# The Scientific Basis of DNA Technology

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A former NSW policeman, Harry Blackburn, was cleared of multiple sex charges on the 11 October 1989, and a Victorian was convicted of rape a week earlier. In both cases DNA (deoxyribonucleic acid) profiling was used. DNA profiling illustrates the most powerful concept in forensic science, the power to **eliminate a suspect**. DNA profiling has a very high **discrimination** power and is therefore very powerful in elimination. Its discrimination power is so high that it also has a major use in *identification*. These two aspects must be kept distinct.

**An example from common experience**

These concepts will be examined with a familiar example:

Four independent witnesses to a crime were asked to identify a suspect in a police line up. They based their identification on the following:

No. 1 The two were indistinguishable in that they both had two legs.

No. 2 The two were indistinguishable in that they both had two arms.

No. 3 The two were indistinguishable in that they both wore clothes.

No. 4 The two were indistinguishable in that they both had dark hair.

The evidence of all the independent witnesses is consistent. Convict!!

How likely is it that a man with two legs will also have two arms? We answer that question from a knowledge of the population of all human beings, so-called population statistics. We do not answer it from a knowledge of criminal suspects.

A knowledge of human populations tells us two things:

- Having two legs is very common. This test will not have much diagnostic value.
- If a person has two legs, then it is very likely that he will have two arms as well. The second witness does not corroborate the first because the two tests are not independent.

**An example from current practice**

A real life example of how statistical parameters are evaluated and presented is provided by the current techniques of paternity testing. This civil procedure has many aspects in common with forensic investigations. For example: It has become of increasing importance locally in the light of changes in the *Family Law Act* with its intention to reduce social security payments by garnisheeing the wages of errant biological fathers (Magnusson & Selinger 1988).

The initial testing normally consists in analysing blood samples for eight independently inherited red blood systems. Unlike arms and legs, these tests are selected and tested to ensure that they are all independent. Given the result of one, the result of another is neither more likely, nor is it less likely.

*Table 1*

**The Process of Paternity Testing**

| 1<br>Red cell System | 2<br>Obligatory Paternal gene | 3<br>Alleged Father x | 4<br>Cumulative x | 5<br>Random Man y | 6<br>Ratio x/y | 7<br>Cumulative Ratio x/y |
|---|---|---|---|---|---|---|
| ABO | $A_1$ | 0.5526 | 0.5526 | 0.1755 | 3.15 | 3.15 |
| MNSs | *Ms* | 0.5000 | 0.2763 | 0.2991 | 1.67 | 5.26 |
| P | $P^2$ | 1.0000 | 0.2763 | 0.5016 | 2.00 | 10.49 |
| Rhesus | *cDE* | 0.4693 | 0.1296 | 0.1444 | 3.25 | 34.10 |
| Kell | *k* | 1.0000 | 0.1296 | 0.9525 | 1.05 | 35.81 |
| Duffy | $Fy^a$ | 0.5000 | 0.0648 | 0.4351 | 1.15 | 41.15 |
| Kidd | $JK^a$ | 1.0000 | 0.0648 | 0.5162 | 1.94 | 79.71 |
| Colton | $Co^a$ | 1.0000 | 0.0648 | 0.9619 | 1.04 | 82.87 |

**Total probability** cumulative x = 0.0648 cumulative y = 0.000782 cumulative x/y = 83

Column 1 names the tests used, starting with the familiar ABO blood grouping. Column 2 states the gene carried by the child which must have come from the father, because it did not come from the mother. The alleged father has all these genes and so none of these eight tests **excludes** the alleged father from paternity. The results are `consistent with' the alleged father being the biological father. However `consistent with' is the dirtiest phrase in forensic science. Consistency must be quantified.

The emphasis shifts to calculating the probability of **inclusion,** that is the actual probability of paternity. (It is assumed that one sperm fertilised the ovum from which the child developed, and that the identity of the child's mother is not in doubt).

Column 3 calculates the probability (according to each of the tests in turn[1]) that the alleged father supplied the sperm that produced the child. Column 4 multiplies the individual probabilities together, to give the total probability for the combination of independent tests.

The more tests that are done the lower the calculated probability that the alleged father is the biological father (column 4). The probability **decreases** as more tests are done. It is down to 6.5 per cent by the eighth test. If more tests were done, it would decrease even further.

