
  - To dispense arrays with same polymer (same or different)
- **Inject/Pause Wizard**
  - To remove bubbles in CEP chamber, channels, and tubing
- **Wash Array Wizard**
  - A part of the recommended maintenance procedures
- **Wizard Menu Wizard**
  - To install or replace a new array

**Electropherograms**

GMID lecture with Lucy 😊
Appendix 4: Genemapper ID Presentation:

GeneMapper ID

Lucy A. Davis
February 2010

Suggested Reading
- GMID Manual
- Chapter 7 – Analyze Data
- Appendix B – Software Genotyping Algorithms
- Appendix F – Transfer of Data
- Recommended
  Chapters 3, 5, 6 and Appendix C

Internal Sizing Standards
- GS300 ROX (Applied Biosystems)
- IL300 G5Q (Promega)
- LT20-209 ROX (Life Technologies)

Size Standard
- Size Standard required 2 points below the fragment of interest and 3 points above the fragment.

Analysis Method Editor

Peak Detection Algorithm
- Basic Mode – uses the Local Southern size calling method uses the reciprocal relationship between fragment length and mobility.
- Classic Mode – Uses five analysis parameter options – ranges, data processing, size calling method, peak detection, and split peak correction. Mac based method
- Advanced Mode - Uses four main analysis parameter options – ranges, smoothing and baselining, size calling method, and peak detection. NT based method
Sizecalling Methods

- **Local Southern** – Type used by forensics. Determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility.
- **2"" or 3"" Order Least Squares** – Uses regression analysis to build a best-fit curve.
- **Cubic Spline** – Forces the curve through all known points.
- **Global Southern** – Similar to the Least Squares but compensates for electrophoresis migration anomalies, creates a best-fit line through all available points.

**Process of Sizing DNA Fragments Using an Internal Standard**

1. Fit a curve through points a, b, and c.
2. Fit a curve through points b, c, and d.
3. Average the calculated base pair size determined by steps 1 and 2, average those 2 numbers together to get the base pair sized of the fragment of interest.

**Allele Calling**

- Under that Panel Manager you identify which chemistry you are using.
Virtual Allele Bins

- Virtual Alleles are alleles that the software will assign an allele call but the allele is not in the allele ladder.
- These are common micro-variants that have been identified.
- CODIS does not allow alleles to be uploaded that are outside of the lowest or highest allele in the ladder.

In Geneflexion, the virtual alleles are in red, while the alleles that are in the ladder are grey.
Smoothing Option

- Reduces the number of false peaks detected by the software.
  - None – If the data has very sharp, narrow peaks of interest.
  - Light – Provides best results for typical data.
  - Heavy – Apply to data from slower runs that have very broad peaks, or to avoid the detection of sharp edges. Might reduce peak area or eliminate narrow peaks.

Baseline

- GMID computes the baseline for each dye color independently.
- GMID baselines an electropherogram by subtracting the baseline from the raw electropherogram.
- The baseline value is the lowest GMID allows you to choose the baseline data point that sets where the software will set the lowest value on the electropherogram.

Baseline

- A small (low) baseline window
  - Causes the baseline to creep into the peaks, resulting in shorter peaks in the analyzed data.
- A large (high) baseline window
  - Causes the baseline to rise too low, resulting in elevated and possibly not baseline-resolved peaks.
Polynomial Degree & Peak Window Size

- Affects the sensitivity of peak detection
- These can be adjusted to detect a single base pair difference while minimizing the detection of shoulder effects or noise
- The peak detector computes the first derivative of a polynomial curve to the data within a window that is centered on each data point in the analysis range

Polynomial Degree

- A polynomial is an expression of length constructed from mathematical equations (i.e. \( x^3 - 5x^2 + 2 \))
- The degree is the number of terms defined in the equation (the above equation has a degree of 3)
- Curves with larger polynomial degrees (more variables in the equation that defines the line) will identify sharper increases in the slope of the line making the peak on the EFG

Polynomial Degree & Peak Window Size

- Using curves with larger polynomial degrees (more variables in the equation that defines the line) allows the curve to more closely approximate the signal and therefore the peak detector captures more peak structure in the electropherogram
- The peak window size sets the width (in data points) of the window to which the polynomial curve is fitted to data. Higher peak window size values smooth out the polynomial curve, which limits the structure being detected. Smaller window size values allow a curve to better fit the underlying data

Constant window size 15

Peak detection Sensitivity

- Controlled by Polynomial Degree & Peak Window Size
- More Sensitive (Increase) Polynomial Degree
- Less Sensitive (Decrease) Peak Window Size
- More Sensitive (Increase) Peak Window Size
- Less Sensitive (Decrease) Polynomial Degree
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Creating a new project

- Once GMID is open, there will be a blank project on the screen.
- From here, the sample files from the run folder will need to be added.
- To do this, go to File > Add Samples to Project, Ctrl K, or the icon.

Navigating to your run folder

- Navigate to your run folder that you want to analyze.
- Move over the entire run file by selecting add to list.
- Each run folder will already be created specific to each case of the D2C.
- When you open the run file, make sure you put your sample files in a folder under your "My Documents" file. Do NOT put them in an external disk or back them up.

Sorting with the Comment

- The Comment column will allow you to sort in the order of injection.
- If you don’t do this, your plots will be in alphabetical order, not injection order.
- Make sure the Comment is selected in the samples table.
- To check this, select the table, and select the Comments is checked.
- Next, go under Edit in D2C, and look by Comments.

Now you can analyze your project

- Be sure the correct analysis parameters are selected.
- Make sure the range is right.
- Be sure to check all size standards.
- Verify sample type column is correct.
- Make sure the file is correct.
- Make sure the panel information is correct.
Analyzing the Project
- Select All (Ctrl A) to analyze the project.
- Highlight the project data.
- Select all files and go to the Size Match Editor.

Problems with Size Standards
- Make sure you have the correct size standard selected.
- If you are missing the 75 or the 450 peak, you will need to reanalyze your entire project with expanded parameters.

What is wrong with this standard?

<table>
<thead>
<tr>
<th>IF...</th>
<th>THEN...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples data made</td>
<td>Size standard used is acceptable, continue.</td>
</tr>
<tr>
<td>Peak heights are more defined</td>
<td>Peak standard (75) may be unacceptable, refer back.</td>
</tr>
<tr>
<td>Ratio (late compared) is only one peak</td>
<td>Ratio (450) may be unacceptable. Refer back.</td>
</tr>
<tr>
<td>Correct additional exist for any peaks</td>
<td>If the incorrectly assigned peaks is needed to determine the total sequence, the incorrect standard is acceptable.</td>
</tr>
<tr>
<td>Standard peak(s) is unable to be identified</td>
<td>Unknown corrects cannot be analyzed to the existing standard. Refer back.</td>
</tr>
</tbody>
</table>
Raw Data Review

- In order to look at raw data, select all sample files to View Raw Data (Ctrl F2)
- You can also use the same function in a simpler fashion to the View Data Editor
- The off-scale value for multi-capillary instruments is not a standard set value, like the 8101 on the 310. It will vary depending on the sample, run, capillary etc.
- In addition, if spike, pull-up, or overlapping peaks are present, it may trigger the off-scale flag for samples less than 8600.
- Remember, OS is a samples list means there is an off-scale peak with the site 121810 in region 174130.
- OS in the genotype tab means there is an off-scale region with the marker range.

Data & size calling range

- Raw points or data points

Off-scale Samples

- Peak height must be within the acceptable range for the instrument being used. For samples with raw data ~7400RFUs on the 3100 data is interpreted with caution.
- Multiplicity of peaks may produce off-scale data less than 7400. Be sure to examine the raw data. The use of the Off-scale (OS) PQV is a helpful indicator of off-scale data within a sample and locus.
- The sample may be interpreted with caution, but it can be re-injected at a decreased injection time.

Process Quality Values (PVQ)

<table>
<thead>
<tr>
<th>PQV Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQO</td>
<td>Size Quality Over-ni</td>
</tr>
<tr>
<td>SFNF</td>
<td>Sample File not Found</td>
</tr>
<tr>
<td>OS</td>
<td>Off Scale</td>
</tr>
<tr>
<td>SQ</td>
<td>Size Quality</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>PVQ Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Allele Edit</td>
</tr>
<tr>
<td>ADO</td>
<td>Allele Display Overload</td>
</tr>
<tr>
<td>AN</td>
<td>Allele Number</td>
</tr>
<tr>
<td>Bin</td>
<td>Out of bin allele</td>
</tr>
</tbody>
</table>

250 Basepair Peak

- The 250 basepair peak for each sample should be checked to confirm the migration is not at 100 bp.
- You can look at this data by going to Display plots and reviewing Orange dye only.
- If the data is outside range – consult Supervision/instructor.
  - Data may need to be re-run or broken into smaller projects.

250 Basepair Peak Evaluation

When you filter on "show select best" the table will show the base pair size of just the 250 peaks.

Displaying Plots

- Highlight the desired sample(s)
- Analyze-Display Plots
  - Samples with low sizing quality value cannot be displayed
  - To zoom in/out, right click on X or Y axis-Highlight Zoom to...enter desired value
  - Apply to all electropherograms
  - Hold pointer over X or Y axis, what fills magnifying glass appears, right click and drag

Deepening Artifacts from the True Alleles

- Blue channel
- Green channel
- Yellow channel
- Red channel

Figure 1. A. Balas (2000). PowerPoint slide showing the effect of artifacts on DNA analysis.
Artifacts
- Peaks may be labeled when they are not actual alleles — Artifacts
- Artifacts may be edited
- Edited peaks meeting specific criteria must be documented

Spikes
- Peaks are approximately the same bp size and may be present in all 5 colors
- A spike in <5 colors can be viewed by overlaying bins
  - If necessary re-run

Pull up
- Spectral overlap
- Pull up peaks can be approximately the same bp size as true alleles in another color
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**AB Validated stutter per allele**

- C3F1PO – simple repeat – [AGAT]n
- D7S820 – simple repeat – [GATA]n
- D18S119 – compound repeat – [TCT]n

**D21S11 – complex repeat**


**Different types/degrees of Stutter**

- If the template strand bugs out during the replication you will get stutter in the -4 position.
- If the complimentary strand bugs out, you will get stutter in the +4 position.
- Excessive DNA template can also lead to stutter in the -8 position.

**Stutter**

- Majority of stutter is n-4 from true allele.
- Interpret with caution if there is an indication of mixture.
- We will use validated stutter values.
- Forward stutter n+4 is usually seen when you look at data 50 RFUs and lower.
- Extremely overloaded samples may have n-8 stutter.

**Stutter (continued)**

- The amount of the shorter (stutter) product is measured as a percentage of the height of the main peak.
- Dividing the RFU value of the shorter signal by the RFU value of the main peak produces the stutter percentage.
- The maximum percentage of stutter product has been identified for each STR locus.
  - QMD will remove the allele label for signals within this maximum percentage.
  - QMD computes the stutter percentage by dividing the RFU value of the stutter signal by the RFU difference in the two peaks.

**STR Alleles with Stutter Products**

<table>
<thead>
<tr>
<th>STR Allele</th>
<th>RFU Stutter</th>
<th>RFU Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>D8S1179</td>
<td>0.2%</td>
<td>0.4%</td>
</tr>
<tr>
<td>D21S11</td>
<td>0.2%</td>
<td>0.4%</td>
</tr>
<tr>
<td>D18S119</td>
<td>0.2%</td>
<td>0.4%</td>
</tr>
</tbody>
</table>
Expected Stutter Values

- Through validation studies, it is possible to determine maximum stutter values expected at a loci.
- GenemapperTD has adjustable stutter filter settings - peaks under max stutter value are not labeled.

Stutter Cut-off Values

<table>
<thead>
<tr>
<th>LOCI</th>
<th>IMMA.RESULTS</th>
<th>% CUT-OFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S1312</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>D2S1301</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>D7S820</td>
<td>98.10</td>
<td>2</td>
</tr>
<tr>
<td>D2S1338</td>
<td>91.00</td>
<td>2</td>
</tr>
<tr>
<td>D5S818</td>
<td>8.50</td>
<td>2</td>
</tr>
<tr>
<td>D2S1301</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>D2S1338</td>
<td>11.10</td>
<td>2</td>
</tr>
<tr>
<td>D2S1312</td>
<td>11.10</td>
<td>2</td>
</tr>
<tr>
<td>D2S1338</td>
<td>98.10</td>
<td>2</td>
</tr>
<tr>
<td>D5S818</td>
<td>8.50</td>
<td>2</td>
</tr>
<tr>
<td>D2S1301</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>D2S1338</td>
<td>98.10</td>
<td>2</td>
</tr>
<tr>
<td>D5S818</td>
<td>8.50</td>
<td>2</td>
</tr>
<tr>
<td>D2S1312</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>D2S1338</td>
<td>98.10</td>
<td>2</td>
</tr>
<tr>
<td>D5S818</td>
<td>8.50</td>
<td>2</td>
</tr>
</tbody>
</table>

Dye Artifact peak

- Caused by unincorporated dye from primers, that can migrate into the range of interest.
- Morphology typically does not resemble a peak (broader and poorly shaped).

Shoulder peak

- Caused by broadening of a peak and appears approximately 1-3 bp smaller or bigger than the main allele.

Raised baseline

- Caused by fluctuations of the electrical current.
- May occur in one or all five colors.
- Edit noting base pair range and color.
Non-template Addition

- Tag polymerase will often add an extra nucleotide to the end of a PCR product, most often an "A".
- Can be enhanced with extension soak at the end of the PCR cycle (e.g., 15-45 min @ 60 or 72 °C).
- Can be reduced with new polymerase.
- Best if there is NOT a mixture of "+-A" peaks.

-A product

- Caused by incomplete adenylation.
- Appears approximately 1 base pair less than true allele.

Artifacts List

<table>
<thead>
<tr>
<th>Type of Artifact</th>
<th>CMR Editing Abbr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stuttered/Goat/Repeater</td>
<td>SDTR</td>
</tr>
<tr>
<td>Allele Flips/Reversals</td>
<td>NAC</td>
</tr>
<tr>
<td>Repeaters</td>
<td>SRP, RP, RPV, RPR, RPRV</td>
</tr>
<tr>
<td>Primer extension</td>
<td>PR</td>
</tr>
<tr>
<td>Spikes</td>
<td>SPS</td>
</tr>
<tr>
<td>Supersaturated</td>
<td>SPSX</td>
</tr>
<tr>
<td>Nonspecific artifacts</td>
<td>NSA</td>
</tr>
<tr>
<td>Non-representative typing result</td>
<td>NRT</td>
</tr>
</tbody>
</table>

Allelic Ladder

- Contains common alleles and some variants.
- The common alleles are taken from a pool of individuals which will result in alleles in the allelic ladder having different peak heights (the more common the allele, the higher the peak in the ladder).
- Combines all loci and dyes in one amplified sample for each system (PPXCO).

Allelic Ladder Formation

Separate PCR products from various samples are amplified and come together in a particular STR locus. Then, they are combined and re-amplified to form the final representatives of alleles spanning population variations.
Allelic Ladder (continued)

- Base pair sizes of the alleles from samples are determined using the internal Lane Size Standard (LSS) and then compared to the bp sizes from alleles in the ladder (also sized with LSS) to obtain the allele call
- Each electrophoresis run must contain a working allelic ladder for each system being used
- Vendors provide amplified DNA of the ladder
- Allelic ladders are included in PCR Kits; will vary vendor to vendor

Discrete Alleles

- Amplification of STRs produce alleles of discrete sizes
- Discrete sized alleles allow for the construction of allelic ladders containing fragments of known (common) alleles at a given locus
- Allelic ladders permit simplified interpretation of results
- Multiplexing STR tool offers unlimited potential for a highly discriminatory test for human identification

Allelic Ladders

Check allelic ladders to assure:

<table>
<thead>
<tr>
<th>P.</th>
<th>THEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele heights are above threshold, and alleles are present and well-resolved</td>
<td>Allele ladder is acceptable</td>
</tr>
<tr>
<td>Peaks below threshold, or allele missing or not well-resolved</td>
<td>Remove ladder; reanalyze if all are bad, repeat CE run (discuss with technician)</td>
</tr>
<tr>
<td>A allele is present, in close proximity to an allele but not in the ladder (known alleles)</td>
<td>Ladder may be used</td>
</tr>
<tr>
<td>A allele is present, in close proximity to two alleles and does affect allele assignment</td>
<td>Remove ladder; reanalyze if all are bad, contact supervisor you may be able to use another ladder</td>
</tr>
</tbody>
</table>

Virtual Alleles

Virtual Alleles what are they?

- Not all known alleles are present in ladder
- The software has sizes programmed in for known alleles that are not in the ladder
- You will get allele calls for these “virtual alleles”
- If your peak does not correspond to virtual alleles or represented ladder alleles
  - You get an OL allele call: may be a variant or a sizing error you have determined which it is

Off-Ladder Alleles

- Alleles detected that are not present in the ladder
- Sometimes called variant alleles
- Off-ladder alleles sometimes result when there are migration differences between alleles injection to injection, this can be corrected by sizing with the allelic ladder closest to the sample in the run
- If the off-ladder is reanalyzed but still not labeled, the sample must be repeated on another CE run or second amplification to show reproducibility
Off-Ladder Alleles (continued)
- If off-ladder allele is reproducible, it will be used for interpretation and designated as follows:
  - Off-ladder alleles within the ladder
    - Designated with improper bases of partial repeat
      - Example: 1X12, 3X14, 6X15
  - Off-ladder alleles outside the ladder
    - Alleles are shorter than the shortest allele in the ladder
    - Alleles are longer than the longest allele in the ladder
    - Designated as improper alleles OR incorrect allele
      - Example: at PCR when a <b>diabetes</b> would be possible
- Allele lengths for real off-ladder alleles will be required in the case file and must be from the same run as the sample.

Negative Controls
- Kit negatives, reagent blanks and LIZ standard
- Select negative controls in display plot
- Zoom the Y axis to approximately 100 rfu
- If the presence of contamination is determined in negative control samples, evaluate all samples. A contamination investigation must be initiated.

Microvariant

Negative Controls
- Kit negatives, reagent blanks and LIZ standard

| IF...          | THEN...
|----------------|-----------------
| No alleles are present | Controls are acceptable, proceed |
| There are potential alleles above threshold | Repeated with other and are not alleles, the control is unacceptable. Further analysis is required |
| After re-loading potential alleles remain | Controls are unacceptable. Re-test and re-setup all samples associated with that template |
| After re-loading potential alleles no longer remain | Controls are acceptable, proceed |

Positive Controls
- Amplification positives
- The alleles of the positive amplification control alleles should be checked against established values.

Positive Controls
- Extraction and Amplification positives

| IF...          | THEN...
|----------------|-----------------
| Peak heights are above threshold | Controls are acceptable, proceed |
| Fragment sizes are established | Controls are acceptable, proceed |
| Peaks are narrower than others or improper substrate allele values | Controls are unacceptable |
| Positive control peaks are not detected | Re-test and re-setup all samples associated with that template |
Sample Assessment

- After controls are deemed okay and recorded:
  - Assess for potential artifact peaks
  - Note sample description (single source, mixture, degraded, allele dropout)
  - Note off scale peaks
  - Assess samples for potential re-runs

---

Have a lot of ‘OL’ alleles?

- Did you add your samples to an already established project?
  - Every time a sample is added to a project that has been analyzed, the software may think the sample is from another run, therefore requiring its own alleles. If you want a sample, delete the sample files and add to a blank project. You will need to account for this when in post-amp and checking your run.

- Do you have the Bins set up in your Allele Tab of your Analysis Parameter?

