

Innovation in DNA Analysis

An innovative method for profiling DNA can speed analysis of samples, reduce costs and allow identification of previously hard-to-identify specimens.

The method, denaturing high-performance liquid chromatography (DHPLC), allows the rapid and accurate separation of mixtures that contain the mitochondrial DNA (mtDNA) of more than one person. It has the potential to benefit criminal and mass disaster investigations requiring timely analysis of mtDNA. For example, the technology is useful in forensic analysis because it can separate mixed samples that contain the DNA of both a suspect and a victim in a crime.

DHPLC technology was originally developed by Transgenomic for medical applications such as screening for breast cancer susceptibility in women, according to Phil Danielson, professor of forensic genetics at the University of Denver's Department of Biological Sciences. Danielson and his team subsequently adapted the biomedical technology to a forensic application, using grants from the Office of Justice Programs' National Institute of Justice (NIJ).

"I had met with law enforcement lab practitioners a long time ago and I was amazed at the number of challenges they faced in trying to carry out standard forensic testing," Danielson explains. "It intrigued me, the rigor and precision required of forensic lab analysts impressed me, and I thought I could apply molecular biology to the field of forensics."

One challenge facing lab analysts at the time was separating mixtures of mtDNA on evidentiary material.

"They could not separate mixtures and even analyzing mtDNA was too cumbersome for most laboratories," Danielson says. "We have a well-equipped lab and after talking to practitioners, we tried to come up with new technologies they could use to rapidly and accurately analyze mtDNA."

The resulting DHPLC method took years of research. With the help of the National Law Enforcement and Corrections Technology Center (NLECTC) and lab

practitioners' advice, Danielson's team developed a funding proposal and obtained their first NIJ grant in 2003. NLECTC is associated with the university, and helped put researchers in touch with practitioners to obtain bone samples from unclaimed remains to use in testing.

"We put together a team of researchers from the U.S., Denmark, Iceland and China, a team of highly skilled people who put their heads together and developed a forensic application for DHPLC," Danielson says.

NUCLEAR VS. MTDNA

DNA (deoxyribonucleic acid) is the basis for an individual's genetic makeup. Nuclear DNA, the DNA found in a cell's nucleus, is a combination of DNA from both one's mother and father. It is a tool for identifying people, but is fragile.

Cells also contain mtDNA, which is inherited only from the mother and is available in larger quantities per cell. Because mtDNA, which is located outside the cell's nucleus, is much more abundant than nuclear DNA, it can provide forensic investigators with a means to test older, damaged, degraded or tiny biological samples. Additionally, mtDNA can be extracted from samples with little or no nuclear DNA, such as hair shafts.

FORENSIC DHPLC APPLICATION

Traditional mtDNA analysis is complex and time consuming. Danielson and his team developed two major forensic applications for DHPLC to expedite processing.

The first is a comparative sequence analysis, in which different samples are compared as a screening tool for mtDNA. It quickly eliminates irrelevant samples, such as hairs from nonsuspect residents of a house where a crime occurred.

"The method is faster, cheaper and easier," Danielson says. "The initial presumptive screening takes seven minutes, compared to hours and hours if not days by the old method. It costs less because it takes less human time to do the test. The presumptive screen is not a replacement for traditional analysis; it's a screening tool. For example,

it allows us to screen 12 samples and determine that two warrant further detailed analysis. We focus the analyst's attention only on the samples that are most informative."

The second application of DHPLC technology is the ability to separate mixtures that contain the mtDNA of more than one person, which was the primary focus of the team's six years of research funded by NIJ. Researchers performed about 27,000 assays, or analyses, to separate mtDNA mixtures and validate the technology and show it works 100 percent of the time. "Everybody was doing assays day and night," Danielson says.

A technological disadvantage is that the technology can only separate the mixtures of two individuals.

"We can do two with 100 percent accuracy," Danielson says. "With a mixture from three people, it is very complex and some are not resolvable. Fortunately, the majority of forensic cases involved the mixtures of just two people."

Another disadvantage is that mixture separation cannot be done by an analyst on his own with a computer spreadsheet because the analysis is very complex, so a software program is needed that will automate it computationally, Danielson explains. NIJ in 2009 provided a grant to hire a software engineer to create a user-friendly program for analysts in the United States. Creation of the software program is underway.

NEXT STEPS

Danielson says the DHPLC technology is ready for practitioner use. They have seen interest from practitioners in China and Holland, where it can be easier to get some new technologies accepted in court than in the United States.

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"We have to find the right test case in the U.S. to apply the technology," Danielson says, and his team is focusing on Colorado.

Other ways analysts could use DHPLC technology is to link unidentified remains to reference samples for missing persons.

"Remains are stored by law enforcement agencies, and every once in a while skeletal remains are discovered," Danielson says. "Depending on the condition, it is not uncommon for there to be very little of the nuclear DNA left, so usually it's mtDNA that you look for, particularly if you have comingled remains or remains from more than one individual.

"You generally use mtDNA when all the other forms of DNA testing fail," Danielson says. "It's DNA of last resort. We can also use it for cases where the only evidence is hairs; if it's a cut hair or a hair without a root, it's difficult to get results from nuclear DNA. For hair analysis, mtDNA is the gold standard."

For more information on DHPLC DNA technology, contact Phil Danielson at the University of Denver at (303) 671-3561 or pdaniels@du.edu.



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