DNA: Collection and Interpretation

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In this second article of a mini-series focusing on forensics, author Tracy Alexander looks at how the use of DNA profiling has been developed and refined, where DNA samples are collected, and how they are used.*

The use of DNA evidence in criminal trials has increased beyond imagination since its first use in the mid-1980's. Deoxyribonucleic acid is a macromolecule generally found within the nucleus of cells. The genome is the entirety of this cellular DNA and encodes all the genetic information that governs an organism's structure and function, and is unique to that organism, except in genetically identical individuals. Analysis of the variable sections of DNA is frequently employed to determine paternity and to resolve immigration disputes, but public perception is focussed on its application as an aid to police investigative processes, to identify victims of crime and, by the transfer, exchange and persistence of traces of DNA, the offenders responsible for crime.

The nucleus is not the only place that has DNA; cells contain tiny sausage-shaped organelles known as mitochondria. These are the powerhouses of the cell specifically designed to make energy. Each mitochondria has between 1 and 10 copies of circular DNA that code for proteins required for energy production and each cell may have hundreds of mitochondria depending on its function (for example, mitochondria are found in large numbers in muscle cells). Hence in adverse conditions when the genomic DNA is too severely degraded to be useful it is sometimes still possible to get some useful genetic information from the mitochondria. This information is not as statistically powerful as genomic DNA but is very useful in identifying family members. The mitochondria are inherited through the maternal line as at fertilisation it is only the sperm's nucleus that is injected into the egg, not the mitochondria.

Whilst all nucleated cells contain DNA, not all nuclear DNA is contained within cells. Like many of the elements that go to make up an organism the DNA is recycled. Dead cells are harvested and the DNA broken down. In the process of this long stretches of DNA can be found in the plasma and, a recent discovery, in sweat. This discovery has far-reaching implications for the evidential value of "touch" DNA, particularly in terms of secondary and tertiary transfer between individuals and touched items.

Sampling

The National DNA Database (NDNAD) holds samples from three sources: personal samples from those arrested or charged, crime scene samples and voluntary personal samples. Before 2004 these were known as Criminal Justice (CJ) samples, and were not admissible as evidence without confirmatory samples being taken on the re-arrest of a suspect. The taking of this second sample could entail a delay of up to two weeks during which time the suspect had the opportunity to abscond or to undertake multiple crimes, knowing their arrest was imminent. However, since 2004 samples are taken under the provision of the Police and Criminal Evidence Act (PACE) and can be used for evidential purposes.

Interpreting DNA evidence in the lab – sample preparation

There are three possible samples that can be taken from arrested persons for search against and inclusion on the database: blood, buccal scrapes (from the epithelial lining on the inside of the cheek against the buccal muscles) and pulled head hairs. Improvements in technology have meant that blood samples which used to be taken from major crime suspects due to the likelihood of obtaining a usable profile, have been largely replaced by the use of buccal scrapes. Pulled head hairs are an option if a suspect refuses to give a buccal sample voluntarily.

From a crime scene there are many sources from which to obtain a DNA profile, with varying degrees of evidential value depending on the circumstances.

Often blood is found, particularly at major crime scenes. The red blood cells, of which there are about 4.5-5 million red blood cells per micro-litre (one thousandth of a millilitre) of blood, have no nuclear or mitochondrial DNA. It is therefore only the white blood cells, of which there are only 5-10 thousand per micro-litre of blood, which can be analysed for a DNA profile.

Saliva does not contain DNA at point of production but epithelial cells from the inside of the cheek regularly slough off and are deposited in the saliva in sputum and on items coming into contact with the mouth, such as cigarette butts, drinking vessels, masks, gags and on licked stamps and envelopes.

Hairs with roots are a good source of DNA but dead hairs that have fallen out (telegen hairs) usually only contain mitochondrial DNA. The source of a hair, i.e. where on the body it has grown, may be identified by examining it in section. Determining the source may add value to an investigation, for example finding head hairs on the boot of a suspect from a victim who has been kicked in the head may indicate the individual responsible for a fatal injury. Chest hairs on a particular knife in a multiple stabbing incident with more than one suspect may indicate which weapon caused wounds to the chest and thereby which suspect is responsible for the chest injuries.