**Why?**

`Your Honour, if you will allow my client, the alleged father, to mate with the plaintiff, (the undisputed biological mother), I will show that this child before the court, of whom he is accused of being the alleged father, will not be produced!'

We have asked the wrong question. Study of only the `suspect' (alleged father), and the `crime' (mother and child), leads to an erroneous conclusion.

The only question that makes any sense is to ask about a **ratio** of probabilities:

Given the fact that this child was in fact produced, how much more likely is it that the alleged father produced it, compared to any other man selected at random from the relevant population? To answer this ratio question, a comparison is made between the chances that a single sperm, carrying all the necessary genes contributed by the biological father, could be produced by the alleged father (this probability is called $x$) and the chances with which such a sperm might be produced by a man randomly selected from the same population as the alleged father (this probability is called $y$). The calculation of $y$ requires an accurate knowledge of population statistics. With values for both $x$ and $y$, it is possible to calculate $x/y$.

The ratio $x/y$ in column 6 gives the number of times the alleged father is more likely to be the biological father than is a random man. It is the odds ratio in favour of the alleged father, over a random father, being the biological father. Column 6 shows that each individual test gives only a relatively small odds ratio implicating the alleged father over the random man, but column 7 shows that the cumulative odds for the independent tests increase as more and more tests are included.

The traditional alternative ways of presenting this result are as follows:

| | |
|---|---|
| Paternity Index: X/Y | 83 to 1 |
| Relative Chance of Paternity: X/(X+Y) | 98.8% |
| Relative Chance of Non-Paternity: 100 RCP | 1.2% |

The need for this ratio occurs again and again in forensic science. It is seen in matching paint samples, in matching glass fragments or pieces of fabrics, in matching tool marks on bullets. It is required every time there is a move from stating a conclusion in terms of exclusion to stating one in terms of inclusion.

The need for a ratio rather than a single probability cannot be over-emphasised.

## DNA Profiling

Although DNA profiling is used in a similar way to conventional blood grouping, for the elimination or association of suspects with a crime, the possible vast increase in discrimination power allows much firmer statements to be made. There is also only a small chance of a false positive result. The greater discrimination power also means that large populations can be screened as an alternative to conventional crime investigation. DNA profiling is complementary to conventional blood grouping in a rape investigation because blood group substances are contained within the seminal fluids, while DNA is contained in the sperm, which can be separated and kept frozen.

DNA profiling must be kept in perspective. It is an extension of technology which allows more precise results. Law cases where the identity of the person is the only substantive issue are in the minority. In sexual assault cases the question of consent is often paramount, in assault the question of provocation and so on.

The science behind the technology will now be looked at.

*Some simple genetics and molecular biology*

Our genes consist of long twin molecules of DNA wound in a double helix. Forty-six of these form the 46 chromosomes found in nearly all of our body cells. Four different bases, represented by the letters C, T, G and A are attached to each strand, and these bases pair off to hold the two strands together.

