Crime Scene Genetics: Transforming Forensic Science through Molecular Technologies

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Crime Scene Genetics: Transforming Forensic Science through Molecular Technologies

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Advances in DNA (deoxyribonucleic acid) technology over the past 25 years have led to spectacularly precise forensic identification techniques, although some applications have also unleashed controversies regarding genetic privacy. Current molecular forensic work is pushing these technologies even further by analyzing extremely damaged DNA and by introducing RNA (ribonucleic acid) techniques to forensics.

In 1986, a British teenager named Richard Buckland admitted under police questioning that he had raped and murdered 15-year-old Dawn Ashworth in Leicestershire, England. He denied, however, any connection to a three-year-old murder that police were convinced had been committed by the same person. Had this happened just a year or two earlier, Buckland may have gone to prison for one or both murders. But a new technique, called DNA fingerprinting, conclusively demonstrated that semen found at both crime scenes did not belong to Buckland.

Leicestershire police and the United Kingdom's Forensic Science Service conducted a mass DNA screening of local men, looking for a match to the genetic profile of the murderer. They found nothing—until a man was overheard saying that he had given a DNA sample in place of his friend Colin Pitchfork. After Pitchfork was tracked down, he quickly became the first person convicted for murder on the basis of DNA evidence.

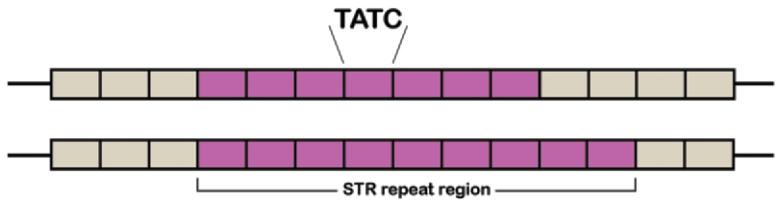
Until the 1980s, such precise identification of a suspect was unheard of. If someone left a drop of blood at a crime scene, forensic scientists could analyze only the person's blood type plus a few proteins that exist in slightly different versions in different people. But neither of these tests is particularly specific: many people share blood types and protein markers, making unique identification from a blood stain nearly impossible.

The course of molecular forensics changed in 1984, when geneticist Alec Jeffreys, of the University of Leicester in the United Kingdom, discovered a new type of marker in the human genome. He found that our DNA contains many noncoding regions in which a sequence of 10 to 100 base pairs is

repeated multiple times. Although the sequence is usually the same at each region in all people, the number of times that the sequence is repeated is highly variable among individuals.

Jeffreys immediately saw the potential for forensic use of these markers, which he called "minisatellites." In less than two years, forensic labs across the world could create DNA "fingerprints" of crime suspects by profiling their unique minisatellite makeup. For the first time, forensic scientists could create genetic profiles so specific that the only people who share them are identical twins.

DNA fingerprint techniques evolved subtly over the next several years, until the polymerase chain reaction (PCR), developed by Kary Mullis, was introduced into forensic work. By allowing the selective amplification of any desired stretch of DNA, PCR ushered in unprecedented sensitivity in low-level DNA detection at crime scenes. All of today's forensic genetic methods are based on PCR.



The short tandem repeat, or STR, is the standard genetic marker used in forensic cases worldwide. At the locus shown, the sequence TATC repeats seven times in the top allele and nine times in the bottom. The number of repeats varies widely between individuals. By analyzing the STR number at multiple loci, investigators can be confident that no two individuals could share an STR profile. Graphic: Melissa Phillips.

STR profiles and databases

The standard genetic forensic test used in crime labs across the world assays an individual's profile of markers, called short tandem repeats (STRs), which are genetic sequences similar to minisatellites, although the repeating DNA sequence in STRs is considerably shorter. STRs are equally variable among individuals: with each additional STR locus a forensic scientist analyzes, the odds become vanishingly small that two people will have the same STRs at all loci.