Sexual assaults are often resolved by finding an exchange of material between victim and suspect. Therefore a large number of samples are taken from victims in order to find traces from the suspect, either at a police station, hospital or rape suite. These include internal and external vaginal and anal swabs, plus samples from the mouth and any other pertinent areas, such as where the victim has been licked or bitten. On arrest a suspect will also have numerous samples taken in order to find traces from the victim, including swabs from the glans and shaft of the penis. Exhibits are also collected from a crime scene to identify DNA from all parties involved. The ability to split DNA between semen and vaginal material is vital in the investigation of sexual assault using DNA evidence (the cellular and seminal fractions). Semen is only produced by post-pubescent men with approximately one million spermatozoa per ejaculate. Vasectomised and naturally azoospermic males have a vastly reduced number of spermatozoa per ejaculate but it is often still possible to obtain a profile.

Footwear and clothing are often analysed for 'wearer' DNA as well as trace materials, which may come from cells sloughed off by close contact with the item or from sweat, as with 'touch' DNA. Other items that have come into contact with biological material can also yield a DNA profile. One of the suspects in the Canary Wharf IRA bombings in November 1992 was identified from nasal debris found on a piece of green tissue paper recovered from a van containing a bomb that had failed to explode.

Trace amounts of DNA can be found in urine. They are hard to identify as they are contained only in discarded cellular material washed from the walls of the urinary tract and are diluted by other waste products. Similarly, very fresh faecal material may also yield trace amounts of DNA in mucal secretions on the outside of the stool.

DNA is also used for identification of the deceased. Venous blood is preferable but not always possible, particularly if the victim has bled profusely. Where a corpse is badly burned or heavily decomposed samples may be sought in deep muscle tissue where nuclear DNA has not been exposed to degradation, or, if there is none remaining, DNA may be obtained from bones or teeth.

Processing and Analysis of Samples

In all circumstances a protocol for the collection of samples must be strictly followed to ensure that there is no cross contamination. Officers collecting samples will wear protective clothing, and once obtained, samples are sealed in sterile containers before processing. Crime scene samples and arrestee samples are processed separately, often at a completely different laboratory site.

Arrestee samples are usually buccal scrapes, one from the inside of each cheek. The two samples, 'A' and 'B' are kept separately, with the B swab stored for a maximum of 6 months after the swab was taken, allowing it to be used in the event initial testing fails. Crime scene samples then go to a casework laboratory. Since the samples come in many forms each sample will be treated differently to extract the DNA, and some will require presumptive testing to locate the DNA for profiling and to establish whether a crime scene stain is from saliva, blood or semen.

Stains and samples are then chemically treated to extract the DNA (either manually or robotically) and a process of quantification follows. A fresh sample will almost always provide an adequate amount of DNA. The smallest standard starting template permissible is a one nanogram (one millionth of a gram per millilitre) sample.