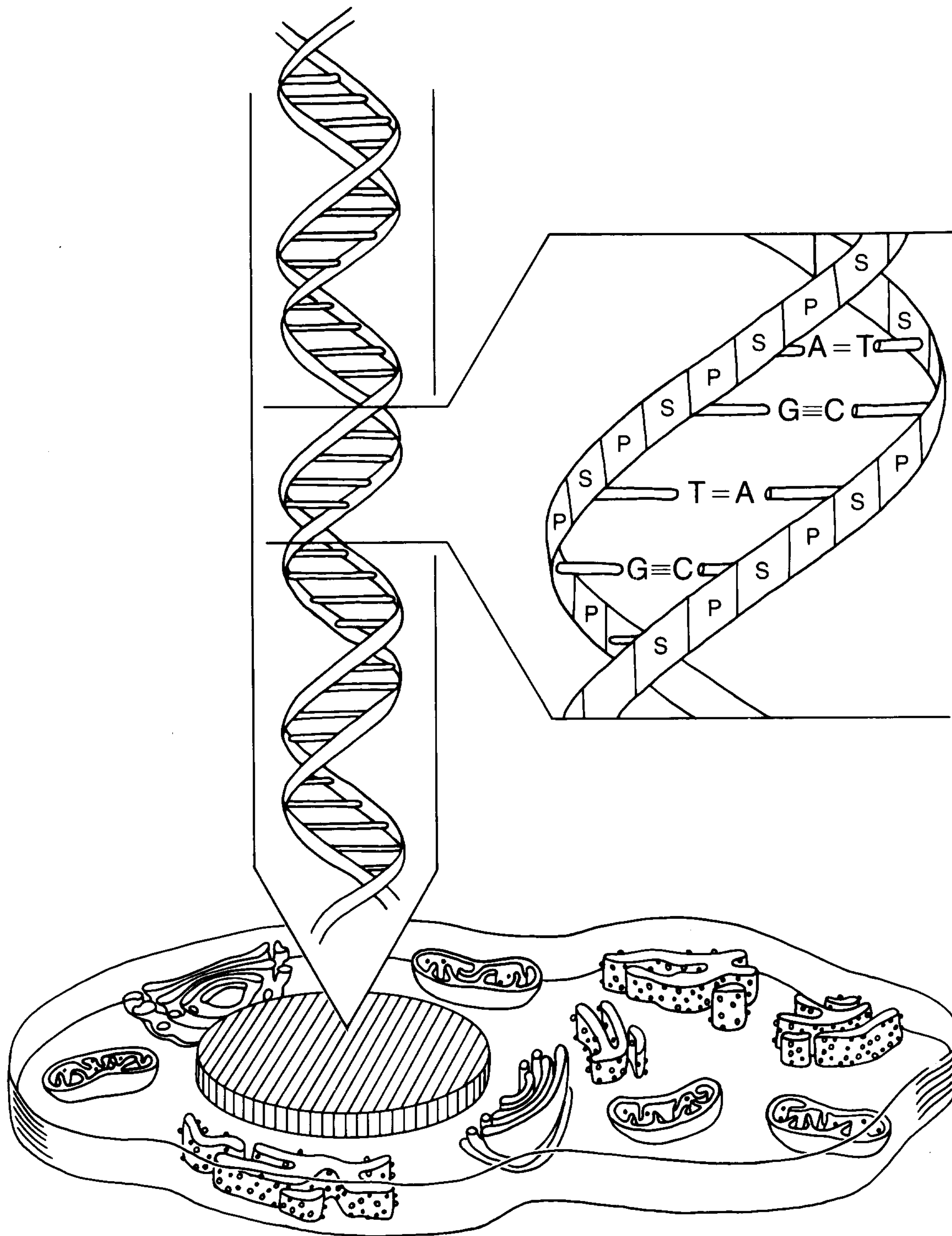
The sequence of base-pairs act as a four unit code, just like a dot and dash form the two unit Morse code. Because there are four bases, these can code for $4^3 = 64$ items, which covers the twenty or so amino-acids that go to making up proteins plus punctuation for starting and stopping production. A section of DNA coding for one protein is called a gene and the length of a gene can vary from 100 to 5,000 base pairs. The human code is approximately seven thousand million units long[2].

The DNA code specifies our genetic heritage by coding for the production of proteins[3]. Our proteins are us. An important set of proteins are the enzymes which make the chemistry of our bodies work. As we all have similar chemistry, much of the code is therefore identical between individuals. Some of it differs. The primary blood grouping is called ABO. It results from the product of a single human gene (defined by carbohydrates synthesized by enzymes). One form of the gene (the A-allele) produces the enzyme which makes A-type blood, while another form of the gene (the B-allele) makes B type blood. The absence of both results in O type blood. The presence of both enzymes makes AB type blood.

Chemical reagents based on an immunological reaction are used to pick up these blood types. Other bases for groupings are also being used as shown in the earlier table. Taken together they narrow down the choice between individuals considerably, but not exclusively. Blood grouping tests can also go wrong occasionally.

There is, however, much more DNA sequence in our cells than is used for coding and switching. United States' geneticists discovered in 1980 that certain regions of DNA did not appear to code for anything in particular and also differed from one person to another (except in identical twins). These nonsense regions of code were called hypervariable regions.

*Figure 1*




Alternating units of sugar and phosphate form the sides of the DNA ladder, while base pairs (A-T and G-C) form the ladder's rungs. T unites exclusively with A, and G unites exclusively with C.

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What was interesting was that within these nonsense regions there were a number of short sequences (about 15 to 20 bases long, for example GGGGGGGAACAGCGACAC), each of which is then repeated many times over. These short sequences are common, but the number of times that the sequence repeats is different between individuals. The number of repeats could vary from 200 to 1400 times, so one is looking at the chance of two individuals picking the same number between 200 and 1400. By using four short sequences one is asking two individuals to pick the same four numbers between 200 and 1400.