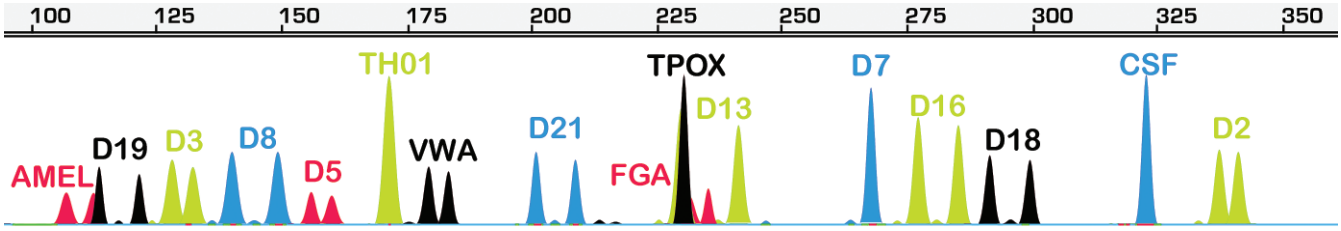
Most human forensic casework is performed with standardized commercial "multiplexes" that assay STRs at multiple genetic loci simultaneously. The ease with which STR profiles can be typed today has led to the development of large national databases containing STR profiles of millions of people suspected or convicted of crimes. In the United States, the DNA Identification Act of 1994 authorized the Federal Bureau of Investigation to create a national DNA database: the Combined DNA Index System (CODIS). CODIS originally consisted of a forensic index, which contains DNA profiles from crime-scene evidence, and a convicted offender index, which contains profiles of DNA samples taken from convicted offenders. In the past several years, CODIS has added indexes for arrestees and missing persons.

Criminal investigators can query CODIS with STR profiles taken from biological samples found at a crime scene. If the crime-scene sample matches a profile in the offender database, this information can lead police to a likely suspect, or exonerate an innocent suspect. By searching the forensic index, investigators can link crime scenes together if they find the same person's DNA at both scenes.

The standard DNA profile collected in the United States and entered into CODIS consists of 13 STR loci plus the amelogenin gene, which is found on the X and Y chromosomes and can establish the sex of unknown sample sources. The probability that two unrelated individuals share the total profile is less than one in one trillion. CODIS currently contains nearly 6 million STR profiles, says John Butler, of the National Institute of Standards and Technology.

National DNA databases have aroused some controversy—whose DNA goes in them, and how is this information used? Laws regulating these databases differ from country to country and, in some cases, between jurisdictions and states. In the United States, STR profiles were originally collected only from convicted sex offenders, but the database has expanded significantly in the last decade, Butler says. Most states now permit DNA profiling of all convicted felons, and some states collect profiles of all arrested suspects.

The United Kingdom's National DNA Database, which contains more than 4



This STR profile shows 15 STRs plus the amelogenin gene (AMEL), which reveals the individual's sex. The length of each amplifed DNA fragment (given in base pairs along the top) reveals how many copies of a particular STR the fragment contains. At loci with two differently sized peaks, the individual has two different STR alleles, while a single peak indicates the same STR copy number on both alleles. Image: John Butler, National Institute of Standards and Technology.

Forensic genetic analyses have been used to solve historical mysteries, such as the identity of bones suspected to belong to the Romanovs, the Russian royal family executed in 1918. In 1994, samples taken from these bones yielded enough intact DNA to prove, by means of STR analysis, that all the bones came from the same family. Researchers then took mitochondrial DNA (mtDNA) samples from living matrilineal relatives of both Tsarina Alexandra (above) and Tsar Nicholas II. These sequences matched the mtDNA sequences extracted from the bones, confirming that they did indeed belong to the Romanovs.

million profiles, collects samples from individuals arrested for all but the most minor offenses. In England and Wales, but not Scotland, profiles stay in the database even if the arrestee is never charged or is acquitted in court. "There's some debate going on at the moment" about this policy, however, says Peter Gill of the UK's Forensic Science Service. Two British men who were arrested for a crime but later cleared are petitioning the European Court of Human Rights to have their DNA removed from the database. "It's possible that some of these profiles may have to come off if the court finds that they're illegal," Gill says.

DNA database rules differ in other countries. For example, DNA samples in the Netherlands are entered only for those who are serving jail sentences longer than four years, and then profiles are removed 20 to 30 years after conviction. Laws also differ concerning preservation of DNA samples themselves. In some US states, only the STR profile is kept; the DNA samples themselves are destroyed after analysis. In many states and in England, however, DNA samples are preserved indefinitely, for possible analysis with technologies not yet invented. In some countries, investigators now search DNA databases not only for full matches but also for partial matches to STR profiles. A partial match indicates that the perpetrator may be a close relative of the person found in the database. Such "familial searching" is "pretty routine in the UK," says Gill.