---

Using the PQVs

- Once analysis is complete, the genotypes table is now accessible to the analyst.
  - This table contains PQVs that may indicate potential problems with analysis.
  - CO, LH, TH, BD, AN, ADD, GO, CC, BIN, and PD are especially useful in quick examination of stutter quality.
  - The CO or PD are flagged to a certain extent the cause of the PD is extremely difficult of using a ladder with a 60 yellow tag, it may lead to incorrect typing.

---

Printing Plots

- When printing evidence and control samples – Select Plot Setting: Casework
  - When printing allelic ladders – Select Plot Setting: Allele Calls
  - Zoom X axis to ~70-465 bp
  - Print all plots

---

Printing Tables

- Under File > Export Combined Table
  > Select One Line Per Sample – name table with run name and analyst initials
  - Export to appropriate folder Click
  Export Combined Table
Saving & Reporting GMID Project

- File > Save project
- Export project
  - Tools > GeneMapper Manager, Projects tab;
  - Export (*.ser)

Exporting/Importing previous analyzed projects

- Moving Projects to a different computer
- Project files will be named .ser
- GeneMapper Manager --> Click on Project tab --> Highlight project to be moved --> Export --> Export to appropriate location
- Import --> Go to the appropriate location of .ser file --> Import

Acknowledgements

- John Platz – University of North Texas
- Joanne Sgueglia – Mass. State Police Forensic Laboratory
- Andy Wilt – Illinois State Police Forensic Laboratory
- Applied Biosystems GMID, Genescan/Genotyper Manuals
Appendix 5: Capillary Electrophoresis Examination:

Capillary Electrophoresis Workshop
Final Exam

Name: __________________________ Date: ____________

Answer the following short answer questions. (80 points total — 2 points each unless otherwise noted)

1. What is the name of the process that describes how the DNA enters the capillary?
   a. Sample Sticking
   b. Electrometnic Injection
   c. Electro-osmotic Flow (EOF)
   d. None of the above

2. Which of the following helps keep DNA denatured?
   a. Urea
   b. 2-pyridinone
   c. Formamide
   d. All of the above

3. The migration of the DNA through the polymer is primarily based on fragment size since it has:
   a. Many A-T base pair sequences
   b. A constant charge-to-mass ratio
   c. A variable charge-to-mass ratio
   d. A high salt concentration in the sample solution

4. Which of the following should be performed at least on a weekly basis?
   a. Change the buffer
   b. Change the capillary
   c. Re-align the laser
   d. Change the polymer

5. Which of the following is performed on an “as needed” basis on the 3130xl:
   a. Spectral calibration
   b. Spatial calibration
   c. POP-4 change
   d. Buffer change

(10)
6. What are the components that are added to the master mix when loading identifier samples onto the CE?
   a. ROX and LIZ
   b. Formamide and LIZ
   c. Formamide and ROX
   d. Identifier Ladder and Formamide

7. What is a function of the formamide during capillary electrophoresis?
   a. Keep the DNA denatured
   b. Provide an internal size standard for each of the samples
   c. Dilute the salts in the sample solution
   d. Both a and c

8. What are the DNA fragments within a sample compared to when determining their base pair sizes?
   a. ROX or LIZ
   b. Allelic ladder
   c. Formamide
   d. Buffer

9. What are the DNA fragments within a sample compared to when determining their allele designations?
   a. ROX or LIZ
   b. Allelic ladder
   c. Formamide
   d. Buffer
Questions 10 and 11 refer to the following electropherograms

10. What is the white arrow pointing to?
   a. Spike
   b. Full-up
   c. PCR artifact
   d. -A

11. What are the black arrows pointing to?
   a. Spike
   b. Full-up
   c. PCR artifact
   d. -A

(4)
12. The following can be used to help monitor the precision of a run:
   a. 150 bp ROX
   b. 200 bp ROX
   c. 300 bp ROX
   d. 250 bp ROX

13. Why is it important that the DNA remain in single strand conformation during electrophoresis?
   a. Better resolution
   b. Better interaction with the POP-4
   c. Less "A" will occur
   d. Both a and b
   e. Both a and c

14. What term best describes the process where DNA fragments from the sample become collected in the beginning of the capillary in a sharp band, before being separated, due to the increased ionic strength of the buffer inside the capillary?
   a. Electrophoretic injection
   b. Sample stacking
   c. Constant charge-to-mass ratio
   d. None of the above

15. What calibration on the 3130xl allows the instrument to map the area of each capillary onto the camera (in other words, it tells the camera from which capillary the fluorescent signal is coming from)?
   a. Matrix
   b. Spectral
   c. Autosampler calibration
   d. Spatial

16. What allows the CE instrument to correct for the overlap in the fluorescence emission spectra of the dyes?
   a. Matrix
   b. Spectral
   c. Spatial
   d. Both a and b
   e. Both a and c

17. What phenomenon could you see if the matrix or spectral calibration is not working properly?
   a. "A"
   b. Stutter
   c. Peak shoulder
   d. Pull-up

(12)
18. What sizing method does Genemapper® ID use to size peaks?
   a. Local Southern
   b. Global Southern
   c. Cubic Spline
   d. 2nd Order least squares

19. Using the following numbered steps, what is the correct order of events that occur on the capillary electrophoresis instrument?
   1. Sample stacking into a precise, focused band
   2. DNA fragments separated by size using the polymer in the capillary
   3. Emissions of the fluorescent tags are captured by the CCD camera
   4. Laser excitation at the capillary window
   5. Sample injected into capillary

   a. 2-4-1-1-5
   b. 5-1-2-4-3
   c. 1-5-3-4-2
   d. 3-4-5-2-1

20. Review the following GeneMapper® ID image. What common PCR occurrence is the arrow pointing to?
   a. Stutter
   b. -A
   c. Dye artifact
   d. Spike
21. (18 points) On the diagram below of the 3130, identify the following parts:
   a. Detection cell block
   b. Buffer and water reservoirs
   c. Anneal buffer reservoir
   d. Pump block
   e. Lower pump block
   f. Oven
   g. Autosampler
   h. Polymer delivery pump (PDP)
   i. Capillary array
22. What factor is the electrokinetic injection dependent on?
   a. Ionic strength of the sample
   b. Ionic strength of the buffer within the capillary
   c. Both a and b
   d. Neither a nor b

23. What does the Local Southern Method use to determine the peak sizes in a DNA sample?
   a. 2 peaks above and 2 peaks below the fragment of interest
   b. 2 peaks above and 1 peak below the fragment of interest
   c. 1 peak above and 2 peaks below the fragment of interest
   d. 1 peak on either side of the fragment of interest

24. What is the most likely reason for the *-12 allele present in the first blue locus on the electropherogram below?
   a. A true off-ladder allele
   b. *A
   c. Stutter
   d. Pull-up
25. Why is it important to have a low salt concentration in your sample solution?
   a. Less competition for the DNA molecules upon injection into the capillary
   b. To lessen the possibility of hypotension in the instrument
   c. It is important for sample stacking
   d. Both a and b
   e. Both a and c

26. Which of the following describe the capillaries used on the 3130xl for forensic purposes, as they are received from the manufacturer? Circle all that apply (4 pts).
   a. 47 cm in total length
   b. 35 cm from the inlet to the detection window
   c. Made of fused silica
   d. Coated on the inside
   e. Polyimide on the outside for added stability

27. What does the “4” stand for in POP-4?
   a. 4% concentration of polyacrylamide
   b. 4” modification of the formula
   c. Concentration of area
   d. None of the above

28. DNA migrates toward the:
   a. Cathode
   b. Anode
   c. Negative electrode
   d. Both a and c

29. Which of the following describes the method of measuring the difference in the excitation and emission spectra of the fluorescent tags on the DNA fragments?
   a. Electrophoretic injection
   b. Sample Stacking
   c. Stokes Shift
   d. Multi-component analysis

30. What temperature is the electrophoresis run at?
   a. 50°C
   b. 55°C
   c. 60°C
   d. 65°C

31. What data is along the left side of the electropherogram
   a. Relative fluorescent units
   b. Data points
   c. Scan points
   d. Base pair size

Page 8 of 9
BONUS QUESTION:

1. What does the acronym LASER stand for? (3)
Appendix 6: Capillary Electrophoresis Course Evaluations with Feedback

**UNIVERSITY AT ALBANY**
State University of New York

**CAPILLARY ELECTROPHORESIS & DATA ANALYSIS**
Jan. 11-15 & 8, 2010

**Attendee Course Evaluation**

Please fill out this evaluation form. Rate each of the aspects of this course by circling the appropriate number. Comment as appropriate.

### Course Overall

<table>
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<th>Excellent</th>
<th>Comments/Suggestions</th>
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<tr>
<td>A</td>
<td>1 2 3 4</td>
<td>5</td>
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(67% 63% 33%)

Geared towards a less experienced crowd - could be translated for the audience. Examples and software time were great. We could have moved faster.

### Speaker: Lucy Davis

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(33% 33% 33%)

Lectures could be more focused.

### Speaker: Jamie Belrose

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<td>1 2 3 4</td>
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(33% 33% 50%)

Great pace and comfortable with material.

### Quality of Audio-Visual

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(66% 33%)

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(50% 50%)

(67% 63% 33%)

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129
Facilities

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Comments/Suggestions:
Was nice having it at our own lab. Did not take away from work for a week.

Please Comment On:

Did the material presented meet your professional expectations?
Yes - 5

What topic or topics did you find most useful to you?
Practice with designing various protocols.
Theory/ background lectures were great.
Trouble-shooting, intro to QMCD-X
All of it.
Theory and interpretation

Would you recommend this course to other scientists?
Absolutely
Yes - 5

General comments:
Thank you.
Thanks for your time and expertise -- lectures were great!
I feel I really learned a lot. The class helped clarify a lot of things I "thought" I knew. I am no longer afraid of the software. Thank you!
The simulated data sets were very good for practice.

Suggestions for future classes:
PCR
Consider shortening to 3 or 3.5 days.

Thank you for your input!
### Attendee Course Evaluation

Please fill out this evaluation form. Rate each of the aspects of this course by circling the appropriate number. Comment as appropriate.

#### Course Overall

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<tr>
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<td>More hands-on with 3106xl, less time devoted to 6800.</td>
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<td></td>
<td>A list of very useful information, very well executed.</td>
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<td></td>
<td>Very informative and enjoyable.</td>
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<td>Flexible enough to accommodate different backgrounds.</td>
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<td>I learned more in this class than I expected to.</td>
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#### Speaker: Lucy Davis

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<tr>
<td>Comments/Suggestions</td>
<td>Very good lecturer, made it very interesting.</td>
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<td>Builds good rapport with students, flexible and interactive.</td>
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<td>Very knowledgeable and friendly.</td>
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<td>Made course adjustments based on needs of the students. Presented the material well.</td>
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<td>Very knowledgeable and patient. Resourceful and very well spoken.</td>
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#### Speaker: Jamie Belrose

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<td>Very well versed and knew and understood the chemistry.</td>
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<td>Fully helped me understand the flow and any of the ideas and instrument.</td>
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<td>Very knowledgeable of the course material, presented the material very well.</td>
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<td>Too fast.</td>
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<td>Top notch instructor and very helpful.</td>
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<td>Extremely knowledgeable and focused. Answered questions clearly and thoroughly.</td>
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<td>Very good instructor: able to explain complicated concepts very well.</td>
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<td>The presentations were very well organized. I really enjoyed the notes, very thorough.</td>
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<td>One overheads were very helpful and aided in the presentation of materials.</td>
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<td>Made it really easy to follow along.</td>
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<td>Made it really easy to follow along and to serve as a reference source.</td>
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This document is a research report submitted to the U.S. Department of Justice. This report has not been published by the Department. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

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Campus was beautiful, I had enough space to work.

Please Comment On:

Did the material presented meet your professional expectations?
Yes: 3
Yes, it was a very good review of my training as well as more detailed in the areas I had issues with. It exceeded my expectations. Unlike other workshops, there was a lot of explanation here and I'm walking out feeling like I know a lot more.

Personally, I expected more hands-on with the 3130i. However, it did benefit greatly from the topics discussed.
Yes, very helpful: 2
Yes, it explained the overall process and the instructors explained things completely and fully.

What topic or topics did you find most useful to you?
RT-POR: 3
CE/3130: 2
Electrophoresis theory: 5
GMDP: 1
Explanation of local southern method and how software allows us to interpret data
As much as I dislike theory, it was educational and interesting.
Quantifier and 3130i lecture helped me a lot too.
Hands-on lab and software
Sample stacking and spikes.

Would you recommend this course to other scientists?
Yes: 5
Absolutely. If I was not employed I'd take the 4-month course.
Absolutely - even the most experienced analysts would learn so much valuable information.
Yes! Especially newcomers that have limited experience. It fills in all the blank spots left over from specific training.
Yes indeed.

General comments:
Very satisfied!
I appreciate the educational opportunity that you provided for me. Thank you. Hope you find more grant money. I think this program provides a beneficial niche in the field of DNA.
Jamie and Lucy are both so knowledgeable and did a great job of explaining everything.
Thank you for sharing your wealth of knowledge.
Lectures on the CE and 3130i very helpful. 3130i lecture was a bonus.
Thank you for the opportunity to take part in the training - it was great.
I found the class to be very helpful. I feel more confident on the subject of CE. Thank you.

Suggestions for future classes:
Have it in the spring?
More hands-on time with 3130i
Instructor and practice with GMDP/IDX
Maybe a side on interpretation of complex mixtures.
I hope this course will be able to continue - very valuable to new analysts like myself & to experienced analysts. The experienced analysts stated that they learned a lot of valuable information.
Maybe first day of computer work. Lucy or Jamie can have us following along with our laptops.
Do a bit set together and then separate.

Thank you for your input!

132
# Attendee Course Evaluation

Please fill out this evaluation form. Rate each of the aspects of this course by circling the appropriate number. Comment as appropriate.

## Course Overall

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Comments/Suggestions: Very specific and would highly recommend this course to forensic biologist. Very thorough and informative. Very informative about theory/practices. In depth coverage and detail.

## Speaker: Lucy Davis

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Comments/Suggestions: NA – death in family.

## Speaker: Jamie Belrose

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Comments/Suggestions: Clear and well spoken. Sufficiently answered all questions. Superb.

## Quality of Audio-Visual

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Comments/Suggestions: Rich in information, very complete with data specific to principles and applications; really enjoyed Jeopardy! Clear and new views of old material provided a nice change of pace.

## Quality of Handouts

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Comments/Suggestions: Followed audio/visuals well. Small font on slides makes it hard to read on handout.
Facilities

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Please Comment On:

Did the material presented meet your professional expectations?
- Exceeded expectations.
- Yes.
- The material presented was very detailed and informative.

What topic or topics did you find most useful to you?
- Electrophorisis and CE theory
- The theory behind high performance capillary electrophoresis
- Theory and concepts of instrumentation and procedures
- All topics were helpful.

Would you recommend this course to other scientists?
- Absolutely.
- Yes.

General comments:
- Good content. The Jeopardy game was fun and helped me to learn.
- Thank you for the on-site visit.
- Very clear, informative, and concise.
- I would like to have this course again, at least for review. But I enjoyed this very much.

Suggestions for future classes:
- None. This was very fun, interactive, and informative.

Thank you for your input!
Appendix 7: RT-PCR Course Information:

Real-Time PCR Theory & Chemistries

Given by: Northeast Regional Forensic Institute
Hosted by: Westchester Co. Crime Laboratory
Funded by: The National Institute of Justice

January 6 – 8, 2010; Spaces Are Limited!

Instructors: Bruce McCord, Ph.D., Lucy A. Davis, & Jamie L. Belrose

Course Description
This 2.5 day course is designed to provide the theoretical and practical background necessary to perform quantitative PCR and data analysis. Today’s advanced technologies have led to an exponential number of cases being submitted to crime labs for DNA testing. For these techniques to be successful, it is imperative that biological evidence be analyzed and interpreted effectively. These already using RT-PCR are welcome to attend the one-day advanced lecture by Dr. Bruce McCord.

Introductory Lecture
Wednesday, January 6, 12:30 – 4:00pm: NERFI
- Theory of Polymerase Chain Reaction (PCR)
- Several Methods of Quantification (Yield Cells, Spectrophotometry, UV-Vis, & SYBR Green)
- How the amount of input DNA can/will impact PCR – adding too much or too little...
- Theory of Real-Time PCR
- Advantages of Quantitative PCR
- Fluorescent Detection (Stokes Shift)

Thursday, January 7, 8:00am – 4:00pm: NERFI
- Kit from Applied Biosystems: Quantifier™ Human, Quantifier™ Y Human Male & Quantifier® Duo
- Taqman® Probes & 5’-Nucleotide Activity
- Fluorescence Resonant Energy Transfer (FRET)
- Instrument Optics (Turbiscan Lamp, Virtual Filters & Charged-Coupled Device Camera)
- Definition: Threshold, Cycle Threshold, Internal PCR Control, Slope, Y-intercept, & Correlation Coefficient
- AB 7500 System Sequence Detection Software (SDS) v1.2.3 and HID Real-time PCR Analysis Software v1.0
- Absolute Quantification (Standard Curve)
- Troubleshooting outlier standards & changes in slope and Y-intercept
- Review of major concepts
- Multiple choice exam and issuance of completion certificates.

Advanced Lecture
Friday, January 8, 8:00am – 4:00pm: McCord
- Multiplex methods for real time detection – Sybr Green, Planar
- The three -rule and other interesting issues with Y STRs
- How RT-PCR could be used to evaluate and decide whether to conduct uninformative vs. Y STRs
- Confidence zones
- Low copy number and real time PCR
- Application of fast PCR for setting laboratory thresholds
- Precision vs. Accuracy, variability with standards, and how labs might incorporate the use of SRMs.
- Validation issues
- Inhibition and mechanisms of how inhibition occurs (causal factors) and how to possibly overcome this dilemma
- BIC Cts for detecting inhibition
- Efficiency shape of the amplification plot for detecting inhibition
- Application of DNA melt curves for inhibition detection
- How RT-PCR could be used for screening samples for probeable value
- Methods to detect DNA degradation by real time PCR
- Short answer exam and issuance of completion certificates.
Appendix 8: RT-PCR Introductory Presentation

Real-Time PCR
NERP / NJU
Presented by: Jamie L. Belose, Lucy A. Davis
Created by: NERP Staff

Purpose of Quantitation
- Wide range of amounts of DNA at crime scene
- Inhibition of samples by either substrate or sample itself
- Narrow tolerance of PCR systems input DNA
  - 1–2 ng DNA typical identifier manual specifies
    - 0.1–10 ng
  - Too little = inefficient amplification
  - Too much = generation of artifacts, inhibition of assay
- Is the DNA human?

Purpose of Human-Specific DNA Quantitation
- All sources of DNA are extracted when biological evidence from a crime scene is processed to isolate the DNA present.
- Thus, non-human DNA such as bacterial, fungal, plant, or animal material may also be present in the DNA extracted from the sample along with the relevant human DNA of interest.
- For this reason, the FBI DNAS Standard 5.5 (4.5 in THOR nucleic acid) requires human-specific DNA quantification so that the approximate amount of human DNA can be included in the subsequent PCR amplification.
- Multiplex STR typing works best with a forty narrow range of human DNA (specific 2.5 to 10 pg of input DNA ISO 2009 with commercial STR kit).