DNA cutting enzymes can recognize specific pieces of code and can be used to cut out the repeating sequences of interest.[4] Jeffreys in the United Kingdom identified two of these short sequences in 1984 and made probes for them.[5] Probes are complementary sequences of DNA which bind with the original one, and if made radioactive, can be used to identify and visualise the sequence on photographic film.

*Experimental technique*

The experimental technique employed is not complicated, but involves a very large number of simple steps, which must be performed meticulously and with understanding of the processes involved. A simplified description in terms of five steps follows, but this is only schematic.

In step 1, the blood sample, swab, semen stain, etc. is taken into solution and the cells are ruptured to release the DNA.[6] An enzyme similar to that used in some laundry detergents is used to digest the protein, which is separated out by dissolving it in phenol. Phenol and salt solution are fairly immiscible (they do not mix to a great extent.)

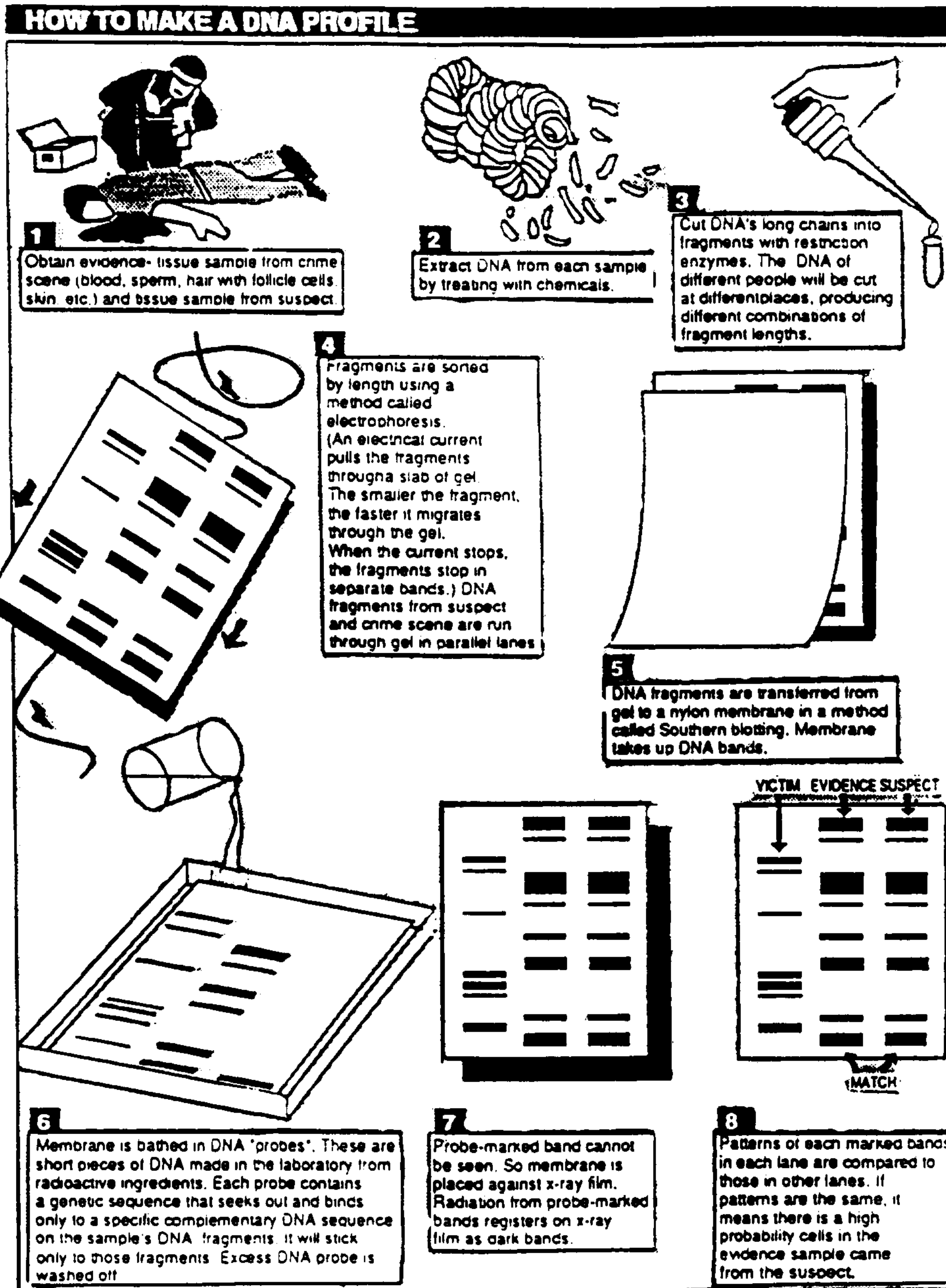
In step 2 the DNA is cut into fragments defined by having the cuts occurring at the same sequence of code every time.[7] The repeated sequences are kept within these fragments.