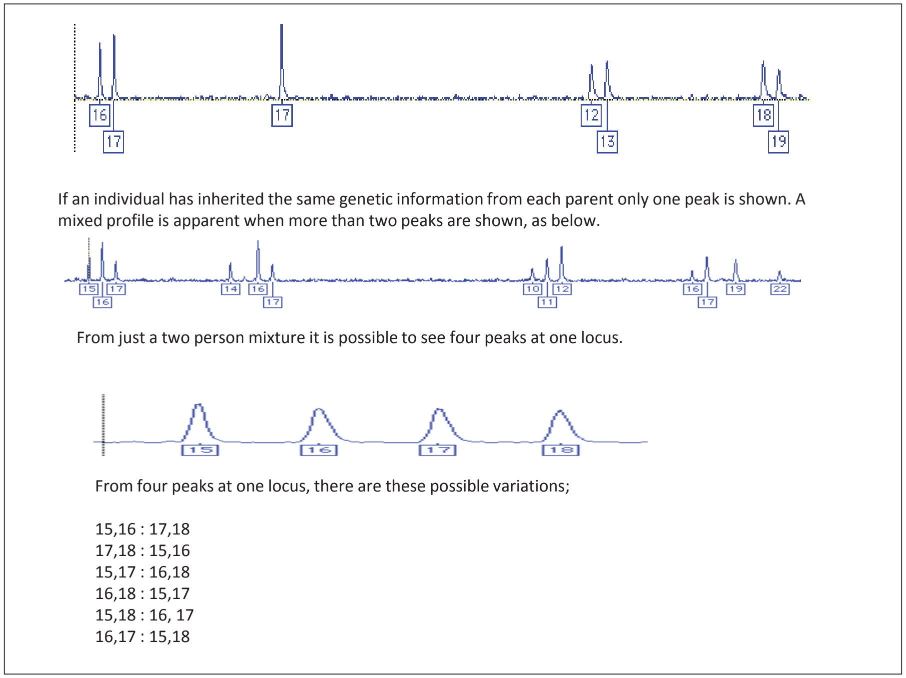

Figure 1. DNA Electropherograms to demonstrate mixture interpretation

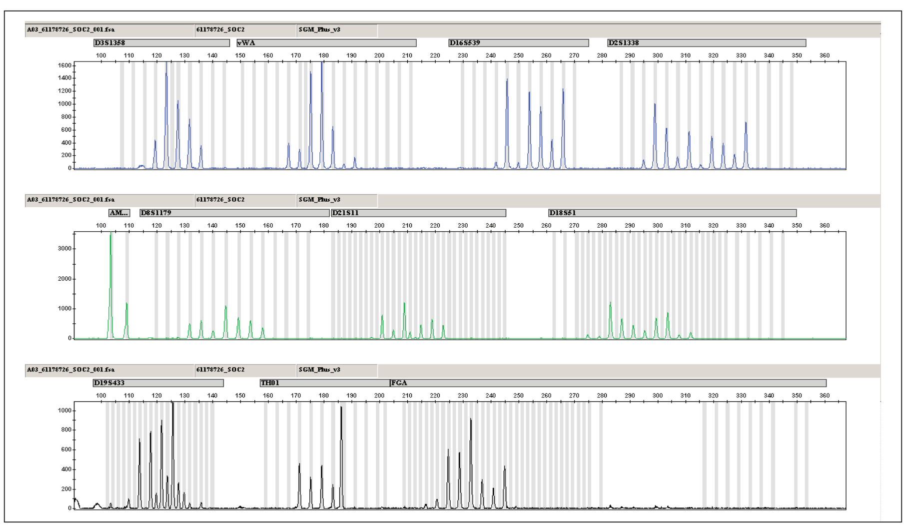

Figure 2. Example of a complex mixture

This is subjected to the standard 28 cycles of polymerase chain reaction (PCR), a process which enables the copying of small amounts of DNA and which then provides sufficient amplified DNA to produce a profile which can be converted into a numerical code. If sufficient information from the profile is obtained, then this is sent to the NDNAD where it can be digitally compared to the millions of subject and crime stain profiles held there. Should there be no match, the profile from the crime scene sample will be held on the database indefinitely in case a match should appear in the future, either person-to-person, person-to-crime stain or crime stain-to-crime stain. However, should the database indicate a match with a profile held on the database, then the information is passed on to the investigating police force and a warrant for the arrest of that person is issued. Provided there is other supporting evidence, this means that the police can proceed with a prosecution.

The limitations of the system

Even with the incredible advances in technology, crime scene DNA profiling can be used to conclusively exclude a person from an enquiry but even though it may provide compelling evidence of association, it is not proof of identity. Interpretation, particularly of complex mixtures, can be affected by many variables from interpretation guidelines differing between forensic providers to operator subjectivity. In addition, it is estimated that the addition of partial crime stain profiles to the NDNADB may mean that approximately 0.1% of matches are adventitious, that is, occurring by chance. The current sensitivity of profiling means that approximately 75% of crime scene profiles generated are mixtures. Finally, despite extensive research in many different areas from collection to extraction to profiling, not enough is known about the propensity of individuals to leave their DNA and how that DNA is subsequently transferred between objects and individuals.

Whilst profiles obtained from the 'Next Generation' technologies will increase the discriminatory power of profiling, the inherent increase in sensitivity will lead to more background DNA becoming part of a profile, so mixtures will be even more common, and the increase in the number of sites will make these mixtures even more difficult to interpret accurately, and will take longer, with inevitable repercussions. Despite public perception, DNA profiling is never the complete answer to solving crime, but continues to assist police forces as a vital aid to the criminal justice process.

Interpretaion of DNA profiles

The next article shows how the use of DNA profiles has contributed to and also changed the Criminal Justice system.

**Article 1 - DNA Technology: 150 years or Research and Development, by Tracy Alexander. ILM January 2014, Vol 39, Issue 1. Tracy Alexander BA(Hons), MSc, Cold Case Review Manager, LGC HQ, Room A018, Teddington, Email: Tracy.Alexander@lgcforensics.com*

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