Impact of DNA Amount into PCR

UV/Spectrophotometer
- Variable wavelength light source
- 260nm DNA
- 270nm phenol
- 280nm protein
- Absorbance detection
  - Can determine quality and quantity of DNA
  - Can determine if human DNA

UV Methodology
- Run calibration standards
- Prepare calibration curve
- Measure unknown 260nm
- Compare to curve
UV Spectroscopy Ratios

- $\text{Abs } 280/260 = \geq 1.8$ protein contamination
- $\text{Abs } 270/260 = \geq 1.2$ phenol contamination

Advantages of UV Quantitation

- Quick
- Accurate if have clean solutions.
- Assessment of phenol or protein contamination

Disadvantages of UV Quantitation

- Needs lots of DNA at least 250 ng.
- Not human specific
- No information on quality of DNA

Yield Gel Quantitation

- Agarose gel electrophoresis
- 1% agarose with ethidium bromide.
- 1x TAE buffer
- DNA phosphate backbone negatively charged

Yield Gel Apparatus

- ~200V for 8 min applied across gel
- DNA travels toward positive anode
- Large fragments move slowest – sieving of agarose gel.
- Ethidium bromide intercalates with double stranded DNA.

Yield Gel continued
Yield Gel Detection

- The more molecules of DNA - the more Ethidium bromide you have associated with the DNA band
- Compare intensity of band with standard bands
- High Molecular weight DNA is quantitated
- Degraded DNA shows up as streak
- Not human specific - viral and bacterial DNA looks the same as human
- Standards are Lambda DNA, not human DNA

Yield Gel Results

Slot Blot Procedure

- Denature DNA
- Transfer DNA to charged nylon membrane
- Hybridize primate specific biotinylated probe
- Add HRP-SA conjugate
- Detection

Slot Blot Apparatus

Slot Blot Quantitation
Hybridization Based Assay using Human Specific DNA probes

- QuantIBlot Human DNA Quantitation System
- Detection methods:
  - Colorimetric with biotinylated oligonucleotide labeled probes with streptavidin-horse radish peroxidase conjugate, color substrates
  - Chemiluminescent with biotinylated oligonucleotide labeled probes with streptavidin-horse radish peroxidase conjugate, light-emitting substrate

Yield Gel Advantages

- Quick and Easy!
- Low cost
- Consumes little sample
- Sensitive to approx. 1 ng of DNA
- Measures quality of DNA
Membrane Development

Slot Blot Results

Slot Blot Advantages
- Consumes little sample
- Works on single stranded and double stranded DNA
- Human specific
- Accurate with degraded DNA to a point

Disadvantages of Slot Blot Detection
- Time consuming
- No indication of DNA quality
- Expensive (cheaper than real time PCR)
- Technique dependant

Real Time PCR Quantitation

Real Time PCR Advantages
- Automated
- Very sensitive and wide dynamic range 0.023 ng to 50 ng/µl
- Evaluates inhibition
- Very reliable and robust
- Minimal sample handling and setup
History

- **RT-PCR (qPCR)**
  - Developed by Higuchi in 1993
  - Uses a modified thermal cycle with a UV detector and a CCD camera
  - A plasmid clone was used as an internal control, as the increased fluorescence increased
  - RT-PCR also reveals reverse transcription PCR, which is used when working with RNA. So real-time PCR is often abbreviated qPCR (quantitative)

PCR Amplification

- **What is PCR?**
  - Polymerase Chain Reaction
    - Uses template DNA, oligonucleotide primers, and nucleotides (dNTPs), & thermostable DNA polymerase to replicate selected regions of DNA
    - Amplifies a specific DNA sequence (one cycle vs. another cycle)

PCR Phases

- Initial denaturation
- Annealing
- Extension
- Product formation
- No more increase in product
- Poor replication of results
- Running out of materials
- Still making more product
- Not exponential and poor replication of results

Real-Time Quantitative (qPCR)

- **What is real-time PCR?**
  - qPCR works the same way as conventional PCR
  - The addition of fluorescent probes to quantify DNA concentration
  - Accumulation of product can be monitored with every cycle = "real-time"

- **How can we measure the concentration of DNA in a sample with fluorescence?**
  - To answer this question, we must know some of the fundamental concepts involved with fluorescence and its detection

Detecting Fluorescence

- Four required components:
  - 1) Excitation source
    - Excitation wavelength - Ability to excite the fluorophore
  - 2) Fluorophore (fluorescent molecule)
    - Fluorescent dye or fluorophore/absorbs a wavelength
  - 3) Wavelength filters
    - Spectral emission filter - absorption of photons
    - Necessary for correct detection levels of the fluorophore of interest
  - 4) Detector
    - CCD (charge coupled device) camera - records the intensity of light emitted

Fluorescence

- Light of a certain wavelength excites fluorophores
- Emission of fluorescent light occur at another, higher (longer) wavelength
- Different wavelengths of light are used to excite intensity of light emitted will change

<http://science.knowledge.net/fluorophore/fluorophore.html>
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**Stokes Shift**
Different dye excite at same wavelength but emit at different wavelengths allowing us to distinguish them.

**Excitation Source**
- Tungsten-halogen lamp emits white light with peaks at varying intensity across the electromagnetic spectrum.
- Filters needed to isolate the wavelengths of interest.

**Filters**
- Optical filters allow certain wavelengths of light to pass through while others are blocked.
- Several nanocry emission filters are used in real-time quantitation because of the multiple dye system used.

**Multiple-Dye Detection**
- Although this graph is of cell staining, the same principle is applied to fluorescently labeling the DNA products from qPCR.

**Forensic Kits Available**
- Applied Biosystems
  - Quantifier™
  - Quantifier™ Y
  - Quantifier Duo™
- Promega
  - Flexor HY™

**Quantifier™ Human DNA Quantitation Kit**
- Amplicon is 62 bp
- Probe is to Human telomerase reverse transcriptase gene (hTERT) chromosome 5
- Nontranslated region (intron)
- Diploid
Applied Biosystems Quantifier™ Kits
Assay: Two Major Components
- Human Target Specific Assay
  - FAM labeled probe (autosomal or Y-chromosome)
- Internal PCR Control (IPC)
  - VIC labeled probe
  - 10,000 copies/μL of synthetic non-human template
  - Inhibitor Detection

Dyes used in Quantifier™
- FAM – blue human DNA reporter dye
- VIC – green IPC reporter dye
- ROX – red passive reference dye
  - Used in all samples – minimizes operating variation and well to well optical differences.
  - Color represented on screen is not necessarily same as dye – can set plate to any color.

Passive Reference Dye
- The Quantifier kit also includes a passive reference dye
  - ROX.
- The information gained from including this dye is used to account for the instrument’s shortcomings.
- The laser excitation across the 96-well plate is not equal.
  - This is due to the plate’s square shape and bulb’s round shape. The wells in the middle of the plate receive more excitation energy than those around the outside.
  - The ROX information is used to normalize the data for all wells of the plate.

Quantifier™ Kits Use TaqMan®
Probe Technology
- Primers used to amplify a particular region
- Fluorescently labeled probe binds to target
- When target is amplified, probe is hydrolyzed, releasing the fluorescent tag
- Instrument measures the cycle to cycle changes in fluorescent signal
- Quantity of fluorescence measured is directly related to target DNA concentration

TaqMan® Probe
- The Quantifier™ probe is autosomal specific and is complimentary for a single copy target within the genome
- The target region is an intron within the human lactase penetrance transcript (LACTPN) at chromosome 9 between the primers
- 5’ end of the probe contains a fluorescent dye – FAM, known as the reporter dye
- 3’ end of the probe contains a non-fluorescent dye, known as the quencher dye
- FRET also contains a minor groove binder

How TaqMan® Probe Works
- The probe has a fluorescent dye attached to the 5’ end (reporter) and a non-fluorescent dye attached to the 3’ end (quencher)
- While the reporter and quencher dyes are in close proximity to one another there is an energy transfer from the reporter (High energy) to the quencher (low energy), resulting in no fluorescence
- This phenomenon is called Fluorescent Resonance Energy Transfer or FRET
How TaqMan® Probe Works

- AmpliTaq Gold DNA Polymerase has 5' nuclease activity. The 5' nuclease activity of the enzyme acts upon the surface of the template to remove nucleotides downstream of the growing amplicon that may interfere with its generation.

- This coupled with the FRET (Fluorescent Resonance Energy Transfer) makes it possible to detect PCR amplification in real-time.

How TaqMan® Probe Works

- The TaqMan™ probe is very short (12-18bp). This allows the PCR amplicon to be very small (52bp). Smaller amplicons mean a more efficient PCR and a greater chance of quantifying degraded DNA.

- The Minor Groove Binder has two additional roles:
  - First, it anchors the small probe to the DNA, it does this by binding in the "minor" groove.
  - Second, it increases the annealing temperature of the probe. The probe needs to have a higher annealing temperature than the primers, otherwise there would be annealing and extension before the probe gets a chance to sit down.

Real-Time PCR Instrument

- The instrument directs light into each well using:
  - Applied Biosystems Prism 7900 Steady-Heat Laser
  - Applied Biosystems Prism 7900 Laser

- The light passes through optical adhesive cover (or strip) on plate and excites fluorescent dyes in each well.

- A system of lenses, filters, and a dichroic mirror focuses the fluorescence into a charge-coupled device (CCD) camera

- Fluorescence emission between 500nm and 510nm is separated based on wavelength into a precisely spaced pattern across the CCD camera.

- The instrument contains 3D5 software that applies algorithms to the fluorescence detected from the CCD camera to determine relative fluorescence units (RFU).
Summary of TaqMan® Probe and Quantitation

- Start with a sample containing an unknown amount of DNA.
- Quantifier™ kit contains primers and probe that will target and attach to the HFK7 gene.
- Through the 5' nuclease activity of the polymerase and FRET, the amount of DNA template will be detected by the instrument based on the amount of fluorescent emission after each PCR doubling of the DNA.
- Using software and the standard curve, the fluorescent emissions are converted to give a quantity of DNA.

Cycle Threshold

**Cycle Threshold (Ct)**

- The Threshold is the level of detection or the point at which a reaction reaches a fluorescent intensity above what is considered to be background.
  - The Threshold is set at 0.2 as per Applied Biosystems.
  - The Threshold line is set in the exponential phase of the amplification for the most accurate reading.
- The cycle number at which the samples reaches this threshold level is called the Cycle Threshold (Ct).

The smaller the Ct... the more initial template DNA you have

**How do we get an actual DNA concentration?**

**Standard Curve:**

- Several known concentrations of DNA (standards) are run on the instrument plate along with your samples and the data is used to generate a standard curve (Ct vs. log concentration).
- The fluorescence measured from the unknown samples is then compared to the standard curve and the initial concentration is interpolated.
Standard Curve

- Made using purified human, male DNA
- Known concentration of 230 ng/µl
- Standard is serially diluted (3-fold difference between each concentration) to give 8 concentrations ranging from 50 ng to 23 pg per al
- Duplicates of each dilution of standard are added to analysis plate
- The Ct value for each dilution of standard is determined based on the number of cycles it takes for the fluorescence to reach the threshold.

Using standard curve to determine unknown concentrations

- Standards:
  - We input concentration of each dilution into the Sequence Detection Software (SDS)
  - Ct value for each dilution is measured by the instrument
  - Ct value for each dilution is determined based on a standard curve

- Determinants:
  - Ct value for each unknown is measured by the instrument and concentration is determined based on the standard curve
  - Software compares Ct values of standards to Ct values of standards
  - Software takes input data of standard concentrations to determine concentration of sample - quantity of DNA

Examining the Standard Curve

- \(C_t = m \log(Qty) + b\)
  - Why use log? Answer to obtain a straight line
  - \(m\) is the slope
  - \(b\) is the y-intercept
  - Qty is the quantity of starting DNA

Examining the Curve

- Slope = steepness of the line
- Slope value (m) indicates amplification efficiency for the assay
  - -3.32 indicates 100% efficiency
  - Range of -2.0 -- -3.3 considered acceptable for Quantifier kit

- Y-Intercept indicates the expected Ct value for a sample with a quantity of mg/µl
  - because log(Ct) = log(1) = 0
  - For Quantifier the y-intercept Ct should be around 20.
You Deserve a Break!

Examining the Curve

- $R^2$ should be $\geq 0.99$ (correlation coefficient)
  - Value of 1 means curve perfectly fits data points
  - $< 0.98$ indicates a problem:
    - Task and quantity entered for wrong detector
    - Incorrect quantity entered for standards
    - Pipetting trouble
    - Other issues?
  - If you see this, don’t worry we will figure it out!

Resulting Curve 1

- Each point is outside the expected region of the standard curve
- Points form a horizontal line

Possible Problem: Wrong Detector

- Task and quantity entered for IPC detector rather than Quantifier™ detector
  - The Quantifier™ Human detector’s Task should be “Standard” NOT the IPC’s Task

Resulting Curve 2

- One or more points lie(s) outside the standard curve

Possible Problem: Incorrect Quantity Entered

- In the diagram on the previous page, 0.062 was entered as the value for the outlier point
- The correct value is 0.02 (circled area would be expected area for point)
Change in Slope

- An acceptable slope of -3.14
- An unacceptable slope of -3.80
- Looking at the same Cq value for a theoretical sample – let’s say 29 – a different quantity of DNA is calculated based on the different slopes in the standard curves.
- What does this mean in terms of our data?

Downstream Issues

- If the unacceptable slope was used to calculate the amount of sample needed for amplification, the quantity would be overestimated to the left of the y-intercept (underestimated to the right of the y-intercept).
- Therefore, less sample and thus less DNA would be needed for PCR amplification and a false negative is possible. (Sample overload if point to the right of the y-intercept -> pull-up in profile)

Overestimation

Underestimation

Resulting Quant values

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<th>Dilution</th>
<th>Concentration (ng/ul)</th>
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<td>0.1</td>
<td>10</td>
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Change in Y-Intercept

- If the slope of the standard curve remains the same, but the y-intercept changes... (both values below are acceptable)
- The whole curve shifts
- The same Cq value leads to a different concentration calculated
- Can lead to the same downstream errors as poor slope
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Y intercept Effects

- Out of Range Slope Causes
  - Generally slope issues are a result of inaccurate pipetting
    - Could be inaccurate pipetting
    - Could be inaccurate pipettor
    - Could be both!
    - Generally a problem with preparation of calibration standards
  - Less likely – something wrong with kit

Y intercept out of range causes

- Could be DNA standard is not at stated concentration
- Could be inaccurate pipetting
- Could be inaccurate pipettor
- Generally a problem with calibration standards preparation
- It is a good practice to establish an acceptable range for Y intercept

Putting It All Together

- Let's go back and talk about the kit

Quantifier™ Kit Components

- Primer/Probe Mix with Internal PCR Control (IPC)
  - PCR primers – one set for HRT, one set for IPC
  - TAM (Quantifier™) and VIC (IPC) labeled "TagMent" probes
  - TopTag™ 2X Universal PCR Master Mix
  - ROX-reference dye
  - DNA standard
    - Human male DNA, 200ng/μl

Internal PCR Control (IPC)

- In the primer mix
- Synthesized DNA strand – sequence not found in nature
- Detects inhibition, instrument malfunction, or problems with assay setup
- Warns of impending PCR and STR analysis failure
Summary of IPC Results

- The failure of both the samples and IPC to amplify most likely means an inhibitor was present in either the sample itself or in the material from which the sample was taken.
- Failure to load the necessary reagents (i.e., Master Mix) would also result in these irregular lines when plotted on the CT.
- Additionally, an IPC failure could also indicate an instrumental artifact due to a sample or the presence of no CT product. An amplification failure in the IPC.
- As you are quickly learning, a failed IPC is not necessarily indicative of just one issue and a sample with a failed IPC should be diluted and re-quantified.

Quantifier™ Kits Procedure

- Universal Cycling Parameters
  - 95°C 10 minutes
  - 40 cycles of
    - 95°C 15 seconds
    - 60°C 60 seconds
  - ABI PRISM® 7500 SDS

Quantifier™ Y Human Male Quantification Kit

- Amplicon 64 bp
- Sex-determining region Y gene (SRY)
- Nontranslated region
- Haploid
Quantifier™ Y Human Male DNA Quantification Kit

Y-chromosome kit useful tool for samples suspected of containing male DNA
- Sexual assault samples
- Crime scene mixtures
- Provides additional information for mixture interpretation
- Provides quant into for Y STR typing

Quantifier™ Y Human Male DNA Quantification Kit Conclusions
- Sensitive
  - Proof of male DNA present on intimate sample
- Offers novel method of mixture detection

Calibrations
- Why are calibrations of the instrument necessary?
  - Because we are processing multicomponent (many dyes) data.
- Background calibration
  - Determines the amount of background fluorescence by measuring the raw spectra generated from a plate with PCO buffer.
  - Background electronic signal
  - Contaminates the sample book
  - Plastic consumables... e.g. plates and caps
  - Poor log calibration
    - Collects and stores spectral data from pure dye standards
    - Regions of interest (ROI)
    - Maps the position of wells on the sample page so the software can trace increases in fluorescence to specific individual wells.

Calibrations
- Raw data is collected between 500 and 600nm.
- During data collection, the SDS software uses algorithms to determine the contribution of each dye.
  - The background component (stored in the B/G calibration file) is subtracted from the raw data.
  - The information in the pure dye file is used to determine the contribution of each dye to the measured spectrum.
    - Measured spectrum = 3(FAM) + 4(MCy5) + 5(ROX) + 4(ROX) + MSE
      - MSE = mean standard error

Calibrations
- The MSE indicates how closely the collective multicomponent spectrum conforms to the raw data.
  - Similar to R² on the standard curve.
  - Are the data be collecting along the lines of what one would expect?
Calibrations

Normalization of Reporter Signals

- The software normalizes each reporter signal by dividing it by the fluorescent signal of the passive reference dye, ROX.
- ROX is present at the same concentration in all wells as part of the master mix.
- By normalizing, the software can account for minor variations in signal caused by pipetting inaccuracies – thus allowing for more accurate well-to-well comparisons.
References

- www.appliedbiosystems.com
- Information also gathered from Power Point slides created by Janice Baruzzo, Lucy Davis, Andy Wilt, Jodi Higgins, Vivian Shelling, and Margaret Trott. Photos by Andy ©
Appendix 9: Advanced RT-PCR Presentations

A comparison between Plexor and Quantifier Duo

Heather Lasse, George Duncan, Bruce McCord, FRI Department of Chemistry
International Forensic Research Institute
Miami, FL 33199
mccordb@fiu.edu 305-348-7543

Introduction

- Quantifier Duo (AD) and Plexor HY (Promega) are two approaches to the analysis of DNA mixtures.
- Both are qPCR kits that determine the relative difference between male and female DNA.
- Quantifier duo is a reverse transcriptase assay that contains a Y probe, an autosomal probe and a control.
- Plexor is a multiplex primer quenching based assay that also contains a Y probe, an autosomal probe and a control.
- The main differences between the two results from copy number, allelic size and the issue of probes vs primers for defining specificity.

Plexor HY Setup

- Plexor probes multi-copy variant CNV (m=20) at locus PMUS on chromosome 17 for autosomal DNA. Amplicon size = 598bp. Detect at 520 nm.
- Because there are 2 copies of the autosomal locus, there is effective equivalence between the number of copies of the autosomal and the Y locus in Plexor.
- Plexor also probes a multi-copy variant CNV (h=10) at locus TSPY. Amplicon size = 333bp. Detect at 560 nm.
- An internal positive control exists at 610 bp. Amplicon size is 130 bp. Detect at 460 nm.
- There is an internal reference IGS.

Duo Setup

- Duo probe a single copy gene. The target is located on the X-chromosome. Promega's HRX assay contains 2 PCR products from the X/autosomal region, one from each chromosome.
- Duo also probes a single copy gene at a single locus with a target length of 496 bp. Detect at 520 nm excitation (PLLEX).
- The internal positive control also has a length of 496 bp, detect at 520 nm.
- Rox is added as a positive reference at 620 nm.
- The common region permits a consistent response to HRX and a consistent correlation to a single minimum sized CNR.