Regulations regarding familial searching currently differ from state to state in the United States, according to Bruce McCord, of Florida International University, although McCord expects that victims' rights groups will push for familial searching in all states and sharing of information between states. "What I think it ultimately comes down to is the rights of the victim versus the rights of the people being tested."

Specialized forensic markers

STR profiling is probably here to stay, at least for the foreseeable future, Butler says. Because national databases already contain so many profiles of STR loci, "it would be very expensive to change to a different set of markers." But "more boutique" markers are sometimes used in special circumstances, he adds.

For example, researchers are now establishing useful markers on the Y chromosome. Such markers do not have as much statistical power as traditional STRs to make unique identifications, as Y sequences are much less diverse between individuals. But because they are found only in males, they are often essential in sexual-assault cases in which forensic samples may contain many times more female DNA than male DNA. "By using something that just targets the male DNA, you can figure out the perpetrator's profile versus the victim's profile," Butler explains.

CODIS currently contains no Y profiles, although it's a possibility for the future, McCord says, because adding Y markers to CODIS would make familial searches among males extremely powerful. Y chromosomes are passed along intact—except for random mutation—from father to son, so all of the male relatives in a family's paternal line share the same Y chromosome. A criminal whose male relatives had profiles in CODIS could most likely be tracked down from a hit on their Y chromosomes.

An especially enticing area of Y chromsome research, according to Gill, revolves around work showing that in some cases, Y chromosomal profiles can predict a man's last name, since the Y is so tightly linked with the patriline. "I was quite surprised, actually, how often the right surname was coming out of these profiles," Gill says. "But you have to choose surnames which are relatively rare for it to be more successful," he adds. "Obviously it's not going to be useful for someone called Smith."

Other specialized genetic markers have now been developed for cases in which DNA is extremely degraded. Damaged DNA samples "are always a problem in forensics," says David Foran, of Michigan State University. Two types of markers suitable for damaged DNA were developed in the aftermath of the September 11 World Trade Center attacks in New York City. From more than 2500 victims of the attacks, NYC forensic personnel tried to extract and analyze DNA from more than 20,000 samples in order to identify the victims' remains.

Almost all the DNA recovered from the site was extremely damaged, says Robert Shaler, then head of forensic biology at the Office of the Chief Medical Examiner in New York. The average length of DNA that they isolated was much too short to be analyzed by traditional STR kits, says Shaler, who is now at Pennsylvania State University. In response to this problem, the Bode Technology Group developed a test for unusually short stretches of STRs. These markers, now termed "miniSTRs," have since been commercialized. They are at many of the same loci as CODIS's standard STRs, which means that they can now be used in any forensics case dealing with fragmented DNA, McCord says.

For the tiniest DNA fragments recovered, even miniSTRs are too large. For these, Shaler and his colleagues contracted with Orchid Genescreen to develop a profile of useful single-nucleotide polymorphisms (SNPs). Because SNPs differ from each other at only one base pair, they are the smallest genetic markers possible.

The primary disadvantage of SNPs is that they do not exist in as many different varieties as do STRs, and therefore their power for making unique identifications is considerably less. Approximately 50 SNPs are required to identify an individual with the same certainty as with the standard 13 STRs. For this reason, SNP use in forensics will probably remain relatively specialized, Butler says.

Mitochondrial DNA (mtDNA) can also be extremely useful for identifying degraded DNA. Mitochondrial DNA is found in the cell's mitochondria instead of its nucleus. Because there are hundreds of mitochondria per cell, there are also hundreds of copies of the cell's mtDNA, which increases the chances that it will survive long time periods or harsh conditions. For this reason, mtDNA analysis is widely used in historical analyses of bones and in cells with little DNA, such as hair shafts.

Mitochondrial DNA is found in both females and males but is inherited only through the mother, which makes it less varied than nuclear DNA. Like Y chromosomal markers, mtDNA "doesn't have nearly the exclusion power" of standard STRs, Foran says. But, "when you can't get anything else, you can often get mitochondrial DNA."

