EVIDENCE TECHNOLOGY MAGAZINE

The magazine dedicated exclusively to the technology of evidence collection, processing, and preservation
Volume 7, Number 2 • March-April 2009

Forensic Entomology

TOPICS IN THIS ISSUE

- Improving recovery of DNA profiles
- P&E room storage and safety
- Designing a crime-scene vehicle
- Interview with a forensic entomologist

BIOLOGICAL EVIDENCE ANALYSIS

How to Improve Recovery of DNA Profiles

Written by Peggy Ho Faix, PhD and Althea Lee

THE ULTIMATE SUCCESS of human forensic identification hinges on careful and accurate handling, processing, and analysis of biological evidence. Physical evidence (such as hairs, bone, teeth) and serological evidence (such as blood, semen, saliva, urine) collected at crime scenes or from individuals can be used to identify crime or catastrophe victims, implicate or exonerate potential suspects, establish paternity and other family relationships, or identify remains. Ideally, there is enough biological evidence to perform genetic analysis. Frequently, the sample is limited to trace evidence, touch samples (i.e. fingerprints), or it is significantly degraded from prolonged decomposition or exposure to environmental factors. Compromised evidence such as this must then be properly collected, transferred to the forensic-analysis lab, and processed further in order to isolate genetic material—DNA and RNA—for genetic analysis.

DNA extracted from trace or degraded evidence is frequently of low quantity or low quality and often is also contaminated with other materials such as heme (blood), indigo dye (denim), or humic acid (soil or plant material) that inhibit identification analysis. DNA degradation over time can contribute to further damage of already compromised sample types, including those derived from ancient or degraded bones or teeth. These and other adverse conditions that impact DNA integrity hamper reliable DNA-typing analysis and definitive individual identification.

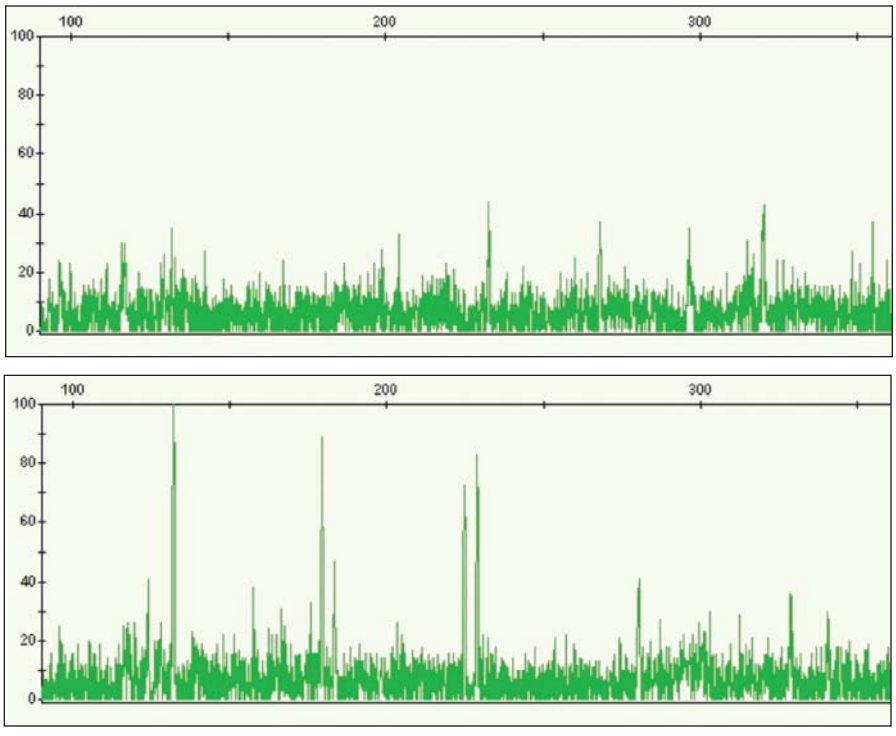

***Figure 1**—These electropherogram panels show the results of STR analysis that was performed on DNA recovered from touch samples (phone swabs) with and without the presence of the Biomatrica STRboost enhancing reagent in the amplification reaction. When the enhancing reagent was added (bottom panel), 15 alleles were detected on the electropherogram, overcoming difficulties commonly associated with significantly degraded DNA samples.*

The early years of forensic genetic analysis

The genetic analysis of biological materials has been utilized since the early 1900s. Initially, scientists utilizied morphological and enzymatic analysis of serological markers such as red blood cells, red blood cell antigen systems, serum proteins, and human leukocyte antigens (HLAs). Many of these older analysis techniques, including blood typing, did not yield definitive identification of an individual and only served to exclude portions of the population.

In the mid-1980s, typing evolved to DNA analysis, or DNA typing. This technology allows for higher power of discrimination between individuals. DNA-analysis techniques include DNA sequencing, RFLP (restricted fragment length polymorphism) analysis, and applications based on polymerase chain reaction (PCR).

Here is a quick, general overview of how DNA analysis works:

❏ Specific DNA regions—called *markers*—are identified in a DNA sample through the binding of a small, unique, complementary DNA sequence —or *probe*—to the target sample DNA.