Duo Calibration Curve

- 50 ng to 23 pg single copy

Plexor HY Calibration Curve

- 50 ng to 3 pg multi copy
An Analysis of Single and Multicopy Methods for DNA Analysis by Real Time PCR

Heather LaSalle
Broward Sheriff’s Office

Introduction

- The goal of this study was to compare results between Quantifier duo and Plexor HR.

- The hypothesis is that Plexor HR, a multicopy system, should produce improved precision at lower levels of template.

- However, it was also expected that the multicopy estimate for Plexor should be less precise than the single copy Quantifier duo due to potential mutations affecting copy number in the genome.

Experimental design

- Initial experiments involved a cross comparison of external standards for each kit.

- Each kit was calibrated with its specific standards and then these samples were run again as unknowns to test reproducibility of the measurement.

- In addition, these standards were run with both chemistries.

Preparation of Standards

- A set of standards is made by serial dilution and run in duplicate.

- The Plexor standards are made from a 50ng/ul pooled standard. The range is: 1.5ng/ul, 1.0ng/ul, 0.75ng/ul, 0.025ng/ul, 0.0125ng/ul, 0.00625ng/ul.

- The Quantifier standards are made from a 250ng/ul pooled standard. The range is: 1.50ng/ul, 1.25ng/ul, 0.75ng/ul, 0.25ng/ul, 0.125ng/ul, 0.0625ng/ul.

- Both the Plexor standards and Quantifier standards were run as unknowns and standards on multiple runs.
Results

- Results show good concordance between the two kits for both the Plexor and quantifier Duo standards at all levels.
- Precision between the two kits was comparable with Duo being a little better at higher concentrations and Plexor better at low concentrations.
- Plexor appeared to have a lower LOD as expected.

Part two:

Validation tests with NIST standards

- Three NIST standards (62172) were examined to check accuracy and sensitivity:
  - Component A is a single source male that should quantitate as 52 ng/ml according to NIST.
  - Component B is a pooled female sample that should quantitate as 33 ng/ml according to NIST.
  - Component C is a pooled mixture of males and females that should quantitate as 54 ng/ml according to NIST.
- Standards were examined directly and also using a serial dilution.
Data from NIST standards

<table>
<thead>
<tr>
<th></th>
<th>Expected from NIST</th>
<th>Quantified Human Sample</th>
<th>Average Human Sample</th>
<th>NAD</th>
<th>Average NAD</th>
<th>NAD Y</th>
<th>Average NAD Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSMM A</td>
<td>62.4</td>
<td>51.82</td>
<td>92</td>
<td>60.69</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSMM A Y</td>
<td>63.6</td>
<td>53.62</td>
<td>90</td>
<td>57.55</td>
<td>NAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSMM B</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSMM C</td>
<td>54.3</td>
<td>70.52</td>
<td>41.375</td>
<td>32.44</td>
<td>NAD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

- The Flexor HY results appear to show an anomalously low human quantitation relative to the male quant, particularly for the single source male.
- The duo results are more consistent for the single source male sample but are just as poor as Flexor for the mixed sample.
- Results for these high level samples however may suffer from inaccuracy – further tests are needed.

To examine these potential differences with more precision a series of dilutions were tested

- NIST Std A (single male) using both Flexor HY and Duo.
- Standard A was diluted in halves starting with 12.1 ng/ul.
- GSMM A = 52.4
  - Dilution order in ng/ml 1.1, 0.55, 3.775, 1.8375, 0.918, 0.459, 0.204, 0.102, 0.051, 0.026, 0.012, 0.0064, 0.0032, 0.0016, 0.0008, 0.0004, 0.0002.
- Standard A was run in triplicate with each chemistry.
- Each diluted sample was amplified with real time PCR once with each chemistry.

Plot of Experimental vs Actual Values

- NIST GSMM A, Duo and Flexor HY

(Duo produces a relatively higher estimate)

Plot of Experimental vs Actual Values

- GSMM A (Duo Autosomal and Y) Results show similar slope.

Plot of Experimental Quantities versus Actual values for GSMM A

(Y trends higher than autosomal indicates extra methylation or loss of autosomal DNA copy.)
To examine these potential differences with more precision, a series of dilutions were tested:

- NIST 516 (pooled female) using both Flexor HY and Duo
- Standard B was diluted in half with values starting with 10 ng/ml.
- GSMR = 53.6
  - Dilution values in ng/ml: 13.4, 6.7, 3.35, 1.675, 0.8375, 0.418, 0.209, 0.104, 0.052, 0.026, 0.013, 0.0065, 0.0032, 0.0016, 0.0008, and 0.0004.
- Standard B was run in triplicate with each method.
- Each diluted sample was amplified with real-time PCR once with each method.

To examine these potential differences with more precision, a series of dilutions were tested:

- NIST 515C (pooled mixture of males and females) using both Flexor HY and Duo
- Standard C was diluted in half with values starting with 10 ng/ml.
- GSMR C = 54.3
  - Dilution values in ng/ml: 13.75, 6.75, 3.375, 1.6875, 0.84375, 0.421875, 0.2109375, 0.10546875, 0.05265625, 0.026328125, 0.0131640625, 0.00653125, 0.003265625, and 0.0016328125.
- Standard C was run in triplicate with each method.
- Each diluted sample was amplified with real-time PCR once with each method.
Sensitivity Experiments

- The sensitivity experiments were continued with amplification of samples A, B, and C in solutions with identifier.
- Quantification results were obtained with respect to actual vs expected quantities and assay parameters.
- Sensitivity quantifying at low values were amplified at 10x total volume and amplification factor was measured.
- Comparison was done with recommended areas of abundance.

### Sensitivity with Plexor

<table>
<thead>
<tr>
<th>Sample</th>
<th>CSRM A (single male)</th>
<th>No amplification</th>
<th>3pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSRM A</td>
<td>100</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>CSRM B</td>
<td>150</td>
<td>75</td>
<td>37.5</td>
</tr>
<tr>
<td>CSRM C</td>
<td>200</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

### Sensitivity with Duo

<table>
<thead>
<tr>
<th>Sample</th>
<th>CSRM A (single male)</th>
<th>No amplification</th>
<th>3pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSRM A</td>
<td>100</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>CSRM B</td>
<td>150</td>
<td>75</td>
<td>37.5</td>
</tr>
<tr>
<td>CSRM C</td>
<td>200</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>
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Sensitivity with Plexor

GSRM C (mixed males and females) - No amplification at 1pg

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plexor A</th>
<th>Duo A</th>
<th>Plexor B</th>
<th>Duo B</th>
<th>Plexor C</th>
<th>Duo C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

No Amplification

Ratio Results (NIST GSRM C A-C)

Quantities from 1pg - 6.5 pg/mL

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plexor A</th>
<th>Duo A</th>
<th>Plexor B</th>
<th>Duo B</th>
<th>Plexor C</th>
<th>Duo C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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<tr>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

No Amplification

Conclusions on sensitivity

- Both kits showed a high degree of linearity over the tested range
- Precision for both kits showed RSD ranging from 7.16% for V ratios and for GSRM C/ experimental of 8% (plexor) and 24% (duo)
- Accuracy was relatively poor and sample dependent, indicating potential sequence effects, neither kit did well in this respect. In particular there appeared to be problems with Plexor (automated) for GSRM A and with Duo for GSRM C (Y)
- Sensitivity was better for Plexor as expected, although duo performed better than expected below its suggested range

Caspework Samples

- To further test the two systems a set of casework samples were investigated
- Quantifier Human, Y Duo, and Plexor HY were compared
- Data is presented below
Conclusions casework

- Results need to be statistically analyzed but generally track each other.
- A two treatment statistical test can be used.
- Further testing is needed to determine the utility of the A/Y ratio in predicting quality of results.

Effect of Amplicon size - ABI kits

Target DNA (kb): 430b - 30000 DNA: 9648-1 copy (DNA = 140, 550)

![Graph showing the effect of Amplicon size on ABI kits](image)

- Chart title: "Effect of Amplicon size on ABI kits"
- X-axis: DNA concentration (ng/µL)
- Y-axis: Amplification Efficiency (%)
Investigation of the effects of sample degradation and inhibition in forensic DNA typing with reference to QPCR

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International Forensic Research Institute
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Large Multiplex Kits provide Efficient and Rapid Analysis of Convicted Offender Samples

But what about degraded DNA?

Such samples present a special challenge

DNA Degradation

1. polymer hydrolyzes (nucleic acids break apart)
2. Pyrimidine dimers (bases X-links)
3. Chemical oxidation (bases become unreadable)

DNA Degradation
Note loss of intensity of larger alleles

Miniplex 1 vs Powerplex 16

Allele Dropout: Standard DNA kit Promega Powerplex 16

Size Range

100 bp  200 bp  300 bp  400 bp

DG  100%  97%  74%  23%  10%
DG2  0%  0%  0%  0%  0%
DG1  99%  98%  69%  23%  10%
DG  0%  0%  0%  0%  0%
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Non-Traditional Samples

- The obvious application for MiniSTRs are samples which are degraded, difficult or of low copy
- We have performed experiments to examine their sensitivity and
  - Capability to amplify DNA from bone and hair
  - Capability to amplify DNA from highly degraded samples
- Big question: How to determine quality and quantity of low level DNA?

- Answer: qPCR

Work in PLU Laboratory- with assistance of Vermont Crime Lab

- Development of miniSTRs for degraded DNA typical case 60-120 bp.
- Size list works poorly on these samples
- So develop a series of different primers to selectively amplify degraded dna

Determination of DNA Quality by qPCR

Aliquots of DNA were amplified using three different sets of primers: 100 bp, 200 bp, and 300 bp. The results were analyzed using qPCR technology, which allowed for the accurate determination of DNA quantity and quality.
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### Amplified Sample – Miniplex

- 100 pg DNA control, 12 μL reaction volume, 35 cycles
- 3 μL H2O extract, 12 μL reaction volume, 22 cycles

### Amplified Sample – Powerplex (Similar results obtained with Identifier)

- 100 pg DNA control, 12 μL reaction volume, 35 cycles
- 3 μL H2O extract, 12 μL reaction volume, 22 cycles

### Results for telogen hairs

- <100 pg: Mini 2 (D5, D6, D15)
  - 0% 1 loc (D9 and D14)
  - 20% 2 loc (D9 and D14)
- 100-500 pg: Mini 2 + Max 4 (D5A, D15, D16)
  - 70% 3 or more loc
  - 20% 1 loc or less
- >500 pg: all 8 locs
  - 70% 5 loc or more
  - 20% 4 loc or more

### The Down Side of MiniSTRs

- MiniSTRs were developed to access degraded DNA.
- They do not solve the inherent low copy limitation of the PCR.
- Instead, because of their sensitivity, they complicate it.
- Better amplification means laboratories can access DNA without pushing cycles.
- At low copy a scientist cannot express a strong opinion about how long a sample has been present.

### Minis show the same effect

In spite of the improved sensitivity, peak balance is poor at low template concentration.

### This amplification needs MiniSTRs

- These do not:
  - Data shows no evidence of degradation
  - Show evidence of degradation
  - Show no evidence of degradation

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**Conclusions**

- MiniSTRs are for degraded DNA
- Validation data reveals a robust and sensitive multiplex amplification
- Virtual yield gel using qPCR helpful for proper analytical results
- Stochastic effects still occur for samples under 10 pg
- Improved results are possible for bone and telogen hair
- Degradation is still a problem

**QPCR and Inhibition**

- Inhibition Mechanisms
- Effects of inhibition on STR profiles
- A real time assay for inhibitors
- The effect of inhibition on Quantifier and duo
- Flexor and inhibition

**Inhibition and degradation**

Which way to turn?
The Problem of Degradation vs Inhibition in DNA typing

The Issue:

- With increasing interest in the forensic community in the interpretation of compromised samples and mixtures, we need to be able to better interpret electropherogram profiles.
- We need to determine the relative effects of DNA degradation and inhibition on peak height ratios.
- We need to understand the combinatorial effects of different inhibitors.
- We need to understand the environmental effects of degradation and soil inhibition.
- We need to analyze the interpretation of DNA mixtures in the presence of a major contributor.

PCR Inhibitors

- Different co-extracted in forensic samples that affect amplification of template DNA.
- Theories:
  - Inhibitors are within the polymerase
  - Inhibitors interact within polymerase by binding to DNA
  - Polymerase is affected during primer extension

Effects of Increasing Humic Acid

- Different levels of PCR master volume, 100ng DNA

MiniSTR Amplification w/ increasing Humic Acid

- Inhibitor Concentration (ng/10µL)
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**MiniSTR Amplification w/ Increasing calcium**

![Graph showing MiniSTR amplification with increasing calcium concentration.]

**The Problem with Mixtures interpretation in the face of set PHRs, inhibition and degraded DNA**

![Diagram illustrating the problem with mixture interpretation.]

**Results – Inhibition Thresholds**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Miniplex 1</th>
<th>Miniplex 2</th>
<th>Miniplex 3</th>
<th>Miniplex 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematin</td>
<td>1 µM</td>
<td>0.8 µM</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>Indigo</td>
<td>320 µM</td>
<td>320 µM</td>
<td>300 µM</td>
<td></td>
</tr>
<tr>
<td>Melanin</td>
<td>0.16 ng/µL</td>
<td>0.1 ng/µL</td>
<td>0.2 ng/µL</td>
<td></td>
</tr>
<tr>
<td>Humic Acid</td>
<td>0.5 ng/µL</td>
<td>0.6 ng/µL</td>
<td>0.6 ng/µL</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>24 ng/µL</td>
<td>32 ng/µL</td>
<td>24 ng/µL</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>1100 µM</td>
<td>1100 µM</td>
<td>800 µM</td>
<td></td>
</tr>
</tbody>
</table>

**PCR Inhibition: Observations**

- Inhibitors act in many ways. The most important is those which contact with DNA.
- These inhibitors produce spurious effects on data, including peak balance problems, false positive detections, enhanced stutter, and poor sensitivity.
- Mechanisms appear to vary with type of inhibitor and sequence information.
- It is important to understand concentration effects and mechanisms so that inhibition cannot be confused with degradation, dropout, and mixture effects.

**Effect of Inhibitors on qPCR**

![Graph showing effect of inhibitors on qPCR.]

**Tests for PCR inhibition using Realtime PCR with high resolution melt**

- Compare inhibition for a single locus (TH01) with primers of various lengths and melting temperatures.
- Determine the effect of length and sequence on PCR inhibition.
- Examine melt temperatures of amplicons in inhibited samples.
- Classify inhibitors by effect on PCR.
- Determine mechanism of different PCR inhibitors.
Experimental Design

- Strep - HUMNTHL STR
- DNA - Heterogeneous 0.37 kb
- Primers
  - 3 primers (100, 200, 300 bp)
  - 5' + 3' primer (5'C
- Amplified product (one product)
- gCt conditions
  - Standard conditions for genotyping (Wickes et al. 2001)
  - No RQ
  - Lower Primer Concentration
  - Reduced Taq
  - Amplification conditions appropriate for Tm (5°C Tm)

Results - Calcium

- No shift in tail cycle
- No change in melting curve
- Efficiency of amplification affected
- No difference for size or Tm

Conclusion: Taq inhibition

Results - Humic Acid

- Effects Ct
- No efficiency change
- Melt curve effects

Conclusion: Inhibits by binding to DNA template

Results - Melanin

- Effects Ct
- Weak effect on efficiency
- Melt curve effects

Conclusion: Inhibits by binding to DNA template, may affect Taq

Results - Hematin

- Strong Ct shift some effects on efficiency
- Same loss of product
- Evidence of binding DNA esp. at high Cts

Conclusion: Inhibitor binding to DNA, may inhibit Taq

Results - Tannic Acid

- Increase in tail off cycle
- No change in efficiency
- Minimal effect on melt curve

Conclusion: Binding effects only at highest concentration, weaker than Humic acid or Melanin
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**Results - Collagen**

- Strange loss in signal with increased inhibitor
- Minimal effect on takeoff cycle
- Evidence of binding DNA and efficiency changes

**Size Effects**

- Inhibition tests examined both concentration and amplicon size of THO, target, for site.
- Hematin showed efficiency changes for short amplicons and CT effects for larger amplicons.
- Melatonin shows CT effects and melt curve effects for larger amplicons.
- Results indicate CT effects are reduced for smaller amplicons for these inhibitors. This indicates a potential advantage for these types of inhibitors with MinSTRs.

**Effect of amplicon length on inhibition** (increasing cos. X: Y)

- Larger amplicons show more inhibitory binding effects.
- Small amplicons show changes in efficiency.

**Important point for these results**

- Just as MinSTRs may be useful in amplifying DNA that is affected by inhibitors, short amplicons may be unable to pick up inhibitors that only affect large STRs.
- Inhibitors that bind Taq will always be detected regardless of amplicon size.

**Transition metal ions**

Metal cations present in degraded samples represent a different type of inhibition

- Zn²⁺, Co²⁺, and Ni²⁺ form DNA-metal ion complexes, termed DNA at pH conditions above 8.
- These cations produce severe effects in CE injection and analysis.

Hennik and McCona, Electrophoresis, 2006
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**Effect of contaminant in reference sample**

- Data problems in subsequent analyses
- Effect is transitory

**Types of Inhibitors**

- Length dependent
  - Largest amplicon most affected by inhibition
  - Collagen
  - Alkaline proteinases possible due to chromatin
  - Possible binding to DNA
- Two-Sequence dependent inhibition
  - Humic Acid - 50% less effective
  - Tannic Acid - 30% less effective
- Change in melting/DNA binding
  - Humic acid, collagen
  - Longer amplicons with melanin and ceramide

**Types of inhibitors 2**

- Taq inhibition
  - Calcium
- Length dependent/ primer extension
  - DNA/RNA polymerase is affected by inhibitors
- Humatin
- Melatin
- Tannic Acid
- DNA modification
  - Modification DNA transcriptional activity (Dr. C.)
  - Certain types

**Conclusions on Inhibitors**

- Preliminary studies demonstrate that inhibition and degradation may produce different patterns of allele dropout
- Inhibition can
  - Reduce Taq activity (Taq activity decrease)
  - Reduce primer concentration (decreased primer effect)
  - Affect specificity/od/niche (insert effect)
  - Affect the possibility to amplicon size effect
  - Interacts DNA (effect CC insertion)

**Clean-Up of PCR Inhibitors**

- Bovine serum albumin (BSA)
  - Protects PCR inhibition by making enzymes more efficient and controlling certain inhibitory compounds
- Low melting temperature agarose/aphenol/filtration
  - Removes PCR inhibitors by capturing large polymers like DNA, releasing smaller inhibitory compounds
- Electrophoretic Purification
  - Inhibits enzymes at different factors under applied fields
- Addition of higher concentrations of Taq polymerase
  - Decreases inhibitors that bind to Taq
- Dilution of Sample
  - DNA/RNA mixture: inhibitors are less concentrated and bind to Taq
- Destruction of inhibitors with NaOH

**Improved Workflow**

1. **DNA Digest**
2. **Extraction**
3. **Amplification**
4. **Analysis**

All Product Literature
Utilizing Quantifier duo as an aid to evidence processing

- Duo gives the quantity of human autosomal DNA permitting proper dilution of sample prior to amplification
- Duo permits the quantification of Y DNA, and reveals its presence
- The internal control sequence permits the determination of the presence of certain inhibitors
- The ratio of male to autosomal DNA permits the determination of the presence of a M/F mixture and the likelihood of success of mixture interpretation. It also indicates if a sample should be processed for Y STRs

### Table 1

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>OneCycler® NEEDED® Duo</th>
<th>No amplification</th>
<th>No amplification</th>
<th>No amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amplification</td>
<td>Amplification</td>
<td>Negative result - no human DNA detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No amplification</td>
<td>No amplification</td>
<td>Inhibit result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No amplification</td>
<td>Amplification</td>
<td>PCR inhibition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No amplification</td>
<td>No amplification</td>
<td>PCR inhibition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Positive amplification occurs when the C<sub>t</sub> value for the detection is <50 (becomes unsuppressible amplification event of DNA, above 60% C<sub>t</sub> value acceptable). The NP&CR reaction sample DNA is added to the reactions at a final concentration. Therefore, the NP<sub>CR</sub> C<sub>t</sub> should range between 29 and 31, with a variation of 0.1 C<sub>t</sub> across the standard curve samples.

