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PROFILE

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Identifying Those Remembered

COVER STORY | New technologies promise to speed DNA identification at disaster sites and in criminal investigations

By Kelli A. Miller

Corbis

Last year, two Denver scientists theorized that a clinical instrument used to spot cancer mutations could speed up the normally tedious DNA identification process. Then the attacks of Sept. 11 occurred, and their work suddenly took on a sense of urgency. When the National Institute of Justice (NIJ) sent out a countrywide plea for information on new technologies that could assist in the investigation at Ground Zero, **Phil Danielson**, assistant professor of molecular biology at the University of Denver, and his laboratory assistant, **Robby Shelton**, immediately stepped up to the challenge.

"[Sept. 11] prompted us to cut to the chase on the research front and show that our approach really would work," says Danielson. "In the absence of that added pressure it probably would have taken one to two years to get to where we are now." In a pilot study using San Jose, Calif.-based **Transgenomic**'s WAVE™ Nucleic Acid Fragment Analysis System, Danielson and Shelton demonstrated that WAVE profiling, one of the most effective technologies for rapidly identifying new genetic mutations potentially involved in the formation of human tumors, could quickly and cheaply perform a kind of DNA analysis called mitotyping. This technique and other advances in DNA forensics promise to speed DNA identification at disaster sites and war zones, and in criminal investigations.

"Our Last Hope"

The most common type of DNA profiling notes differences in the size of specific nuclear DNA regions known as short tandem repeats (STRs). But at many mass disaster sites, including Ground Zero in New York, very little nuclear DNA evidence remains. Many World Trade Center victims were badly burned, with little tissue left intact. Remains found months after the terrorist attacks are in such an advanced state of decay that nuclear DNA analysis is rarely possible.

That is when mitochondrial DNA (mtDNA) comes into play, says **Terry Melton**, president and founder of State College, Pa.-based **Mitotyping Technologies**. Melton is on an advisory panel for the Office of the Chief Medical Examiner in New York that helps the examiner troubleshoot the mtDNA identification process at Ground Zero. Every cell in the human body contains thousands of copies of maternally inherited mtDNA. All maternal relatives have the same mitotype, so forensic scientists can identify a victim's remains through DNA obtained from relatives, even when no body is found. Hair is a robus t mtDNA source, though it rarely contains enough nuclear DNA for STR analysis. "We use mitochondrial DNA when there's nothing left. It's our last hope," admits Danielson.

Conventional mitotyping is a laborious process that requires extensive postelectrophoretic sequence analysis. It works, but it takes "10 to 100 times longer than STR analysis in terms of time and materials," says DNA identification expert **Keith McKenney**, a professor of microbial genomics and diversity at George Mason University in Fairfax, Va.

An Instant Snapshot

Danielson's technology represents a radical departure from existing mitotyping techniques. Armed with forensic samples provided by **Greggory LaBerge**, a forensic scientist at the Denver Police Department, and Transgenomic's WAVE system, LaBerge, Danielson, and Shelton designed a method that completely eliminates the need for direct DNA sequencing and the time-consuming interpretation of results. Their approach takes an instant snapshot of the entire mtDNA molecule in just one step. "We're taking molecular biology and combining it with forensics to do something that's going to have a major impact," explains Danielson.

The DNA WAVE analysis method relies on denaturing high-performance liquid chromatography (DHPLC), a relatively new method of separating DNA on the basis of very small differences in base sequence. DNA molecules are dissolved in an aqueous solution and pumped into a steel column where they bind with microscopic plastic beads. As the chemical composition of the DHPLC solution is changed, the DNA melts and quickly releases its grasp on the beads. Molecules that differ in base sequence fall off the column at different times, producing a DNA-melting profile.

"You basically are taking two different DNA molecules and watching how quickly they melt," says Danielson. "The more sequence differences you have, the more readily DNA will melt." When two samples match, all the molecules in the mixture bind and fall off the column at exactly the same time.

Like traditional STR analysis, Danielson's approach requires PCR amplification of the DNA sample. But his method reduces the total analytical run time. "Right now, sequencing takes 85 minutes to run one sample and analysis and interpretation can take hours to days," says Shelton. "The WAVE instrument completes the analysis in as little as four minutes."

Danielson compares the new technique to how some foods are packaged and weighed. To determine if a canister contains the correct number of potato chips, for example, one could open every can and count each chip. That is basically how the old mtDNA-sequencing method works. A faster approach would be to weigh the container. Since all the chips are uniform in size, a canister above or below the target weight would indicate that it contains the wrong number of chips.