Forensic genetic techniques are sometimes applied to DNA samples from nonhuman animals, plants, and microorganisms. For example, researchers have used DNA fingerprinting methods to prove that a valuable cultivar of strawberry plant was being grown by someone other than the patent holder. Forensic DNA tests have also been developed to identify the source of cannabis samples, and the identification of a particular strain of moss has even been used as evidence in a murder case. STR and mtDNA analyses of hair from pet dogs and cats have been used to tie suspects to crime scenes, and interest in genetically identifying microbial strains has increased since five people died in the United States in 2001 after handling mail laced with anthrax bacteria.

Controversial techniques

In the past few years, researchers have developed several techniques for analyzing particularly small amounts of DNA. It's now possible to retrieve an STR profile from the DNA contained in a fingerprint using these so-called low copy number techniques. Typically this term is reserved for profiles created from the DNA in about 15 or fewer cells, Butler says.

Low copy number techniques have been plagued by some disagreement in the forensics community about their reliability. Because there's so little DNA, contamination becomes a serious concern, Butler says. "The lower the amount of DNA that you work with from the evidence, the more likely you are to contaminate it from yourself or from a police officer or whoever happens to walk by the crime scene." It also becomes more likely that researchers won't be able to get a full STR profile from low copy number analyses, McCord adds.

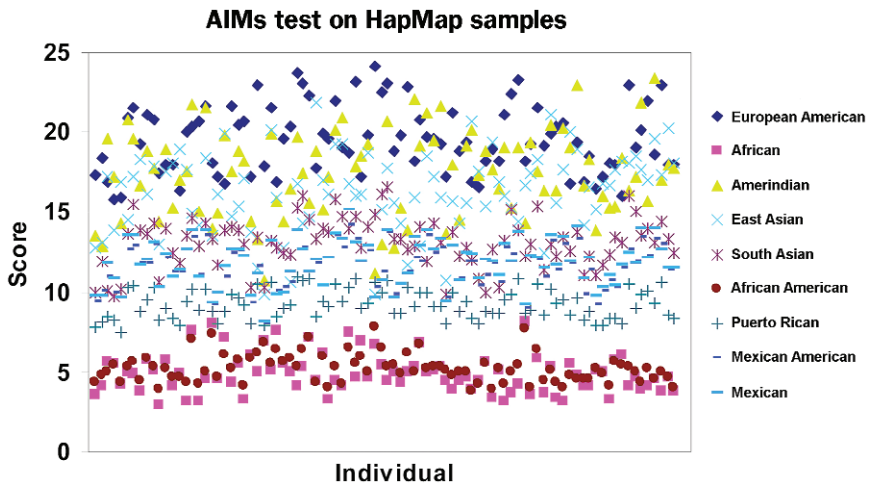
The Office of the Chief Medical Examiner in New York City is currently the only US forensics lab that routinely uses low copy number techniques. In the United Kingdom, low copy number analysis made headlines in December 2007, when Sean Hoey, who was charged with murdering 29 people in a bomb attack, was acquitted after the trial judge threw out DNA evidence based on low copy number techniques. After the ruling, these analyses were briefly suspended in UK crime labs, but the Crown Prosecution Service reviewed the technique, decided that it did not appear problematic, and lifted the ban in mid-January 2008.

According to Gill, the issues with low copy number analyses lie not with the techniques themselves but with the way DNA evidence is sometimes handled in court. It's important to be more cautious when interpreting DNA evidence retrieved from an "invisible stain" like a fingerprint than from a speck of blood, he says, since the blood is very likely connected to the crime itself, whereas the fingerprint may have appeared at another time.

"There are various possibilities for how a DNA profile might be transferred," Gill says. "It doesn't actually tell you that someone committed a crime," even if the profile is analyzed using traditional methods, he points out. "I don't consider that there is any difference between low copy number and conventional DNA profiling. It's all a continuum."

New directions

One of the newest frontiers in forensic genetics involves analysis of a different type of genetic material: RNA. DNA profiles are ideal for individual identification, but because every cell contains the



Researchers are now using new data on human DNA sequence variation—such as that from the International HapMap Project—to identify DNA variants that can distinguish individuals of different ethnicities. In this graph, researchers are looking at the effectiveness of 16 ancestry informative markers (AIMs) in determining likely ethnicity. Using a DNA sample taken from a Nigerian person, the AIMs test would conclude that the individual is most likely to be African or African American (indicated by a low test score) and least likely to be European American, Amerindian, or East Asian (indicated by a high test score). The researchers are currently perfecting these tests so that they can be used to help police predict likely ethnicities of suspects. Image: Ray Miller, Washington University in St. Louis.

same genome, DNA has nothing to say about what type of tissue or fluid is present. RNA, on the other hand, is perfectly suited for this type of analysis. DNA is transcribed into messenger RNA (mRNA) only when that RNA is needed in the cell, so different mRNAs are found in different cell types.