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### Hematin Inhibited Samples

**Correlation with Identifier® profiles**

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>C&lt;sub&gt;t&lt;/sub&gt; Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
</tr>
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<td>5</td>
<td>85</td>
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<td>6</td>
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</tr>
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</tr>
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<td>8</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>110</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
</tr>
</tbody>
</table>

**Effect of increasing concentrations of hematin acid on the amplification of Identifier long template DNA**

**Conclusions**

- MiniSTRs are for degraded DNA. They work especially well for hair and bone.
- Similarly QPCR amplifiers are sensitive to degradation.
- Realtime PCR combined with DNA melt curve analysis can be used to detect PCR inhibition.
- Some inhibitors are amplification size sensitive. This may cause trouble in detecting inhibition using QPCR internal positive controls.
QPCR and Low copy template

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FIU Department of Chemistry
International Forensic Research Institute
Miami, FL  mccordb@fiu.edu  305-348-7543

QPCR and LCN DNA

- Introduction to LCN
- Drop out and drop in
- qPCR assays for LCN detection
- Sensitivity issues

Spinal Tap Video

- The problem of instrument sensitivity
- Exists everywhere and is fundamental to the concept of signal to noise

Amounts of DNA Required

<table>
<thead>
<tr>
<th>Amount Required</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ng – 1000 ng</td>
<td>RFLP/VNTRs</td>
</tr>
<tr>
<td>0.5 – 2 ng</td>
<td>PCR/STRs</td>
</tr>
<tr>
<td>&lt;0.1 ng</td>
<td>LCN/STRs</td>
</tr>
</tbody>
</table>

LCN extends the range of samples that may be attempted with DNA testing

Setting thresholds for the ABI 310/31000

- Where do current ideas on instrument thresholds for the ABI 310/3100 come from?
- How do I set these values in my laboratory?
- Why might they vary from one instrument to the next?
- How do these thresholds affect data interpretation?
- How to deal with issues in a way to ensure a conservative result?
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**Stochastic Statistical Sampling**

Copies of allele:
- >20 copies per allele
- 6 copies per allele (LCN)

Possible contaminator:
- Resulting allelic frequency
- OK
- Fail

**Comparison of STR Kit Amplification SOP with ICN using the Same DNA Source**

**MinSTRs show the same effect**

In spite of the improved sensitivity, peak balance is poor at low template concentration.

**This sample needs MinSTR:**

**The ones that do not:**

**Interesting effects with low copy data**

<table>
<thead>
<tr>
<th>Table 1: Result of analysis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of single units analyzed</td>
<td>226</td>
</tr>
<tr>
<td>Number of samples</td>
<td>226 (99.5%)</td>
</tr>
<tr>
<td>Number of samples with failed STR profile</td>
<td>114 (50.9%)</td>
</tr>
<tr>
<td>Number of samples with acceptable profile (monomorphic, +1 peak)</td>
<td>112 (49.1%)</td>
</tr>
<tr>
<td>Number of samples with fewer profile peaks</td>
<td>82 (22.7%)</td>
</tr>
<tr>
<td>Number of samples with more profile peaks</td>
<td>82 (22.7%)</td>
</tr>
<tr>
<td>Number of samples with zero allele drop</td>
<td>82 (22.7%)</td>
</tr>
<tr>
<td>Number of samples with more than one allele drop</td>
<td>82 (22.7%)</td>
</tr>
</tbody>
</table>

*Additional description or comments:

1. Additional data may be low copy.
2. Data may be low copy if not all peaks are observed.
3. Data may be low copy for samples with more than one allele drop.

**Another problem is stutter**

- Stutter increases at low copy

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Next determine the dynamic range

1. Perform a series of amplifications of 5 different samples with 5.00, 2.00, 1.00, 0.50, 0.25, 0.10, 0.06, 0.05 ng DNA.
2. Use your laboratory quantification system, your thermal cycler, and your PCR.
3. Determine the average and standard deviation of each set of samples.
4. Your dynamic range is the range of concentrations that are not overloaded. Overload point is where peaks fall too flat (this can be checked by ex

Limit of Linearity (LOL)

- Point of saturation for an instrument detection: at least higher amounts of analyte do not produce a linear response in signal.
- In ABI 130 or ABI3100 detectors, the CCD camera saturates leading to non-reproducible peaks.

So how to set thresholds?

- First determine the analytical threshold for your particular laboratory using the signal intensity from one or several CE systems.

Analysis threshold for this instrument is approx. 30 RFUs.
LOD is approximately 10 RFUs.
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### Sensitivity of Detection

- Different ABI instruments have different sensitivities; determination of analytical threshold should be performed following instrument calibration.
- Variations in quantitation systems.
- Variations in amplification efficiency.
- Variations in sample preparation.
- Peaks with height below the threshold should be interpreted with caution.
- Caution should be used when interpreting results.
- Amplification cycling.
- Electrolytic conditions.
- Peak heights are also a function of sample condition / PCR, extraction, inhibition, degradation.

### Sensitivity Study: Profiler Plus

- **Observation:** Peak height variation increases with concentration.
- Therefore, it is difficult to assess the quantity of DNA sample by peak height.

### Next set the stochastic threshold

- **Stochastic threshold:** The signal intensity at which a particular quantity of DNA can no longer reliably be detected.
- Reliability can be defined by an increase in the standard deviation of peak height intensity or an increase in the standard deviation of signal intensity or both.
- The stochastic threshold should be greater than the limit of quantification.

### Sensitivity Study

- **Debbie Nelson-PB**

#### 25 Individuals
- 55 pg to 1 ng amplifications with Profiler Plus and Cofiler.
- Amplification run on five 3130s.
- GeneScan Analysis threshold sufficient to capture all data.
- GenoType: category and peak height.

#### Import data into Excel
- Peak height ratios determined for heterozygous data at each locus.

### Heterozygote Peak Height Ratios

- **Observation:** Peak height ratio variation inversely proportional to input DNA.
**Fuzzy Logic in Data Interpretation**

- The ADI-2020 is a dynamic system.
- Sensitivity varies with:
  - Assay kit
  - Injection volume
  - Input DNA
  - Instrumentation
  - Presence of PCR inhibitors
- The interpretation must be conservative and data from these studies yields guidelines, not rules. These guidelines must be based on in-house validation. In addition, the interpretation and its significance cannot be dissociated from the overall fact of the case.

---

**qPCR Assays Are Also Impacted by Stochastic Sampling in the LCN Region**

Note the larger spread in these dilution series using for the LCN samples (16 pg) because of stochastic sampling.

---

**TECHNICAL NOTE**

**STR Profiles from DNA Samples with “Undetectable” or Low Quantifier™ Results:**

---

**Difference in DNA Quantitation Capability vs. STR Typing Sensitivity**

- Graph showing quantification limits and sensitivity.
- Note: You cannot do PCR if the low level of DNA QLoD was 2.5pg.
- Summary: qPCR is a better way to assess quantity but be careful.
- Sample quantity outside the calibration range. The number has no meaning.
Conclusions

- Be conservative in interpretation
  - Set thresholds based on signal to noise and stochastic amplification (2 thresholds). Base these numbers on controlled in-house experiments.
  - Understand that different instruments may vary in sensitivity – set thresholds high enough to encompass this variation.
  - Understand that even with such guidelines issues such as degradation and inhibition can skew results.
- Leave room for the facts of the sample in your interpretation.

Challenge with Being Able to Go Lower In DNA Quantitation Measurements

- Multi-copy marker (e.g., Alu asain) will be better than a single copy (e.g., Quantifiler) with qPCR of low quantity DNA samples.
- qPCR enables measurement of lower amounts of DNA but...
  - Going into the low copy number realm introduces new challenges:
    - Interpretation of mixtures
    - Defining thresholds for different assays and amplification systems
    - Defining the difference between investigative data and reliable "court-worthy" data.

qPCR for DNA Quantitation

- Will it lead more labs into LCN?
  - or
- Are we already there and about to find out?

When properly used real time PCR can provide clearer information on absolute quantities of DNA.

Why?

- Most laboratories will use amplification results to provide information on DNA thresholds.
- But: The reason qPCR was developed is that using endpoint detection to determine quantity is notoriously imprecise.
- Depending on amplification conditions, it is possible to produce full profiles from a single cell - 8pg.
- So wouldn’t it be better to use qPCR to do so?

What else can go wrong?

- Most validation studies are performed on pristine samples derived from clean sources.
- DNA degradation will result in dropped alleles from larger sized amplicons.
- DNA inhibition will result in dropped alleles from any location and the effects are difficult to predict.
- Inhibition and degradation can produce stochastic effects – peak balance issues and allele dropout.

Fuzzy Logic in Data Interpretation

- The ABI 310/3100 is a dynamic system.
- Sensitivity varies with:
  - Allele size
  - Injection solvent
  - Input DNA
  - Instrument temperature
  - Presence of PCR inhibitors
  - Ge matrix
- Thus, interpretation must be conservative and data from these studies yield guidelines, not rules. These guidelines must be based on in-house validation. In addition the interpretation and its significance cannot be dissected from the overall facts of the case.
**Instrument factors**

1. Because only signal is measured (RT-qPCR) in format DNA analysis, many labs find that one instrument or another is more sensitive.
2. There are also differences in sensitivity based on reaction parameters, capillary exposure (single vs multiply) and laser intensity.
3. Lastly the variation in qPCR sensitivity affects the output of any system.
4. These differences should be corrected by proper setting of threshold parameters and/or adjustment of qPCR results.

**Additional issues**

- Threshold (Abs)
  - 310: 50 RFU/m
  - 3100: 30 RFU/m

- Stochastic
  - 310: 102 RFU/m
  - 3100: 30 RFU/m

- Dynamic Range
  - 310: 450
  - 3100: 3000

**Bottom line**

- Validate each class of instrument and expect differences in sensitivity/signal to noise.
- Compensate for differences by choosing appropriate thresholds.
- Validate at 2 or more injections levels to stop injection time can be increased, remembering that longer injections risk shifting into LQ range.

**Issues with Data below the Stochastic Threshold**

- PCR artifacts and stutter become prevalent
- Low levels of bleed through are possible
- Instrument noise is more prominent
- No peaks may appear
- Dyna plots become more significant in overall graphics
- Low level 2nd contributors may show peaks

**So why examine low level data at all?**

- Detection of straddle data in which one allele is above threshold and the other is below
- Detection of the presence of low level mixtures
- Close to the presence of inhibited samples or poor injections
- Aids in determination if a suspect is excluded as a contributor

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Meatloaf Principle

- You see an allele twice in 3 runs
- What if the 4th measurement shows no allele?
- Is seeing an allele 50% of the time a measure of reliability? Is 66% ok?

Typical LCN Procedure

- Extract DNA from blood
- Create amount of DNA Present
- Perform a separate PCR Amplifications
- Develop consensus profile (based on repeat consistent results)

Catch 22

- Note the Catch 22. Are two amplifications of 50 pg better than 1 of 100 pg?
- Are 3 amplifications of 17 pg better than one of 50?
- Data shows that the lower the amount of DNA amplified the more likely allele dropout and false alleles occur.
- This somewhat calls into question the idea that a sample should be split and run multiple times.

Problems with Obtaining Correct Allele Calls at Low DNA Levels

- Titers (e.g., 30 -> 12.5)
- Problems (e.g., 15, 10, 5, 2.5)
- Percent Typing

Replicate LCN Test Results from FSS

<table>
<thead>
<tr>
<th>Sample</th>
<th>0.001</th>
<th>0.002</th>
<th>0.005</th>
<th>0.01</th>
<th>0.02</th>
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<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample E</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Low Copy Number Limitations (cont):

- Tissue source cannot be determined
- DNA may not be relevant - casual contact/transfer
- If victim and suspect have any common access...
- Old cases may not be viable - handling
- Not for post conviction analysis


It was to designate that allele dropout of a known allele cannot be discontinued when only a single allele is observed (DCS 2013 2e)

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A Precautionary Tale

What does this mean?

What to look for in the CPCR results:

Examine these results YFiler. Are these true alleles?

The Data

- Nuclear Results
  - Quantifier
    - Data omitted below calibration
    - Failed
    - No result
  - PCR
    - One peak
    - Poor PCR concentration—2 alleles

The report

- No nuclear profile due to low incidence or excessive degradation
- Suspect is excluded based on results for 3 of 15 YF primer
- Yes!!!
- My comments:
  - 1. The result clearly a low copy
  - 2. The pattern of alleles is not consistent, with degradation as the cause of failure.
  - 3. All the copies in evidence contain a strong peak about low DNA counts, but this is where there is concern. The DNA could not easily come from the air, so it could come from the suspect.
- Bottom line: Why was this sample even run?
The bottom line:

1. Low signal levels are bad because:
   a. They may indicate low copy DNA = incorrect or misleading results – if you can’t determine when the DNA was placed
   b. They often coincide with peak imbalance
   c. PCR and instrumental artifacts appear at these levels

2. Relying on signal level to determine DNA quantity can be misleading:
   a. There is wide variation in signal strength of amplified DNA, likely the worst occurs at higher levels
   b. Inhibitors and mixtures complicate interpretation
   1. peak imbalance can occur even in single source samples due to inhibition and degradation
   2. Instruments can vary in sensitivity

May 12, 2006: DNA Security Report

Other Issues – Real Time PCR

1. Real Time PCR follows the same rules as LCQ DNA. Low levels of DNA will suffer from stochastic fluctuations, reducing precision, both for the sample and the calibration curve
   2. PCR’s exponential DNA suffers more from LCQ as there is exactly half as much DNA present in each cell
   3. Fluorescent real time PCR will provide lower detection thresholds

Conclusions

- Be conservative in interpretation
  - Set thresholds based on signal to noise and stochastic amplification (2 thresholds). Base these numbers on controlled in-house experiments
  - Understand that different instruments may vary in sensitivity – set thresholds high enough to encompass this variation
  - Understand that even with such guidelines issues such as degradation and inhibition can skew results
- Leave room for the facts of the sample in your interpretation
What if there is a mixture with a large amount of female DNA and a small amount of male DNA?

- Differential extractions may not work if
  - Large differences in DNA quantity between male and female exist in rape cases
  - Vaccinated males
  - Saliva samples
  - Such samples cry out for a male specific marker

Role of Y-STRs (and mtDNA) Compared to Autosomal STRs

- Autosomal STRs provide a higher power of discrimination and are the preferred method whenever possible
- Due to capabilities for male-specific amplification, Y-chromosome STRs (Y-STRs) can be useful in extreme female male mixtures (bite marks, touch samples, etc.)
- Y STR typing can be very sensitive as the kit is designed to detect mixtures of DNA
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**Important Questions Can Be Answered with Y Chromosome Tests...**

- How can we use the Y-STR to analyze paternity issues?
- What is the significance of the Y chromosome in forensic science?

---

**Y Chromosome STRs**

**Advantages:**
1. Specific to males (inherited solely through the male line).
2. Low recombination rate.
3. No maternal input.
4. High genetic diversity.
5. No mtDNA is needed for identification.

**Disadvantages:**
- Limited sampling.
- False positives can occur.

---

**When will Y testing be used?**

1. When regular STR testing fails.
3. When a mixed sample has been collected.
4. When fingerprint evidence is present.
5. When DNA from a bloodstain is available.

---

**Traits found on the Y-Chromosome**

- **Y chromosome:**
  - **Size:** 1.5 million base pairs.
  - **Location:** Between the centromere and the telomere.
  - **Function:** Contains genes that affect male fertility.

**Issues surrounding the Y chromosome**

- **Y chromosome deletion:**
  - **Fraction:** Approximately 1 in 500 males.
  - **Significance:** Can cause infertility.

---

**The Human Y Chromosome: An Evolutionary Markers of Age**

- **Markers:** STRs, SNPs.
- **Applications:** Forensic genetics, paternity testing, evolutionary biology.

---

**Analysis of Y-STR haplotypes and the genetic diversity of Y chromosomes.**
Classes of sequences in the Y chromosome

MSY Region: The euchromic region - 2 Mb segment of 15.44 Mb
- Chromosome
  - 8 Mb identical to Xp11.22. 2 coding genes on 2 portion of the short arm (Xp:11.22)
- Adenovirus
  - 5 Kbp distal to Yp11
- Three sequences reflect the ancient common origin of the two chromosomes and possible evidence of a transposon decay over time
- Amy locus
  - Sense and antisense sequences, some of which are palindromes. Manifested in male sperm
  - The similarity of these sequences caused difficulties in the human genome project
- Metacentric region - regions of tightly wound DNA, not expressed or sequenced

<table>
<thead>
<tr>
<th>PCR products</th>
<th>Core Y-STR Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>DYS385 a/d</td>
<td>STK Marker</td>
</tr>
<tr>
<td></td>
<td>Position (Mb)</td>
</tr>
<tr>
<td></td>
<td>Repeat motif</td>
</tr>
<tr>
<td></td>
<td>Allele range</td>
</tr>
<tr>
<td></td>
<td>Minimum size</td>
</tr>
<tr>
<td>DYS385 a/d</td>
<td>17</td>
</tr>
<tr>
<td>DYS385 a/d</td>
<td>19</td>
</tr>
<tr>
<td>DYS385 a/d</td>
<td>12.24</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>12.93</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>12.91</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>12.84</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>12.81</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>12.79</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>0.53</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>0.57</td>
</tr>
<tr>
<td>DYS385 b/d</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Promega PowerPlex® Y Allelic Ladders

U.S. Core Loci + DYS385

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Difficult Questions...

- Which database(s) should be used for Y-STR profile frequency estimate determination?
- Are any of the current forensic Y-STR databases truly adequate for reliable estimations of Y-STR haplotype frequencies?
  - Some individuals share identical Y-STR haplotypes due to recurrent mutations, not relatedness.
  - Is the database a random collection reflecting Y-STR haplotype frequencies of the population?
  - Is the Y-STR haplotype frequency relevant for the population of the suspect?

The Meaning of a Y-Chromosome Match

Conservative statement for a match report:

The Y-STR profile of the crime sample matches the Y-STR profile of the suspect (let x be number of loci examined). Therefore, we cannot exclude the suspect as being the donor of the crime sample. In addition, we cannot exclude all patrilineal related male relatives and an unknown number of unrelated males as being the donor of the crime sample.

Problems with Most Common Type (MCT)

Most common type in Europe occurs about 3% of the time. 330 out of 13,468 samples. Other types can be rare.

www.ystr.org
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**Using Y quantification in casework**

- Use it as a presumptive test for the presence of male DNA. Find and amplify the most probabilistic samples.
- Use it for an estimate of amplification success. If Y ratio is above 10%, then Autosomal STR analysis may provide a useful result.
- Use it to estimate the amount of DNA template to get a useful Y STR result.