In step 3 the cut DNA is sorted according to size. The most convenient way of doing this is to make the fragments move through an electrophoresis gel under the influence of an electric current. The smaller the fragment, the faster it moves.[8]

In step 4 the identification process is based on the ability of single strand DNA to pair with a second strand of complementary sequence. As a first step the DNA is made single stranded, that is the double helix is unwound. The single strand DNA is transferred to a plastic membrane and it is ready to pair with DNA which has a complementary sequence. The probe which is used to pair is a synthetic piece of DNA which has several atoms replaced by radioactive isotopes of the usual atom. The probe is complementary only to the repeated sequences of interest. The probe is washed over the membrane to allow pairing to take place. The positions at which the probe has reacted has revealed photographically because the radioactivity exposes the film. This produces an autoradiograph.

*Figure 2*

**DNA: The Vital Witness**




(Steps do not correspond to text)

Source: *The Age*, 12 October 1989. Reproduced by permission.

### *Interpretation*

As in most forensic work, the interpretation relies upon a direct comparison of the profiles obtained from the crime scene and the controls. If the band patterns match then there is evidence that the samples are of common origin. The degree of matching depends mainly on the number of bands present in the profiles. It will be assumed that the likelihood of a band occurring by chance, in a second individual's profile is 1:5 (This depends on how accurately bands can be located, that is on their thickness and reproducibility) and that the position where bands occur is independent. Then the chance of two bands matching is 1:25. Statistical independence is a critical technical requirement.

At this point there are distinct differences between the Jeffreys ICI-Cellmark approach on the one hand and the Lifecodes - Genetic Technologies approach on the other. The former single multilocus probe is best suited to paternity testing because it provides more information per plate. Some bands may be missing, but this does not invalidate the paternity match. The latter single locus probe is better for forensic work, because there is far less chance of having missing bands (that is unexplained dissimilarities). However, the odds ratio has to be built up with the use of a number (4 to 5) of separate probes, and this requires more work and more standardisation. The comparison of profiles is best carried out when all the samples have been analysed on the same gel, to remove variability between gels and running conditions. This is not always practical even under normal conditions and impossible if the sample size is large. When different gels are used, internal standardisation is required. A known standard is run several times on the same gel, so that slight variation between gels is reflected in the movement of the standard. This correction and assessment is currently done by eye, but may be able to be computerised.

If there is insufficient DNA, or it is partially degraded, then only a partial profile will be obtained, and the odds ratio will be lower. Thus, in current attempts to identify the remains of Josef Mengele, the infamous doctor of Auschwitz, from DNA in his alleged bones by comparison with the DNA profile of his son, the problem is in the degradation of the DNA (Joyce & Stower 1989). Everything depends on the quality of the sample. If the amount is too small, then it can be amplified by a technique called polymerase chain reaction (PCR). While this technique is useful for exclusion, its power to identify is low.

The convicted Victorian rapist (G.G. Kaufman) initially refused to give a blood sample, so an analysis of blood from his estranged wife and daughter provided a control for half his DNA profile. Eventually DNA profiling linked five of the rape victims to the rapist (Nolan 1989).

Lawyers are wary of DNA profiling for a number of reasons, some good and some not. DNA profiling is the most powerful break yet to appear with the English common law tradition of the right against self-incrimination. Lawyers do not like `machine' evidence, where a scientific technology in effect dominates a verdict. However, DNA profiling is not a foolproof technology in the strict sense of the word.

In the hands of a skilled operator it provides incisive results. In the hands of a fool it provides rubbish. Its major strength is that the rubbish is obvious, it will not produce a false positive result from a sample. (A false positive is the conviction of an innocent party). However, the process is still only as reliable as the sample it receives, so if there is a careless or fraudulent collection of sample from the crime scene or suspect, then the result will be wrong independent of the accuracy of the science. If the sample is degraded, then the odds ratio will have less probative value.