Biological fluid analysis in particular is of great interest to forensic scientists. Researchers have recently shown that RNA profiles of blood can differentiate between menstrual blood and blood found in blood vessels, a distinction that may be important to investigators trying to determine the origin of blood stains in someone's home. Messenger RNA markers have also been identified for saliva, semen, and vaginal secretions. By profiling mRNA contributions from a crime-scene sample, investigators should be able to tell what type of fluid they're working with. In sexual assault cases, this knowledge "might be very important to determine if a sample is coming from semen or from saliva or blood or skin," McCord says.

Basic researchers are also developing other RNA-based methods for forensic use. Two studies have shown that RNA expression profiles can reveal the age of a bloodstain, because different RNA degrades at different rates. If this technique pans out, it will be extremely valuable to forensic scientists, Foran says, as there is currently no method to determine whether a bloodstain is one week or two years old.

With current technologies, a common problem in crime labs arises when investigators have no tenable suspects and crime-scene stains get no match in CODIS. "If there isn't a hit and they have no other evidence to go on, it's really kind of a dead end," says Raymond Miller, of Washington University. Miller is collaborating with several local forensic labs to find out whether they can glean information about a person's ethnicity solely from DNA sequence.

Recent work in human genetic variation suggests that researchers may be able to extract information about a person's ancestry from certain key genetic markers. Although all humans are very similar genetically, people from different geographical populations tend to be slightly more different from each other than are individuals from the same ancestral group.

Over the past several years, scientists have identified a number of ancestry informative markers (AIMs) that are particularly suited to distinguish different ethnic groups. Miller's group is working to develop a panel of about 16 AIMs that can distinguish ethnicity well enough to assist police officers in investigations. Although such markers will probably never be able to pin down a particular ethnic group, much less give an accurate perception of what someone looks like, "if we could provide any kind of information from that DNA sample, then that might help investigators," Miller says.

A major challenge in this area is finding markers that can deal with heavily admixed populations, or those in which people of very different ancestries have blended together. "My suspicion is that it's going to be really tough," Miller says, although he's hopeful that AIM profiles in such populations could at least rule out some individuals.

Some researchers believe that someday DNA profiling may go one step further than revealing someone's ancestry by revealing what a person actually looks like. There are better markers than ancestry for physical appearance, including genes that control pigmentation levels, height, and facial features. Although some studies have been fairly successful at identifying traits based on gene sequence—a study from 2001 found that 90 percent of people with particular variants of the human melanocortin 1 receptor gene have red hair—many studies have shown fairly unreliable correlations between known gene variants and physical features.

"To date, the genes discovered are fairly crude," Gill says. "There's a long way to go with this kind of analysis."

Adopting new technologies

For now, the forensics community confronts more immediate hurdles. Getting new technologies developed by researchers into forensic labs for use in

For more information, visit these sites:

www.fbi.gov/hq/lab/labhome.htm
www.bodetech.com/services/overview.html
www.orchidbio.com/services/forensics.asp

actual casework is a slow process, Foran says. "Unlike all the rest of science, in forensics you can't just change something because you heard that it works better," he says. Every new technique, protocol, or piece of equipment must be validated by a series of different kinds of tests, and validated by each individual lab using it.

"I think the forensic community is conservative, and rightfully so," McCord says. But because it's so time- and labor-intensive to validate new techniques, "nobody is going to bring these techniques online unless there's some sort of crying need for it."

The result is that some more-specialized techniques, like mtDNA typing, are performed by only a few labs in the country. If another lab needs mitochondrial analyses, they simply contract an mtDNA lab to do the work for them. But with advances that are both extremely powerful and technically fairly simple, such as analyzing Y chromosomal markers in rape cases, "there's a big push to get something like that validated in the laboratory," McCord says.

"There are so many new people coming into the field [that] I think the biggest challenge right now is getting everybody trained," Butler says. "In terms of technology, things are constantly improving and developing. It's how well people get trained and educated in those things [that determines whether] the new techniques can be incorporated into the lab."

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