---

**Improved Workflow**

![Improved Workflow Diagram]

---

**Y quantification using YprimA**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Male DNA (YprimA copy number)</th>
<th>Female DNA (YprimA copy number)</th>
<th>Male/Female DNA Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Reference</td>
<td>0.324</td>
<td>0.236</td>
<td>1.41</td>
</tr>
<tr>
<td>Eicophor Sample</td>
<td>0.328</td>
<td>0.507</td>
<td>0.65</td>
</tr>
<tr>
<td>Female Reference</td>
<td>0D</td>
<td>0.217</td>
<td>0.01</td>
</tr>
</tbody>
</table>

From: "YprimA: a novel, highly sensitive assay for quantifying male DNA using a novel, highly sensitive assay for quantifying male DNA."

---

**So the Big Question:**

**When to use Y? And how will QPCR help?**

**Modern QPCR:**

- Ct - Quantifier Duo and Aero
- Rotor-Gene Q: qPCR in combination with other sensitivity thresholds.
- viatro will vary.

1. What is the question? Is male DNA present? Is a mixture present at some level?
2. Type of sample: differential extraction, communication, single donor, touch single digital template preparation
3. No cDNA is the only exception. In actuality, no ratio can be compared to any chromosomal DNA ratio in the absence of a reference.
4. Viability and sensitivity YSTR typing in presence of reference? Would you not perform an autosomal STR profile?
5. Precision and accuracy of estimate. How much accuracy would you expect from the methodology? Would you expect more or less accuracy than when assessing mixed DNA samples?

---

**BASE-qPCR: sensitive and specific detection of Y-chromosome derived DNA**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Male DNA (Ct)</th>
<th>Female DNA (Ct)</th>
<th>Male/Female DNA Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Reference</td>
<td>12.3</td>
<td>31.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Eicophor Sample</td>
<td>10.2</td>
<td>29.1</td>
<td>0.35</td>
</tr>
<tr>
<td>Female Reference</td>
<td>15.0</td>
<td>30.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

From: "BASE-qPCR: a novel, highly sensitive assay for quantifying male DNA using a novel, highly sensitive assay for quantifying male DNA."

---

**Conclusion:**

Y primers can be used to quantify male DNA in forensic casework. The accuracy and precision of the method should be evaluated in future studies.
From the Duo validation paper and Manual

Calculation of Male to Female DNA Ratio

The Quantifiler® Duo kit provides the quantity of human and canine DNA in biological samples. From these results, one can calculate the ratio of male and female DNA using the following equation:

\[
\frac{\text{Male DNA}}{\text{Female DNA}} = \frac{\text{Male DNA} - \text{Human DNA}}{\text{Male DNA}}
\]

or:

\[
= \frac{\text{Male DNA} - \text{Canine DNA}}{\text{Male DNA}}
\]

These ratios are in the form of ng/μL. This ratio does not mean the extent of the mixture, which is useful for making the choice of STR analysis method: ancestral STR or Y STR.

Conclusions

- The Y Chromosome is a complex and interesting piece of DNA
- Y STR typing while not as valuable as autosomal can provide results and statistics based on the counting method
- Y DNA quantification can be used as a presumptive test for evidence screening
- to determine mixtures and if autosomal DNA will work
- to determine the quantity of male DNA for Y STR analysis

Acknowledgements

- Heather LaSalle
- Silvia Zoppis
- George Duncan
- Eric Buel
Appendix 10: RT-PCR Introductory Examination

NERFI: Introductory Real-Time Workshop

Name: ______________________________
Date: ______________________________

1. What is the purpose of the quantitation step?
   5 pts

2. What are the components of the Quantifier Kit?
   5 pts
   - Reaction Mix
   - Human DNA Standard
   - Human Primer Mix

3. Define C\text{\textsubscript{T}}?
   5 pts

4. What is the relationship between C\text{\textsubscript{T}} and the concentration of input DNA?
   5 pts

5. What can the IPC tell you?
   5 pts

6. In the real-time quantification, a sample shows no amplification curve for either the sample or IPC. What could this indicate? How would you proceed?
   5 pts
   - A. No DNA in sample - either re-extract more sample or call the analysis done.
   - B. Forgot to add IPC - re-quant with IPC added
   - C. Inhibited sample - dilute out inhibitors with water or TE and re-quant
   - D. DNA in sample is degraded - end analysis on sample

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7. In the real-time quantification, a sample shows no amplification curve for sample, but the IPC amplification curve is fine. What could this indicate? How would you proceed?
5 pts

A. Amplification failure – check 7500 and reagents, rerun
B. Sample is inhibited - dilute out inhibitors with water or TE and rerun
C. There is no human DNA in your sample - rerun quant or re-extract different part of sample.
D. Standards made incorrectly - remake standards and rerun.

8. The minor groove binder associated with the Quantifiler probe plays three roles, what are they?
5 pts

1. 9. What region of genomic DNA is amplified using the AB Quantifiler Human Real-time PCR Kit? Where is this region located? What size are the resulting amplicons?
5 pts
10. Draw a standard curve and answer the following questions. 10pts

- Label where the 50ug standard is located?
- And the 25pg standard?
- Describe the three criteria used to evaluate the standard curve:
  - Slope:
  - Y-intercept:
  - Correlation Coefficient:
- What values are \textit{actually} measured by the real-time instrument?
- How is the concentration determined for an unknown sample?
Appendix 11: RT-PCR Advanced Examination

Assessment Advanced issues in Real Time PCR Name____________________

1. Explain the issue of PCR efficiency and its effects on real time measurement.

2. Why are calibrations based on the log of the concentration of template?

3. Explain the difference between Plexor and Quantifier Duo

4. What is the effect of amplicon size on the ability to perform real time PCR

5. Explain two different applications for real time PCR melt curves
6. For what particular casework are Autosomal/Y ratios particularly useful.

7. Explain the issues of Y STR sensitivity. Why are Y STRs assumed to be more sensitive if Y based real time results are poorer?

8. Explain this statement: Inhibitors affect PCR melt curves, efficiency and Ct and final product concentration.

9. Why can't you quantitate DNA below its calibration curve?

10. Under what circumstances could a real time PCR result produce misleading results?
Appendix 12: RT-PCR Course Evaluations with Feedback

**UNIVERSITY AT ALBANY**

**State University of New York**

**REAL-TIME PCR THEORY & CHEMISTRIES**

Jan. 6, 7 & 8, 2010

**Attendee Course Evaluation**

Please fill out this evaluation form. Rate each of the aspects of this course by circling the appropriate number. Comment as appropriate.

<table>
<thead>
<tr>
<th>Course Overall</th>
<th>Poor</th>
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<th>2</th>
<th>3</th>
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<td>50%</td>
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**Speaker: Lucy Davis**

<table>
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<td>45%</td>
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**Speaker: Jamie Belrose**

<table>
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<td>70%</td>
<td>15%</td>
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**Quality of Audio-Visual**

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<tbody>
<tr>
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<td>19%</td>
<td>35%</td>
<td>4%</td>
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**Quality of Handouts**

<table>
<thead>
<tr>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
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<td>4%</td>
<td>35%</td>
<td>45%</td>
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</table>
Facilities

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<th>G</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Comments/Suggestions</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>19%</td>
<td>02%</td>
<td>19%</td>
<td></td>
<td></td>
<td>Thank you for coming to see us.</td>
</tr>
</tbody>
</table>

Please Comment On:

Did the material presented meet your professional expectations?
- On only – Not particularly -- I thought the material was covered in a fast too hurried or a pace to learn anything, followed by a test too difficult. Exam based on how the material was presented.
- Yes, all information was provided.
- Yes, exceeded. Great information.
- On only – Yes, I enjoyed McCord's lecture.
- Yes, I do.
- Yes, a refresh on the fundamentals of qPCR and this worked quite nicely.
- Yes, very helpful information.
- Yes, at time a bit over my head – but informative.

What topic or topics did you find most useful to you?
- The information on qPCR inhibitors.
- Melting curves, Primer vs. TaqDuo, Inhibition – everything really.
- The inhibition (IC50, etc.)
- The function of the RT-PCR machine for real-time and other possible uses of the system.
- Determining the level of RT-PCR results & downstream applications.
- Understanding why it is necessary.
- The levels of different protocols on the RT-PCR instrument.
- Troubleshooting, advances for future, current hot topics like low-octane.
- LNQ topics for different interpretations of DNA profiles, mixtures and how to approach samples that fall outside the dynamic range of detection.
- Diagnostic explanation.
- The theory of RT-PCR.
- It was interesting and applicable points in each of the talks.
- Real Time software.
- Although there wasn’t many – I enjoyed actual case examples to relate to the data and findings better.

Would you recommend this course to other scientists?
- Absolutely; I would attend again too.
- Yes.
- Depends on their level of education – first 2 days only good for those just entering the field. Day 3 good for everyone.
- Yes, good mix of overview and new topics.
- Yes, it is very up to date.

General comments:
- Lots of information in a small amount of time.
- Worth the trip from Albany!
- Thank you.
- I don’t think the tests need to be short answer. They should be short and multiple choice.
- Perhaps a little more time to finish the material. One of the talks jumped around a bit making it difficult to follow.
- McCord’s lecture could have been longer and the introductory materials shorter.
- Very informative.

Suggestions for future classes:
- More interpretation knowledge.
- Thank your audience.
- Maybe some time for troubleshooting and QA issues / casework.
- More information and chemistry.
Appendix 13: Leadership Assessment Announcement

Leadership Assessment: Developing the Next Generation of Leaders

Two-day Forensic Science Manager Workshops

March 18-19, 2010  April 1-2, 2010

Sponsors: Northeast Regional Forensic Institute (NERFI)
National Institute of Justice (NIJ), Grant Numbers: 2006-DD-BX-E031, 2006-DNBX-173

Instructors: Dr. Wendy S. Becker
Dr. Edward J. Pavur, Jr.

Location: Northeast Regional Forensic Institute, Albany, NY

Method: Group discussion, case study, brainstorming, role play, readings, review of literature, surveys, integration of ideas, next steps

The ASCLD-LAB accreditation requires leaders to respond to a multitude of management issues that arise in labs. But many labs have limited opportunities and resources to provide leader development and training.

The purpose of this session is to provide leaders with an overview of tools and guidance for assessing and developing employees for supervisory and management positions. The interactive session is targeted to lab directors, supervisors, technical leaders and QA/QC managers.

Session facilitators will draw upon "best practices" research in the assessment, development and retention of forensic lab personnel. Topics include talent acquisition and retention, and staff development. Specific examples will be drawn from the facilitators' research including a national survey of public sector labs, a case study of laboratory recruitment and retention, use of a forensic advisory panel for development of forensic intellectual capital, and examples developing human resource metrics in labs. The session will be highly interactive and focused on topics of interest to session participants.

For reservations, contact John Hicks: johnhicks08@aol.com
Space limited to 10 for each workshop.
Appendix 14: Leadership Assessment Curriculum

Leadership Assessment: Developing the Next Generation of Leaders
February 18-19, 2010
Sponsored by the NorthEast Regional Forensic Institute and NIJ

Dr. Wendy Becker and Dr. Ed Favur, Instructors

Agenda

Day 1  9:00 am – 5:00 pm
- Welcome – John Hicks, NERFI Director
- Introductions
- A Case Study of Forensic Scientist Turnover – Breakout and discussion
- Break
- Job Analysis: From Scientist to Supervisor – PP Presentation & discussion
- Lunch
- Challenges and Opportunities -- Discussion
- Managing Intellectual Capital – Breakout and discussion

Day 2   8:30 am – 3:30 pm
- NYSP FIC tour
- Leadership Skill Development – PP Presentations
- Lunch
- Leader Influencing Behaviors

Cited Works:


Department of Labor. http://online.onelcenter.org/
Appendix 15: Leadership Assessment Presentations

How Does the Supervisor or Manager of the Lab Differ from the Scientist?

Discussion Draft
Tables Derived in Part from the O*NET Database

Table 1. Forensic Analyst Activities

1. Documenting/Recording Information
2. Identifying Objects, Actions, and Events
3. Updating and Using Relevant Knowledge
4. Getting Information
5. Interacting With Computers
7. Communicating With Persons Outside the Organization
8. Interpreting the Meaning of Information for Others
9. Evaluating Information to Determine Compliance with Standards
10. Scheduling Work and Activities

Table 2. Forensic Lab Supervisor Activities

All of the activities for the analyst, plus:
11. Analyzing Data or Information: Principles, Patterns, and Facts
12. Communicating with Supervisors, Peers, or Subordinates: Receiving and Providing Information
13. Processing Information: Compiling, auditing, verifying
Leadership Assessment:
Developing the Next Generation of Lab Managers

Dr. Wendy S. Becker
Dr. Edward Favur
Northeast Regional Forensic Institute (NERFI)
February 18-19, 2010

Leadership

Is leadership an attribution or impression; or is it related to effectiveness, emotion, or morality?

Leadership means

helping your group be more successful.

Leadership in the broad view has two aspects:

- initiation of structure, or concern for production;
  and
- consideration, or concern for people.

Skill Practice

We will use Leadership Challenges we have faced to identify approaches to leadership problems that will be productive.

Skill Practice Methods

- Case studies
- Analysis
- Practice (role-plays)
- Integration of Principles
<table>
<thead>
<tr>
<th>What common challenges do leaders face in the crime lab?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Selecting new group members</td>
</tr>
<tr>
<td>• Evaluating performance; certifying competence</td>
</tr>
<tr>
<td>• Contributing to planning and review for activities, objectives, and budgets.</td>
</tr>
<tr>
<td>• Implementing unpopular decisions.</td>
</tr>
<tr>
<td>• Making decisions.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What common challenges do leaders face in the crime lab?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Listening to concerns; reacting to requests.</td>
</tr>
<tr>
<td>• Resolving conflicts.</td>
</tr>
<tr>
<td>• Helping the group meet objectives.</td>
</tr>
<tr>
<td>• Developing talent.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What common challenges do leaders face in the crime lab?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Balancing mentoring with lab caseload, backlogs, and efficiency.</td>
</tr>
<tr>
<td>• Coordinating lab work with the objectives of outside agencies.</td>
</tr>
</tbody>
</table>

| What other common challenges for leaders have we missed? |
Attitudes and Performance

Are they related?

Influences on Work Behavior

- Culture
- Behavior
- Values
- Attitudes

Attitudes and Performance

- Work attitudes have a reliable relation to job performance.
- Judge 2001, Psychological Bulletin analyzed 25 years of research, including 11,195 articles.
- Results showed that job satisfaction predicts individual performance.
- \( r = .18 \text{ raw}, .30 \text{ corrected}, n = 54,417. \)

Individual Employees

<table>
<thead>
<tr>
<th>General Work Attitudes</th>
<th>Job Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>satisfaction</td>
<td>efficiency</td>
</tr>
<tr>
<td>involvement</td>
<td>quality</td>
</tr>
<tr>
<td>commitment</td>
<td>productivity</td>
</tr>
<tr>
<td>engagement</td>
<td>loyalty</td>
</tr>
</tbody>
</table>

Engagement and Business Unit Performance

- Reviewed results from 7,939 business units and found engagement related to composite business unit performance.
- \( r = .22 \text{ raw}, .54 \text{ to } .63 \text{ corrected}. \)

Engagement and Business Unit Performance

  12: The Elements of Great Managing
- Used the Gallup archives, 1930s to 2006
- 10 million interviews from 124 countries, in 45 languages.
- Identified 23,910 business units with measures of engagement and business unit performance.
First, they found key differences in engagement.

- Engaged: These people look out for you.
- Not Engaged: These people coast.
- Actively Disengaged: These people get even.
- Over time, people become less engaged.

### Engagement and Tenure

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<thead>
<tr>
<th>Tenure: 0 years</th>
<th>Tenure: 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engaged</td>
<td>39%</td>
</tr>
<tr>
<td>Not engaged</td>
<td>49%</td>
</tr>
<tr>
<td>Actively</td>
<td>12%</td>
</tr>
<tr>
<td>disengaged</td>
<td>20%</td>
</tr>
</tbody>
</table>

Second, they found key differences in managers

- Effective managers get the work done with the people they have.
- They don’t try to change their personalities, but they capitalize on the skills that exist, not on what the managers wished their employees could do.

Effective Managers

- Effective managers focus on work elements that the manager can influence.
- For example, rather than trying to make Mary a more conscientious person, the manager recognizes Mary’s best work.

Third, they found some work conditions were related to employee engagement.
How does this apply to the forensic lab?

- Forensic Lab Managers might be able to influence the attitudes held by some of the employees in their labs.
- As a Manager, you can focus on a few high-impact areas for the Forensic Scientists who work for you.

For Example?

- Initiate structure
  - Quality
  - Progress
  - Self development
- Consideration
  - Recognition
  - Valuing opinions
  - Caring about employees as people

“My Coworkers are Committed to Doing Quality Work.”

Encourage Coworkers to be Committed to Doing Quality Work.

“We value the same things, and those things lead to quality results.”

What examples do you have?

What opportunities exist in your lab for improving attitudes?
Leadership Perspectives

Leadership means
helping your group
be more successful.

Leadership in the broad view
has two aspects:

- initiation of structure,
or concern for production:

  and

- consideration,
or concern for people.

Leadership and Results

Grow from Compliance to Quality to Innovation

Classic Leadership Viewpoints

- Henri Fayol
  — Administration performs five Functions.

- Mary Parker Follett
  — “Responsibility resides in the situation,
    not in people.”

- Frederick Taylor
  — Management is responsible for
    systematic operations.
This document is a research report submitted to the U.S. Department of Justice. This report has not been published by the Department. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

Most Influential
Three specialist groups ranked 71 key contributors to US Management. Specialist affiliations:
1. Business History and Economics,
2. Academy of Management, and
3. Management History.


Who contributed most to American business and management thought and practice in the past 200 years?
Number of first place votes:
51 Frederick Taylor
9 Chester Barnard
6 Thomas Edison
4 Henry Ford

Situational Leadership Theory
Tannenbaum and Schmidt (1973)
Hersey and Blanchard (1988)
A contingency theory that focuses on followers’ readiness; the more “capable” the followers (the more willing and able) the less the need for leader support and supervision.

General Leader Functions
Gary A. Riki (1987)
• Covariance among managers’ behaviors (factor analysis)
• Similar content/purpose of managers’ duties (judgment classification)
• Theory of managers’ functions (deductive analysis)

Political Behavior
Influencing or attempting to influence the allocation of rewards in an organization.

• Legitimate
• Illegitimate
Illegitimate Political Behavior

- Deliberate, ruthless manipulation
- Extreme pressure
- Games
- Debasement

Games are a repeated series of exchanges used by an actor on a target, in which the surface intentions differ from the underlying intentions. The exchanges seem plausible, but contain a hidden agenda. The actor may exert influence in order to manipulate, control, weaken, or retaliate against the target.

What Makes Some Kinds of Influence “Illegitimate”?

- Malevolence
- Ambition outweighs judgment
- “My own interests are more important than those of the organization.”

Power, Influence

- The ability to get someone to do something you want.
- Bases of Power:
  - Formal, Legitimate
  - Informal
  - Coercive, Reward
  - Expert
  - Referent (charisma, status)

Influence Tactics

1. Rational Persuasion
2. Pressure/Assertiveness
3. Upward Appeal
4. Exchange
5. Ingratiation
6. Coalition Building
7. Inspirational appeal
8. Consultation

Influence Tactics

Long-term Effectiveness in the US

- Higher:
  - Legitimacy
  - Rational persuasion
  - Inspirational appeals
  - Consultation
- Mid to Lower:
  - Exchange
  - Personal appeals
  - Ingratiation
  - Pressure
  - Coalitions
Loyalty and The Management Role

Managers owe a high degree of loyalty to the organization.