❏ A series of probes bound to a DNA sample creates a distinctive pattern—or *profile*—for an individual.

❏ By comparing DNA profiles, forensic scientists can link specific sample evidence to a suspect or victim. A marker, by itself, is not unique to an individual; however, if two DNA samples are alike in several regions, then the odds are increased that the sample did, indeed, originate from the same person.

Short tandem repeat (STR) technology is a PCR-based application that has gained widespread use for forensic genetic-identification analysis.

The more probes used in DNA analysis (typically four to six), the greater the odds for a unique pattern and the less likely there is a coincidental match. Unfortunately, using additional probes for analysis significantly adds time and expense to testing.

One early DNA typing technique —RFLP—relies on the pattern of specific DNA markers. RFLP utilizes restriction enzymes to cut DNA at specific sequence-recognition sites to generate variable lengths of DNA. These lengths of DNA are then separated using gel electrophoresis. Just as with DNA sequencing, these segments are then visualized through radioisotopes, but the RFLP process follows up with a step where the DNA fragments are immobilized in gel and hybridized with DNA probes that bind to a complementary DNA sample sequence. The presence or absence of a sequence-recognition site dictates whether the enzyme will cut the DNA to generate a fragment of specific size. It is this unique pattern of various DNA fragment lengths that permits genetic typing of an individual.

Moving forward to multiplex STR technology

RFLP has since been replaced by technologies that are based on PCR. These techniques are more sensitive and accurate, do not require large amounts of DNA, and are more cost-effective for routine analysis.

PCR is one of the most prominent molecular-biology techniques and has

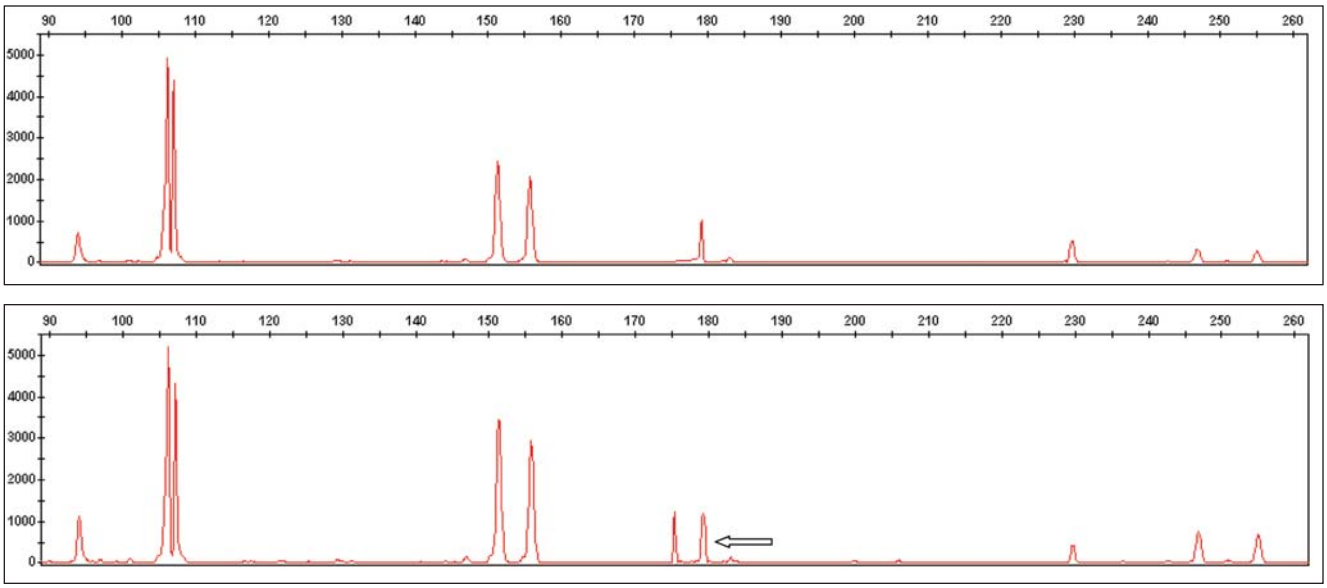

Figure 2*—By comparing the top and bottom panels of this electropherogram, you can see that the addition of the Biomatrica STRboost enhancing reagent to a sample of neat DNA on denim with 100 percent indigo dye (bottom panel) overcame the effects of indigo dye, a commonly encountered inhibitor that is present in forensic source samples. The bottom panel shows enhanced peak definition and recovery of an additional allele (indicated by the arrow) compared to the peak definition shown in the top panel. The top panel illustrates the results when no enhancing reagent was added.*

been incorporated into a myriad of applications in biological and life-science research and diagnostics, including forensics work. PCR is used to make millions of exact replicas of DNA from a biological sample containing nucleic acids. It is highly sensitive and allows for successful analysis and identification of DNA derived from highly degraded or minute quantities of biological samples.