Cutting Costs

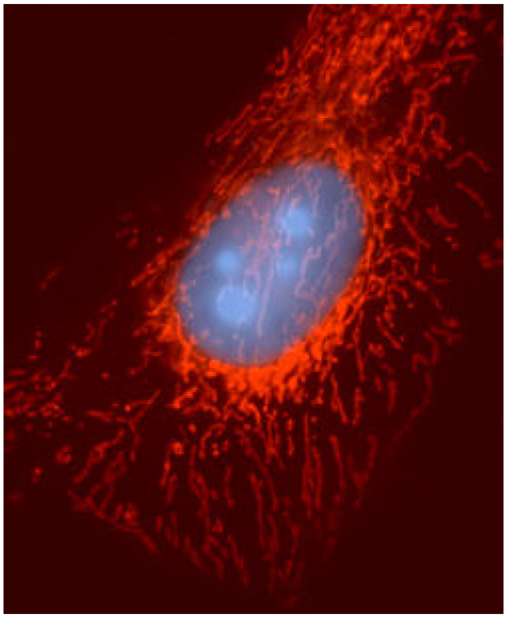
Police departments have been slow to adopt mitotyping; it is just too expensive. "DNA mitotyping work is off the charts as far as expense goes in this day and time," says **Troy Krenning**, law enforcement program manager at the National Law Enforcement and Corrections Technology Center in Denver, a branch of the NIJ. It was Krenning's request that helped kick Danielson's research project into high gear.

Now, Danielson's mtDNA method could dramatically cut costs. "[We] improved the sensitivity of some FBI methods by orders of magnitude, while cutting costs from $2,500 per sample to less than $10 per sample," explains Danielson. And, they did so by using samples much smaller than those required nowadays. Forensic labs usually request at least a 2-cm hair sample; Danielson and Shelton obtained usable DNA mitotypes from hair samples as small as 0.01 cm.

For the initial study, Danielson's team had to quickly prove that the DHPLC technique could accurately identify a mother-son relationship. Working with an incredibly short deadline, the researchers scrambled, asking coworkers and friends to serve as guinea pigs and contribute hair samples. "Rush study that we did, we proved that this is actually viable," Danielson says. "We have not noted an error rate yet."

Extracting DNA

Courtesy of BD Biosciences-Clontech



Cellular ID Cards: A HeLa cell teems with mitochondria (red), each of which contains a molecule of DNA that investigators can use to identify remains when nuclear DNA (blue) is lost or degraded. Such is often the case at disaster sites, such as New York City's Ground Zero.

Streamlining and accelerating DNA analyses can improve DNA profiling, but what if usable DNA samples cannot be found? The remains of many WTC victims consist of degraded bones, and extracting DNA from such specimens has historically been difficult and time-consuming.

The **Bode Technology Group**, the lead private laboratory involved in the DNA identification effort at Ground Zero, has developed a novel extraction method and sample tracking system that allows their scientists to process 10 times the normal capacity of degraded skeletal samples. Laboratory director **Mitch Holland** would not detail Bode's proprietary method, but he did acknowledge that the lab is developing methods to sample human remains as quickly as possible by reducing the required size of an extracted sample.

"We've gone [from] doing 1,500 samples in many months to doing 1,000 samples a week," says Holland. "We've simplified the process to make it more elegant and to allow for higher throughput while maintaining quality." Holland is one of the most recognized experts in using DNA technology in mass disaster investigations. His team of 35 scientists has received over 20,000 samples from Ground Zero so far: 18,000 bone and soft tissue samples from victims and 2,000 from their families. These latter samples are typically buccal swabs, but may also be a "personal effect," such as a comb or toothbrush—anything that might contain identifying DNA.

Boosting the DNA Database

Forensic scientists such as Holland say law enforcement will benefit from the faster typing methods being developed today. These techniques will help build up the Combined DNA Index System (CODIS), which is the national DNA database launched in 1998 to help state and local law enforcement agents compare DNA samples collected at crime scenes nationwide.[1] "Bigger databases will allow thousands of crimes, particularly sexual assaults, to be solved much, much faster," Holland explains.

But current gaps in the database have made it useless in some instances. "There's no question [that] an enormous number of people ... have not been entered into CODIS," says **Clay Strange**, assistant district attorney for Travis County, Texas. Strange was the original director of the American Prosecutors Research Institute, DNA Legal Assistance Unit, in Alexandria, Va., where he was responsible for training prosecutors in the use of DNA evidence. He says the backlog stems from a lack of both manpower and money. "There need to be more people doing this work and far more federal money. [Individual] states, with the exception of Florida and Virginia, just aren't going to devote the time and money needed to boost the databases."

In the meantime, criminal cases remain unsolved, particularly rapes. "We have hundreds, thousands of sexual-assault collection kits ... that have never been typed because there's [no] suspect. It's too expensive to type them," says Strange. Cheaper, faster, and portable DNA technologies, combined with a more robust databank, might someday allow the police to walk away from a crime scene with the name of the perpetrator. "That is the real impact that DNA profiling will have in the next five years," says Holland.

In New York, the DNA identification process continues. According to Melton, "DNA identification of the WTC victims is a massive project, unparalleled in size." Numerous companies are lending their expertise to the effort. Utah-based **Myriad Genetics**, for instance, is using proprietary DNA profiling to map victims' information as well as the genetic makeup of the family members of the missing. Information is stored digitally and returned to New York investigators for comparison.

Danielson says that the federal government's response to his research has been encouraging. His team is now waiting for the thumbs up to fully develop and forensically validate the use of DHPLC and WAVE profiling for criminal investigations and the DNA identification of human remains at disaster sites. "Identifying the victims from the World Trade Center will likely take years," he says. "[We hope that] in the middle of this, WAVE profiling will be approved [and will] dramatically speed things up."

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1. T. Hollon, "Reforming criminal law, exposing junk forensic science," ***The Scientist*, 15[17]:12, Sept. 3, 2001**.