Faithless Servant Doctrine

"[a]n agent is held to uberrima fides in his dealings with his principal, and if he acts adversely to his employer in any part of the transaction, or omits to disclose any interest which would naturally influence his conduct in dealing with the subject of the employment, it amounts to such a fraud upon the principal, as to forfeit any right to compensation for services." Murray v. Beard, 102 N.Y. 505, 508 (1886).


The chief executive committed misconduct that included tax evasion, widespread sexual harassment of employees, and attempts to cover up the harassment.

Initial Case

A MA Superior Court jury on January 30, 2005 found Lars P.E. Bildman liable to his former employer, Astra USA, Inc. (Astra), for fraud, conversion, waste of corporate assets, breach of fiduciary duty, and sexual harassment of Astra employees, and awarded Astra damages in the aggregate amount of $1,040,812.

Result of Appeal

Supreme Judicial Court Boston, MA (SJC-10361) on October 5, 2009, granted Astra recovery of compensation it paid to Bildman during the period of his disloyalty — $5,599,097 in salary and $1,180,000 in bonuses.
Leadership

Decision Making

Vroom-Yetton-Jago
Normative Decision Model

Should my employees participate in this decision?

Participation

- Can increase acceptance of decisions
- Can uncover resistance
- Can improve quality of decisions
- Requires time and effort
- Can create conflict
- Can result in frustration
- Can be stressful

Decision Features 1-5

- Is technical quality important (QR)?
- Is employee commitment important (CR)?
- Do you have the information to make a high quality decision on your own (LI)?
- Is the problem well structured (ST)?
- If you made the decision alone, would employees accept it (CI)?

Decision Features 6-8

- Do employees share the organizational goals in solving the problem (GC)?
- Are employees likely to be in conflict about solutions (CO)?
- Do employees have enough information to make a high quality decision (SI)?
This document is a research report submitted to the U.S. Department of Justice. This report has not been published by the Department. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

<table>
<thead>
<tr>
<th>Decision Making Style</th>
<th>Description</th>
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<tbody>
<tr>
<td>Autocratic I (AI)</td>
<td>Leader solves the problem alone using information that is readily available to him/her.</td>
</tr>
<tr>
<td>Autocratic II (AII)</td>
<td>Leader obtains additional information from employees, then makes decision alone. Employees don’t supply or evaluate solutions, and may or may not be informed of the decision.</td>
</tr>
<tr>
<td>Consultative I (CI)</td>
<td>Leader shares problems with employees individually, and asks for information and evaluation. Employees do not meet as a group; leader makes decision alone. Decision may or may not reflect group influence.</td>
</tr>
<tr>
<td>Consultative II (CII)</td>
<td>Leader shares problems with employees as a group, but makes decision alone. Decision may or may not reflect group influence.</td>
</tr>
<tr>
<td>Group II (GII)</td>
<td>Leader meets with group to discuss situation. Leaders focus on direct decision, but does not decide. Group makes final decision. Leader accepts group decision.</td>
</tr>
</tbody>
</table>
Leadership Assessment: Developing the Next Generation of Lab Managers

Dr. Wendy S. Becker
Dr. Edward Parson
Northeast Regional Forensic Institute (NREF)
February 18-19, 2010

Purpose and Importance

- ASCLD-LAB accreditation requires leaders to respond to a multitude of management issues that arise in labs.
- Labs have limited opportunities and resources to provide leader development and training.

Purpose and Importance

- To identify management tools to help develop managers of technology-intensive jobs
- Participants: lab directors, supervisors, technical leaders, QA/QC managers
- "Best practices" research in the assessment, development and retention of forensic lab personnel. Topics include talent acquisition, retention and development

Agenda

- Thursday, Feb 18
- Friday, Feb 19
A Case Study of Forensic Scientist Turnover

Critical Human Resource Issues: Scientists Under Pressure
Managing Intellectual Capital
Strategic Human Resource Management

Case Study of Forensic Scientist Turnover

- Large Northeastern state forensic lab system
- Implemented new staffing model
- 53 forensic scientists hired year of study
- Looked at retention of new recruits

Selection System Phases

1000+ applicants
750 previous job experience/relevant degree
400 interviews
300 background check
150 drug, polygraph, integrity tests
53 hires

New 2-Tiered Staffing Model

- Scientists train apprentice-type program
- 38 of 53 positions filled by technicians
- Within one year, 16 new hires left organization

Proposed Savings: $14m
Turnover Costs: $850k (conservative)

- Reviewed exit interviews
- Re-contacted lost employees

Reasons That Forensic Scientists Leave

- Personal reasons (spouse, family issues)
- Salary
- Career advancement
- Pursue advanced degree
- Better facilities elsewhere

Lessons Learned

- Employee turnover is costly
- Realistic Job Previews (RJs) critical
- What are strategies for employee retention?
Skill Practice

Critical Human Resource Issues: Scientists Under Pressure

National Survey
Document basic staffing issues in public crime labs
Retention strategies
Lab capacity / level of outsourcing
Performance pressure on scientists / capacity

Method

- Web-based survey
- 286 American Society of Crime Laboratory Directors (ASCLD)
- All items grouped into sections: demographics, caseload, recruitment, turnover, retention, and performance issues
- Reminder email two weeks after initial request
- Follow-up phone interviews

Survey Results

- 55 responses (22%)
- Populations served range from 50,000 to 12 million
- Average size 5.4 labs
- Pay (non-supervisor scientists): $23,100-116,000, average 55.9k
- Average age of oldest case: 6 to 408 months, average 23 months

Survey Results...

<table>
<thead>
<tr>
<th>Question</th>
<th>Mean</th>
<th>SD</th>
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</thead>
<tbody>
<tr>
<td>Do you assess workload?</td>
<td>2.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Provide lower-level development opportunities?</td>
<td>2.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Recruitment to increase analysts and quality?</td>
<td>2.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Salary level?</td>
<td>2.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Average length of second opinion?</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Average number of cases before second opinion?</td>
<td>2.9</td>
<td>0.7</td>
</tr>
<tr>
<td>New analysts in a lab?</td>
<td>3.3</td>
<td>0.6</td>
</tr>
<tr>
<td>New analysts in a firm?</td>
<td>3.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Are analysts utilized?</td>
<td>2.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Are analysts utilized at different levels?</td>
<td>2.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Are analysts utilized for criminal investigations?</td>
<td>2.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Are analysts utilized for training?</td>
<td>2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Are analysts utilized for research?</td>
<td>2.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Pressure to Perform Increases With # Cases

Forensic scientists unprepared for complete cases is actually manner .32*
Forensic scientists unprepared for complete cases unexpectedly .30**
Forensic scientists unprepared for a particular result .23*

*p Correlation is significant at the 0.05 level (2-tailed)
**p Correlation is significant at the 0.01 level (2-tailed)
Lessons Learned

- First national survey to attempt to identify critical staffing issues faced in public forensic science labs
- Serious staff shortages
- Labs need additional scientists to meet our standard of one scientist per 30,000 population.
- Relationship between current staff capacity and amount of outsourcing cases to private labs.

Discussion

- Two major reasons for scientist turnover
  - Personal reasons and salary
  - This is cause for concern, as public labs typically have extensive recruitment phases; in larger organizations, recruitment and selection take as long as 12 months to cycle through.
  - Identified retention strategies
    - Hire people with a link to the industry
    - Provide flexible work hours
    - Human relations
    - Size projects in multiple disciplines
    - Opportunity to transfer to other labs

List of possible lab measures

- Create lab-based programs
- Implement computer systems
- Use forensic data, and establish an identifier for forensic data
- Establish quality control and validation of test results
- Establish policies, standard operating procedures, and protocols
- Implement protocols for the handling and storage of evidence
- Implement protocols for the handling and storage of evidence

Most Common Leadership Problems

- Public Sector

  - Cost cutting/staff reduction
  - Workforce increase and force reduction
  - Benefits and cost analysis
  - Training program evaluation
  - EEO compliance
  - Affirmative action diversity programs
  - Fire management resistance
  - Computer reporting programs
  - Competitive salaries in technical areas

  (Source: Bernardi, 2010)
Skill Practice

As Leaders We Are Not Always Successful

- Attempted leadership – Person A attempts to change person B’s behavior
- Successful leadership – Person B changes his/her behavior as a function of person A’s efforts
- Effective leadership – Person B changes behavior as a result of person A’s efforts; person B more satisfied, better rewarded and attains a goal of mutual importance to person A and B

(Source: Bass, 1969)

Think of the differences between a time …

- … when you attempted to lead a person or group
- … when you successfully changed the behavior of a person or group
- … when you effectively changed the behavior of a person or group

Leadership – More Complex than Managing or Supervising

- "Manager" and "Supervisor" are job titles
- Imply tasks or duties
- Describe "what" needs to be done
- Leadership refers to social-psychological aspect of the role of supervision/managers
- Describes "how" tasks/duties carried out
- Implies "change in behavior"
- Depends on "followers" executing plans

Most people can become effective leaders in the right circumstances

- Leadership is "more than" possession of specific traits
- Modern theories of leadership blend duties of managers with effective leadership

18 Broad Dimensions of Managerial Responsibility

Building, directing, motivating individuals
Training, coaching, developing subordinates
Delegating
Influencing/selling
Maintaining good working relationships
Coordinating subordinates and other resources to get the job done
Planning & organizing
Decision making and problem solving
Staffing

Communicating effectively and keeping others informed
Representing the organization to the public
Persisting to reach goals
Handling crises
Organizational commitment
Monitoring and controlling resources
Technical proficiency
Administration and paperwork
Collecting and interpreting data

(Source: Bommer & Goshen, 1993)
Leadership/Management Connection: Broad Dimensions

- Note that 6/18 dimensions related to leadership - Effectively influencing others
- Berman & Brush (1993) research based on wide range occupational settings: police departments, manufacturing, hospitals, universities, armed services, high-tech firms

Leadership/Management Connection: Skill Requirements

- Strategic:
  - High-level, visionary skills needed to see a systems perspective to understand complex issues, also with empathy on a cultural level
  - Skills, problem-solving, planning, identification of drivers for change, problem identification, scenario planning, objective evaluation
- Business:
  - Business-oriented skills related to specific functional areas
    - Management of material, personnel, financial resources, operations planning
- Interpersonal:
  - Interfacing with and influencing others
  - Skill in social development negotiation, coordination, negotiation, persuasion
- Cognitive:
  - Analysis of information
  - Skills, solving, processing, disseminating, analyzing, learning (teaching, observing, learning), writing, reading, comprehension, adapting, critical thinking

Leadership/Management Connection: Skill Requirements

- As one moves up the managerial ladder:
  - Cognitive and business skills become less important
  - Interpersonal and strategic skills become more important
  - Both business and strategic skills increase with experience

Managing Intellectual Capital

- If you can Measure it, you can Manage it
- Forensic Advisory Board

Recommendations

Employee Performance Measures

1. Cases/claims analyzed per scientist per project team/study laboratory
2. Ratio of total, state, and federal budget dollars to the National Integrated Database (NIDB) in the National Forensic Laboratory
3. Ratio of state, fiscal, and federal budget dollars to the National Forensic Laboratory
4. Ratio of state, state, and federal data sites in the Combined DNA Index System (CODIS)
5. Ratio of technical support personnel per capita of state service region
6. Total cost of analyses per state to support state service region (CDMS) cost
7. Total cost of analysts, for example, (CSLI cost)
8. Total cost of employees, for example, (CSLI cost)
9. Quality control measures, including:
   a. Number of cases
   b. Number of cases per year, per state
   c. Number of cases per year, per state
   d. Number of analyses per year, per state
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**Forensic Advisory Board**
- Simulates private sector board, creates surrogates for natural management
- Steve Kier (Goldman Sachs) moved managing directors onto boards; created joint venture with Harvard to give people board training, set up action experiments, job shadowing (e.g. Naultamy-Fairstead)
- Intellectual capital from experienced active and retired professionals in various disciplines & academics, regular audits using ASCUD/LAB clients
- Review meetings as follow-up, corrective action, regular phone calls, emails, creates new organizational culture of collaboration between staff and advisory group

**Implications/Future Research**
- Increased reliance on private labs raises several critical questions:
  - Trends toward outsourcing and privatization impact public trust
  - Will public sector retain and routine, redundant cases or will continued budgetary analytics bring about privatization or even the demise of public labs?
  - Should a different pay structure be implemented for DNA analysis that takes these issues into consideration?
  - Should a price be put on DNA analysis that can include or exclude a defendant or false convicted offender?
- We hope to continue dialogue with the forensic science community on these important issues.

**Effective Empowerment in Organizations**

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<th>Employees share stocks or company, increases organization's success</th>
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**Guidelines for Participative Leadership**

How to diagnose decision situations:
- Evaluate how important the decision is
- Identify people with relevant knowledge or expertise
- Evaluate likely cooperation by participants
- Evaluate likely acceptance without participation
- Evaluate whether it is feasible to hold a meeting
How to Encourage Participation

- Encourage people to express their concerns
- Describe a proposal as tentative
- Record ideas and suggestions
- Look for ways to build on ideas and suggestions
- Be tactful in expressing concerns about a suggestion
- Listen to dissenting views without getting defensive
- Try to utilize suggestions and deal with concerns
- Show appreciation for suggestions

What to Delegate

- Tasks that can be done better by a subordinate
- Tasks that are urgent but not high priority
- Tasks that are relevant to a subordinate's career
- Tasks of appropriate difficulty
- Both pleasant and unpleasant tasks
- Tasks that are not central to the manager's role

How to Delegate

- Specify responsibilities clearly
- Provide adequate authority and specify limits of discretion
- Specify reporting requirements
- Ensure subordinate acceptance of responsibility
- Inform others who need to know
- Monitor progress in appropriate ways
- Arrange for the subordinate to receive necessary information
- Provide support and assistance, but avoid reverse delegation
- Make mistakes a learning experience

General Guidelines for Empowering Managers

- Involve people in decisions that affect them
- Clarify goals and objectives and explain how the work is related
- Delegate responsibility and authority for important work activities
- Take into consideration individual differences in ability and motivation
- Provide access to relevant information
- Provide the resources needed for new work responsibilities
- Formalize management systems compatible with empowerment principles
- Remove bureaucratic controls and unnecessary controls
- Express confidence and trust in people
- Provide coaching and advice on a timely basis
- Encourage and support initiatives and problem solving
- Recognize important contributions and achievements
- Ensure that rewards are commensurate with new responsibilities
- Ensure accountability for the ethical use of power

How Leaders Enhance Organizational Learning

- Question traditional methods used for more effective methods
- Discourage innovating without gaining support for innovations
- Encourage subordinates to acquire new skills
- Help develop new models and modify what is learned
- Encourage subordinates to introduce new ideas
- Help people recognize their potential for learning new skills
- Link external support with major initiatives
- Encourage experimentation
- Encourage leaders to act as facilitators
- Encourage recognition when innovation is fallible & should be accepted
- Create decentralized accounts with authority


Influence Tactics

- Rational persuasion
- Exchange
- Inspirational appeal
- Legitimacy
- Appraising
- Pressure
- Collaboration
- Ingratiation
- Consultation
- Personal appeals
- Coalition

(Adapted from Full, 2006)
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References


Biological Anthropology.


Appendix 16: Leadership Assessment Course Evaluations with Feedback

Northeast Regional Forensic Institute (NERFI)
Funded by NIJ

Leadership Assessment: Developing the Next Generation of Leaders
A Two-Day Workshop for Forensic Managers
January 14-15, 2010

Participant Event Evaluation

Please fill out this program evaluation. Rate each of the aspects of this workshop by circling the appropriate number. Comment as appropriate.

9 Responses

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Please comment on:

Did the material presented meet your professional expectations?
Yes – Very helpful
Yes – great, I would highly recommend it to the other labs.
The material did, for the most part, meet my expectations.
Yes
Yes. I'll give me more options, to implement some of the ideas in my work place.
Yes. But I am sure the material will improve with further courses.
Yes. I'm walking away armed with some valuable management tools to use. I have optimism they will work.
Yes. The speakers, Dr. Wendy Becker and Dr. Ed Pavur were most effective and professional speakers. Enjoyed the interaction with the participants. Great experience.
Yes. Wish there was a little more time on 2nd day.

What topic or topics did you find most useful/interesting/novel?
All of it.
Leadership topics and solutions to the management issues/challenges brought forth by the participants.
Gary Yukl's theory
I can use these in my work.
The last role plays.
Role play
Visit to Albany lab was excellent! Thanks for re-arranging the workshop schedule to accommodate the tour.
Material on influencing others

General comments:
I truly enjoyed the training.
Friendly staff, great workshop.
This course could be very helpful to people aspiring to be a leader/manager instead of more experienced managers.
The tactics list should be provided & discussed after the role plays. Such discussion would be more useful.
Group discussion was very helpful.
I'm going to recommend this to my colleagues.
Thank you to Dr. Becker and Dr. Pavur. Thank you, John & Katharine. Thank you, NJU for the opportunity given. Is there a Part II? We'll come back.
Keep tour of crime lab included! Like group projects/interaction & to hear from each other, like smaller group size. Could use a management class each year as a refresher & for general discussion.

Suggestions for improvement:
Thanks
Turn up the heat!
Another day/24 hours addition to this workshop will work better.
Add 1 day. More on evaluating employee Performance.
Having more practice & leadership examples/situations with suggestions on the best way to handle certain situations.
Temperature of room: Prnt agenda. X-tra paper for notes or tell people to bring paper/pens

Thanks for your input!
Leadership Assessment: Developing the Next Generation of Leaders
A Two-Day Workshop for Forensic Managers
February 18-19, 2010

Participant Event Evaluation

Please fill out this program evaluation. Rate each of the aspects of this workshop by circling the appropriate number. Comment as appropriate. 10 Responses

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Please comment on:

Did the material presented meet your professional expectations?
Yes x 7
Absolutely. The workshop was way above my expectations.

What topic or topics did you find most useful/interesting/novel?
- Being able to network and discuss issues that other state, county, private labs face
- The tools for discussing situations on how to get what you need (rational persuasion, etc.) Influence tactics
- Having the chance to hear firsthand the issues of other managers and how they lead in their department was very educational/informative
- Types of ways to influence people
- Psychology of being a leader, role playing
- Interactions with other participants was very useful when discussing work issues
- Information on influence tactics
- Retention of employee/morale
- Influence tactics & role playing with the group

General comments:
It was very helpful that so many in the class were in a similar situation to me. Thank you!
Excellent source, would like to see similar offerings.
Great two day workshop.
Wendy [Becker] is an excellent active listener. The focus on the issues the attendees are personally facing makes the class more pragmatic but less instructional/theory/content based.
Loved the interaction with other people & learn how they deal w/ situations, as well as techniques used on their jobs.
Breakout sessions – great time to have one on one’s & share experiences.

Suggestions for improvement:
More time. Maybe add a day – many issues will be generated by the participants & more time given to problem solve these issues.
Less break-out sessions – more presentations
 Longer

Thanks for your input!
Appendix 17: First Responder Training for Schenectady Police Department

Schenectady Police Department
Crime Scene Analysis Evidence Collection & Packaging

Instructors: John Hicks, Lucy A. Davis & Jamie L. Belrose

Course Description
This course is designed to give police officers and other law enforcement professionals the basic knowledge of crime scene analysis and review of forensic evidence including biological fluids they need to keep up with today’s technological advances. Today’s advanced technology has led to an exponential number of cases being submitted to the crime lab. For this technique to be successful, it is imperative that the evidence be collected efficiently. Misssteps in this first stage can and most likely will, have deleterious effects on the laboratory analyses. By properly collecting and packaging evidence you can increase the probabilities of obtaining a successful forensic result.