Short tandem repeat (STR) technology is a PCR-based application that has gained widespread use for forensic genetic-identification analysis. STR technology is used to evaluate specific regions (loci) within DNA. Variability between different STR regions can be used to distinguish one DNA profile from another.

STR analysis has been shown to be highly sensitive and useful for nuclear-DNA, mitochondrial-DNA, and Y-chromosome analysis. The Federal Bureau of Investigation (FBI) currently uses a standard set of 13 specific STR regions of nuclear DNA for the Combined DNA Index System (CODIS), which is a centralized U.S. government-sponsored computer software program that operates local, state, and national databases of DNA profiles of convicted offenders, unsolved crime-scene evidence, and missing persons.

The statistical odds of two individuals having the same 13-loci DNA profile, or "DNA fingerprint", is about one in a trillion, making STR analysis a powerful tool for individualizing genetic evidence. Laws in many of the states have been expanded to include obtaining DNA profiles from all arrestees and from detainees of certain crimes.

Enhancing reagents that are now available on the market can help you obtain sufficient DNA profiles from degraded samples.

Hurdles involved with STR technology

Despite technology advances, STR is still a highly-specialized assay that involves multiple steps, including: DNA extraction; quantification; simultaneous amplification of alleles at multiple loci using fluorescently labeled probes (multiplexing); and subsequent separation, detection, and fluorescent analysis by capillary electrophoresis.

Although almost every aspect of the assay is now provided as standardized kits (such as Applied Biosystem's Identifiler and Promega's PowerPlex 16 Systems) that are used on automated genetic analyzers (such as the ABI 3100 PRISM Genetic Analyzer), multiplex STR analysis still has some substantial challenges.

For example, degraded (low-quality) or low-quantity samples do not consistently yield sufficient DNA profiles for identification purposes, nor do samples that contain PCR inhibitors such as heme, indigo dye, humic acid, melanin (hair or skin), bile salts (feces), collagen (tissues), urea (urine), and myoglobin (muscle tissues). Until now, researchers have tried to overcome difficulties with performing multiplex STR analysis by either re-running the reaction or switching to another kit—but both of these solutions are costly and time-consuming.

Boosting your odds of obtaining multiplex results

STRboost from Biomatrica, Inc. was recently developed to enhance the amplification of challenging genetic samples encountered in forensic casework. STRboost substantially enhances multiplex PCR amplification reactions used for human-identification applications, resulting in improved accuracy, sensitivity, and precision.

Adding this enhancing reagent directly into multiplex reactions may help to resolve stochastic effects that are typically associated with sample degradation or inhibition, including allele dropout and suboptimal peak-height definition. Analysts can continue using their own cycling conditions and equipment. The reagent is compatible with commercially available genetic identification kits and does not interfere with automated detection systems.

"STRboost has been demonstrated in our labs to increase the number of detectable alleles by improving allele call rate, enhancing peak height, and overcoming the inhibitory effects of humic acid and indigo," said Dr. Steven B. Lee, director of Forensic Science and professor for the Justice Studies Department at San Jose State University in California. "STRboost makes multiplexing a more robust analytical procedure and reduces the time previously spent on optimizing STR analysis, thus improving our ability and efficiency to successfully recover DNA profiles."

During the development of the STRboost product, Lee worked in conjunction with a forensic-analysis lab to successfully use the enhancing reagent to recover DNA profiles from low copy number DNA samples recovered from touch samples taken from phone swabs. Multiplex STR reactions were performed using the PowerPlex 16 System from Promega, either with or without the STRboost enhancer present in the amplification reaction (see Figure 1 on Page 28).

Results of this work indicate that when the STRboost enhancer was present in the amplification reaction, all alleles were successfully recovered from the touch DNA sample. Comparatively, no alleles were recovered in standard reactions without enhancer.

Additional analysis has demonstrated that including STRboost in amplification reactions of samples containing indigo can also overcome inhibitor factors that would otherwise hamper recovery of a useful DNA profile for identification purposes (see Figure 2 on Page 29).

STRboost is ideally suited for generating DNA profiles from difficult samples such as those containing inhibitors, degraded samples, or from samples that are derived from limited sources such as trace evidence and low copy numbers.

"The ability to directly add STRboost into amplification reactions without having to further optimize reactions and cycling conditions makes it easy to incorporate STRboost into existing workflows and standard laboratory protocols," said Lee. "It also tends to increase the likelihood of definitive individualized identification of forensic genetic evidence."

Technological advances that improve the sensitivity and reliability of applications used for genetic-identification analysis should prove to be particularly useful for successfully recovering DNA profiles from compromised sample types.

About the Authors

Peggy Ho Faix, PhD is a Project Manager at Biomatrica, Inc. (San Diego, California) and Althea Lee is Director of Marketing at Biomatrica. Both of the authors are involved in the management of the forensics program at Biomatrica. Faix can be reached via e-mail at:

pfaix@biomatrica.com

Lee can be reached via e-mail at:

alee@biomatrica.com

FOR MORE INFORMATION

To learn more about the STRboost product, you can go to Biomatrica's website: **www.biomatrica.com/strboost.php**