Course Outline
8:00 AM   Welcoming and Introductions
8:15 AM   Crime Scenes & Crime Scene Evidence: Things to look for and what is most helpful to the crime lab.
9:15 AM   Break
9:30 AM   Crime Scenes & Crime Scene Evidence - continued
11:15 AM  Crime Scenes & Crime Scene Evidence - continued
12:00 AM  Lunch

References:

Crime Scene Investigation: A Reference for Law Enforcement June 2004, Technical Working Group on Crime Scene Investigation; NCI 200160
Appendix 18: First Responder Presentations

Evidence Preservation
- NERF, etc.

Sources of Biological Evidence
- Blood (no nuclear DNA in red)
- Semen (sperm and other cells)
- Saliva (from cheek & other cells in mouth)
- Bone (mostly in DNA)
- Hair (mostly in DNA)
- Skin (usually in form of trace DNA)

Collection: Personal Protective Equipment
- PPE must be used
  - Gloves
  - Face Mask
  - Hair cap
  - Booties
  - Disposable suit
  - Protective eyewear

Packaging
Proper ID → Proper Chain of Custody
- Items must be air-dried before they are packaged
- Place evidence at room temperature
- Use safe, leak-proof packaging (especially for sharps)
- Identify with unique numbers
- Date, sign, initial to maintain chain of custody
- Source of touch DNA (any object that has small amounts of DNA from regular touching) can be swabbed and dry sealed package

Importance of DNA Integrity
- Safety
  - Protect self from unknown biological fluids
  - Prevent
    - Contamination!
    - DNA technology cannot distinguish between DNA in sample and DNA from contamination
    - Destruction
    - Erase

Safety
- Protect yourself from biological evidence
- The source of your evidence may be unknown
- If you don’t know who the fluids come from, you don’t know whether the fluids contain diseases!
- Blood can contain hepatitis B, hepatitis C, and HIV

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What we’re talking about today

- Evidence
  - Collection
  - Crime scene
- Types of evidence
  - Biological
    - DNA
  - Trace
    - Hairs and Fibers

Forensic Science

- Public interest
  - Edgar Allen Poe, Sherlock Holmes
  - Quincy
  - And now CSI, etc.
- Intrigued by darker side of humanity
- Puzzles, clues, solutions

Role of the Forensic Laboratory

- To use science to support or deny the victim or suspect’s story
  - The laboratory needs to know the total history of what happened.
  - Number of suspects, previous sexual intercourse, exact times involved, time between assault and examination.

We’re all ‘Pig Pen’

- Like ‘Pig Pen’, we carry our micro-environments with us wherever we go.
  - When our environment comes into contact with another, transfers occur.
  - These transfers are indications of association.

Example of transfer

You own a dog. You pet the dog. You go to work.

You sit in your chair.

Transfer Example

Direct Source (A to B)

Indirect Source (A to B to C)
Crime Scenes
- Confirm what is involved
  - Always try to take a minimum of 2 people
- Inventory consumables in crime scene kit
  - Evidence bags, batteries, solutions, film
- Try to write the final report within 24 hours of completion of the scene

Planning
- Stop
- Look
- Listen

Do not go in without a plan. Take a methodical approach

Collection of evidence
- From body outward
  - Good for outdoor scenes
  - Take the search in grids or circle patterns
  - Let the evidence found lead you further
- Toward the body
  - Better for enclosed areas
  - Preferred if trace evidence is critical
  - Better if scene has been secured prior to you arrival

Photographs
- General to specific
- 90 degree angle
- Without scale
- Then with scale
- Remove lens cap and check roll advance
- Document what is on each roll

Drawings
- Best way to document scene
- Be specific with measurements
- Note directions
- Can be used with legends of collected exhibits
- Does not have to be high tech

TWGSCI Guidelines
- Report can be downloaded from the web: www.ncjrs.org
- Call 800-651-3420
Footwear
- Everyone wears them
- If they don’t, they’re leaving footprints
- HUGE variation in styles, designs, and types
- Quality often dependent on substrate
  - Material in which impression is made
  - We all walk differently—creates wear
- Outside materials wear differently—creates patterning
- What we walk on varies and damages sole uniquely

Soles vary radically

Finding footwear impressions at the crime scene
- Be aggressive in searching for them
  - Often overlooked or damaged
- Failure relates to
  - Thinking none will be found
  - Incomplete searches of scene
  - Searches after trampling
  - Shoe and surface combination not conducive to leaving impression
  - Intentionally destroyed by culprit
  - Environmental damage (rain, snow, etc)

Factors of wear on the sole
- Person’s foot type
- Occupation
- Habits
- Weight and body type
- Shoe construction
- Style
- Materials
- Surfaces walked upon

Left and right shoes don’t wear the same

Types of impressions
- Dry
  - When shoe track across a dirty surface, the bottom of the sole will accumulate a coating of residue. If they then track on to a relatively clean surface, that residue will be deposited in the form of footwear impressions
- Wet
  - Made when a shoe sole is wet or damp constitute another category of impression.
- Depression
  - The term ‘depressed mark’ is used to describe the evidence an impression of footwear leaves when making contact with a deformable surface, such as soil, sand and snow. It is synonymous with the term three-dimensional impression.
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**Collection—taking casts**

**Oblique lighting**

**Electrostatic lifting**
- Applies electric charge to plate
- Lifts dust/dirt/debris
- Retained through surface charge until treated
- Can be powered through stun gun

**Electrostatic lifting**
- Print lifted
- Document before

**Results of examination**
- Individualization
- Elimination
- Linking to crime, other scenes
- Location of impression
- Corroboration or rebuttal of alibis
- Classification, shoe size
- Number of perpetrators

**Class characteristics**
- Nike Air Dynamic Flight shoes
  - USA total all sizes 227,844
  - Size 13, Black 6,136
  - White 7,032
  - Total 15,168
- Distribution to Georgia
  - All sizes 403
  - Size 13 403

Can't use as statistics but good example
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### Tires

- Design
- Tread
- Rubber composition
- Tyre size
- Track width
- Wheelbase
- Turning diameter
- Retreaded/replacements

### Tire print examinations

- Tread design
  - Thousands of designs (~11,000)
  - Passenger vehicles, trucks, off-road, motorcycle, etc.
- Tire size
- Track width
- Wheelbase
- Turning diameter
- Retreaded/replacements

### Trace evidence - Hair and Fibers

- Can be easily lost or missed
- Get on your hands and knees and look
  - Document location each are collected
- After search is completed you may go back and do vacuum sweeping or tape lifts

### What can be determined from a hair?

- Human vs animal
- Race
- Body area
- Damage/Disease
- Comparison to a known sample

Not all tire tracks are left on pavement
What area of the body is it from?

- **Head**
- **Pubic**
- **Facial**
- **Limb**
- **Chest**
- **Axial (armpit)**

These carry the most information for microscopic comparisons.

What is the person’s ancestry?

- African ancestry
- Asian ancestry
- European ancestry

Estimation of ancestry based on hair characteristics may not correlate with genetic or self-identity.

Why Fibers Make Good Evidence

- everywhere (readily available)
- we’re in constant contact with them
- designed for a purpose (variation)
- fungible (life-span)
- fashionable (variation)
- easily tested (limited characteristics)
- nearly infinite combinations of traits
- especially color

Examples of fibers

- **Natural**
  - Wool, cotton, silk, linen
- **Manufactured**
  - Rayon, acetate, lyocell
- **Synthetic**
  - Nylon, polyester, kevlar
Fiber characteristics

- Cross-section
- Color
- Polymer type
- Optical properties
- Voids/inclusions
- Diameter
- Other properties (metamerism)

Perception/detection of color requires:

- Light source
- Object
- Observer (human or instrumental)

Simultaneous Contrast

- The eye generates contrast to heighten perceptual borders
  - Important evolutionarily
- Simultaneous contrast changes apparent lightness and chromatic color in a direction opposite to those of the inducing background
  - Complementary colors

Changes due to washing 50x

Significance

- Examples to think of...
  - Department store
  - Public transit
- What are the chances of two non-associated textiles matching?
Textile Labeling

- Manufacturer Identification
- Fiber name and % composition
- Care instructions
- Country of origin

What Is Soil?

- Mixture of organic and inorganic material
- May range from 100% inorganic (sand) to nearly 100% organic (peat)
- Inorganic part is minerals
- Organic part is decayed plant and animal material and is sometimes called humus
- Many labs put soil in a big generic class and say, "It's all the same."

Glass

- Tempered
  - Auto windows
  - Shower doors
- Non-tempered
  - Laminated glass
  - windowed
  - Architectural
  - architectural
- Headstones
- Containers

Forensic Significance

- 700 types of glass in common use today
- Can be individualized if fracture match can be made
- Tiny particles are class evidence
  - Analytically classified
    - Optical properties
    - Elemental content

Types of Analysis of Glass

- Physical Match
  - non-tempered
- Comparison
  - Physical properties
  - Chemical properties
  - Optical properties
- Impacts
  - Direction of blow
  - What caused the damage
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**Broken Windshield Fracture Match**

**Glass breakage**

Most of the glass is projected forward in the direction of the blow. Some of the fragments will be projected backwards, towards the person breaking the window. Up to a distance of 1 meter - known as blastback. Anyone standing close enough when the window breaks will be covered in tiny fragments of glass which will stick to their clothing and their hair.

**Introduction - Paint**

- Chemistry of paint very complex
- Forensic role is to compare paint evidence from scene to known source
- Individual evidence only in cases where large enough paint samples exist to physically match

**Paint Cross Section**

**Unknown Paint Chip**

**Known Paint Chip**

The physical layer structure of paint is important.

**Tape**

- May be source of fingerprints
- Hairs and fibers may adhere to the adhesive portion
- Chemical composition of tape adhesive and backing
What happens at a crime scene?

- **Identification of blood**
  - Documentation of scene and examination performed
  - Photograph everything
  - Use drawings, charts, everything
  - Evaluation of stains location
  - Use of alternate light sources or chemical enhancers
  - Very important in blood spatter interpretation
  - Field identification of blood
  - Presumptive tests

Collection of blood samples

- **Collect it all**
  - Call the laboratory if there is a question
  - Evaluate the location of the stain and presumptive value
  - If in doubt of method of collection, cut it up and bring in the entire object
  - Always use clean utensils when collecting anything

Collection of blood samples

- **Scrapping**
  - Use a piece of paper, then fold the paper
  - Do not scrape directly into an envelope
  - Be cautious of type of collected due to aerosols, and hazardous materials

- **Swabbing**
  - Keep the stain concentrated
  - Collect applications (Clots)
  - Doffer to collect it and not use then lose the sample

Collection of blood samples - Controls

- **Reagent controls**
  - Use distilled or sterile water
  - Bottle water will do - only if there is nothing else available
  - Do collect the sample of whatever you use

Collection of blood samples - Controls

- **Substrate controls**
  - Collected from area adjacent to stain

Packaging, Transportation, Shipping

- Air dry all samples
- Liquid blood standards
  - EDTA - purple top tube
- Store in paper bags
- Keep it cool
  - Frozen, refrigerator, air condition
- Transport to laboratory as soon as possible
- Documentation
Chemical Tests

- Testing of visible stains
  - Evaluation of results
    - These tests say that the stain is blood only - no species identification
    - A non-reaction does not mean that blood was not there

Chemical Tests

- Testing of invisible stains
  - Luminol
    - Reacts better the older the stains
    - Be cautious of false positives
  - Amido black
    - Permanently stains the object
  - Fluro-scene

- Evaluation of results
  - Presumptive tests - not definitive identification
  - Sensitive for small amounts of blood
  - Very good to lead you to larger stains

Luminol

Can you test blood after it has been fingerprinted?

- Chemical/substrate combinations
  - Super glue
    - Used on glass, paper, metal and plastic
  - Ninhydrin
    - Used only with paper
  - Super glue + Rhodamine
    - Used on glass, plastic and metal
  - Super glue + Rhodamine + black powder
    - Used on glass, plastic and metal

What is Blood

- Red Blood Cells - RBC
  - Approximately 4 million in a single drop of blood
  - Basic cell of analysis in conventional serological testing
  - Does not contain DNA

What is Blood

- White Blood Cells - WBC
  - Approximately 7000 in a single drop of blood
  - Does contain DNA - is the cell of that analysis
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Species Origin
- Is it animal or human?
  - Interesting point in special cases
- How much do I need
  - Conventional semenology
  - DNA analysis

Semen Identification
- Seminal Fluid
  - The liquid portion of semen
    - Chemical enzymatic test can be performed to identify this
    - This can be used to screen for the presence of semen
    - Can be visualized by and alternate light source
      - UV light source, Woods light

Semen Identification
- Spermatozoa
  - The solid part of semen
    - Can be used to differentiate between human and different animals
    - The portion of semen that contains DNA

Identification of Semen
- Primary indicator of sexual activity
- Begin with the vaginal swabs and work our way out
  - Vaginal swabs, external swabs, underpants, sheets, bedding, other
- With successful analysis of semen, limited further analysis will be performed

Different issues in cases

You never know what the story may be!
**Forensic Evidence Collection**
- Penile swabs
  - Can be collected if male is victim or suspect
- Is used to detect foreign DNA on penis
- Collect a minimum of 4 swabs

**Saliva**
- Fluid from oral cavity that is identified by the presence of the chemical amylase
  - Can be used to identify oral sodomy
  - Can be detected AT TIMES from swabbing of genital areas, breast area, bite marks, penile swabs
  - If it is not detected, it does not mean that something did not happen
  - Epithelial cells contained within this fluid could be used for DNA analysis - by PCR testing

**Saliva Collection**
- Bite marks or breast swab
- Wet one swab and swab area of mark
- Allow that swab to air dry
- Take a dry swab and swab original area on skin to collect left over solution on skin

**Saliva (Buccal) Swabs**
- Known Buccal Standard
  - Can be used as a known standard
- Only collect 2 swabs
- Swab the inside of the cheek area
- Allow to air dry

**Urine**
- Fluid - varies in concentration
  - Can be detected AT TIMES when available in concentrated amounts by chemical analysis
    - If it is not detected, it does not mean that it is not present
  - Only conventional serological analysis is by ABO typing if available in concentrated amounts
  - Epithelial cells contained within this fluid could be used for DNA analysis - by PCR testing

**Perspiration**
- Body fluid that cannot be chemically identified
  - Epithelial cells will be present that may be identified by DNA analysis
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Other Physiological Fluids
- Feces
  - If it looks like it, it smells like it, it possibly is.
  - Very little you can do to identify or type.
- Tissue
  - Can be identified microscopically.
  - Can be typed by conventional enzymatic testing.
  - Can be typed by DNA analysis.

DNA
- Deoxyribonucleic Acid
  - Fundamental building block of all living organisms.
  - Very stable chemical molecule.
  - No two people except for identical twins have the same type.

Egg
- 23 pieces of DNA (Chromosomes)

Sperm
- 23 pieces of DNA (Chromosomes)

Single Cell
- Contains 46 Chromosomes

The cell copies its DNA and divides into 2 identical cells
- Then repeats the process over and over.
Baby Rapist is born

The DNA in every cell of his body is identical to the DNA that started in that first sperm and egg combined.

Definitions

- DNA – Deoxyribonucleic Acid
- Locus – A single location (site) on the DNA chain
- Loci – Plural of locus
- D7S820 – A location on the DNA chain of Chromosome 7. It was the 820 site identified sequentially
- vWA – Another location on the DNA chain that is referred to by its common name, and not designation

Differential Extraction

- Vaginal swabs will contain both the female’s epithelial cells and male’s sperm cells
- Separates sperm DNA from the female epithelial DNA
- From one piece of evidence we now have two DNA samples

Different types of Evidence

- Condom thrown in trash, on floor, in toilet, in pocket

Match at Locus D5S819

Autorad of the of DNA profile at D5S818.
The male fraction DNA profile matches
the suspect's DNA profile at locus D5S818

The frequency of occurrence of that match is
1 person out of 25

What do those numbers mean?

\[10 \times 30 \times 19 \times 15 \times 18 \times 25 \times 12 \times 6 \times 10 \times 15 \times 20 \times 13 \times 27\]

equals

3,889,360,800,000,000

1 person in 3.8 Quadrillion

What do those numbers mean?

\[10 \times 30 \times 19 \times 15 \times 18 \times 25 \times 12 \times 8 \times 10 \times 15 \times 20 \times 13 \times 27\]

equals

3,889,360,800,000,000

6,000,000,000

(world population)

Best Samples to use for identifying unknown bodies or bloodstains

- Father of the missing person
- Mother of the missing person
- Brothers and sisters of the missing person
- Children of the person accompanied by a sample from the other parent of the children

RFLP - Restriction Fragment Length Polymorphism

- Used in the United States since 1987
- Accepted in every state's legal system
- The technique has been found to be very reliable and stable
- Only a couple of private labs can do
PCR
Polymerase Chain Reaction
- Make copies of the DNA until you have enough to analyze
- Based on the same procedures that your body uses when it copies the DNA so the cell can divide
- Applied to forensic cases in 1991

Biological specimens you can analyze with PCR:
- Very small blood stains
- Semen from vasectomized males
- Stamps and envelopes
- Cigarette butts
- Hairs with a root end
- You name it, it's worth a try

Types of analysis by PCR:
- DQAlpha - PolyMarker
  - DQA1-PM
- Short Tandem Repeats
  - STR's

DQAlpha - PolyMarker
Frequencies of occurrences will be between
1 person out of 1000
to
1 person out of 100,000
DQAlpha - PolyMarker

- Has been fully validated
- Used on forensic cases since 1991
- Interpretation of mixed samples
- No laboratory can to this type of analysis any longer

DQAlpha - PolyMarker

- Interpretation of mixed samples
- This is where a lot of the overturned DNA cases are coming from

STR’s

- Easier to visualize mixed samples
- The standard DNA analysis used by all forensic laboratories
- Fully accepted in the courts
- Allows you to analyze up to 15 different genetic loci simultaneously

Techniques are rapidly advancing.

Report Formats - Match

<table>
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<th>Sample</th>
<th>Victims</th>
<th>Suspect</th>
<th>Victim not-sperm</th>
<th>Suspect not-sperm</th>
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<td>8, 11</td>
<td>14, 14</td>
<td>8, 11</td>
<td>12, 14</td>
</tr>
</tbody>
</table>

Y – STRs

- DNA testing for samples that contain a mixture of male and female DNA
- Only the male DNA type is identified, therefore mixtures can be resolved
- Statistics are lower than standard STRs, like mitochondrial statistics
Mitochondrial DNA Analysis

- Highly specialized analysis
- Good for bones and hair shafts
- Only a few laboratories perform the analysis - FBI, Armed Forces laboratory, and a few private labs
- Regional Public Labs – Arizona, Minnesota, New Jersey, Connecticut
- Cost: $2000
- Maternally inherited

Maternal Inheritance

Contamination

The curse of the Forensic Laboratory DNA Section

What can you do to help prevent Contamination

- Be aware
- Gloves and protective clothing
- Clean everything
- Collect controls
- Documentation

What can you do to help prevent contamination

CODIS
Database Statistics
As of June 2009
- Total number of profiles: > 7,409,920
  - Total Forensic profiles: > 272,452
  - Total Convicted Offender Profiles: > 7,137,468

http://www.fbi.gov/lab/casework/codis/codis1.htm

How does it work?
DNA profile is developed from the victim sample. Profile is placed in the CASEWORK database. Search is performed to see if any samples with the same profile exist.

Match between 2 casework samples
Unsolved case to unsolved case
Unsolved case to solved case
Date rape cases

How does it work?
DNA profile is developed from sample collected from a convicted offender. Profile is placed in the OFFENDER database. Search is performed to see if any unknown samples with the same profile exist.

90x156 to 522x706