The process involves a very large number of small steps, each of which has to be done correctly. The major source of error is in mixing up transfers of material from one step to the next. Housekeeping has to be impeccable.

The technique is also unique in the way that it has become commercially valuable property as current patent challenges testify (Lifecodes Corporation 1985).

Open scientific techniques are self-correcting because they are in the open literature and information is exchanged. Scientists check each other's work and are quick to comment on it critically, as the episode on cold fusion has demonstrated. The problem with such commercial secrecy is that critical aspects of the technique may not be available to the operators. A lawyer could choose to ask a well-placed question in court on just such a point. The question may have no bearing on the matters of substance, but this has never yet inhibited a lawyer out to score points by embarrassing a witness.

But there are real questions of substance in DNA profiling in forensic cases, which must be explored by counsel. To this end we have been developing our forensic inference chart concept[9] to empower lawyers to ask the `right' questions to obtain the truth. It will be a shame if they merely abuse this and seek only to win their case.

## Conclusion

Like many forensic techniques, it is the power of DNA profiling to exclude the innocent that is its greatest value to society. It also shows a quantum jump in its ability to convict the guilty. Justice demands a full understanding of the technology by the courts, the jurors and general public.

## Footnotes

1. Personal communication: D.S. Ford, National Blood Group Reference Laboratory, Commonwealth Serum Laboratories, Parkville Victoria.

2. However, most of the bases in DNA (approximately 90 per cent) do not actually code for proteins. Some of these extra sequences are responsible for switching protein production on and off (for example growth hormone production), some tell the cell whether to secrete a particular protein or to locate it in a particular compartment within the cell or in the cell membrane, and some are required simply to maintain the structure and function of chromosomes. (As every cell in our body (except the germ cells) contains the same code, the differences between cells (from hair to liver) depend on what part of the code is activated. Even the function for activating the start of some cancers appears to lie in the genetic code. There is some evidence that the universal invariance of the DNA in all cells is not quite true and this could have dire consequences for DNA fingerprinting.

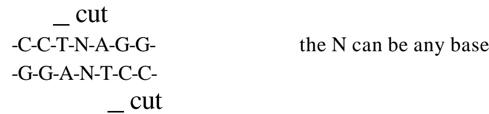
3. Genetics is the science of diversity. What distinguishes us from the Orang-utan, or from one another? Very little, if the overall DNA code is looked at. The variability between individuals is called polymorphism (from the Greek `many forms'). Small differences in the code in our genes between individuals can lead to gross differences in our bodies. On the other hand, because more than one codon can code for a particular amino acid, we can have a difference in a gene which causes no difference in function at all.

4. The chromosomes are of different length, but together they contain about $7 \times 10^9$ base pairs. This corresponds to $7 \times 10^{-12}$g, or seven millionths of a millionth of a gram of material, but stretched out this DNA would be about two metres long. We thus have a large amount of DNA in our cells. To find out how a particular section varies from one person to another the DNA needs to be cut up into manageable bits and then the particular piece of interest identified. The molecular biologist cuts the cell DNA up into bits using what is called restriction enzymes. These are molecular scissors which cut whenever they see a piece of specific code in the DNA. A cutter which cuts when a particular 4 base sequence occurs can be used. This will be on average every 44 = 4096 bases. What sequence will code the cut can be chosen from a selection of about 500 cutters. The size of the cut bits is also controlled by how long the process is allowed to occur. The shorter the time, the larger and more random the bits. You can imagine the mess in such a soup. It has been likened to working in the US Library of Congress with no catalogue and the books randomly arranged.

5. Conceptually the simplest, and increasingly the preferred method of production of the probe is by organic chemical synthesis. Because there are many synthetic steps involved in the process it is critical that the efficiency of each step is very high. Automated DNA synthesizers are available commercially which have >99 per cent efficiency per base added to the probe and a very rapid throughput. A probe of 17-20 bases can be prepared in unlimited amounts for approx $250 in 3-4 hours of automated synthesis. The probes are labelled by simply attaching a radioactive atom (called a label) on one atom (the amount of radioactivity per gram is very high for short probes and weaker for large probes because there is one label per molecule).

6. DNA from semen can be detected, on average up to 20 hours after intercourse, but this depends on drainage, sperm count, etc. It is possible to separate the DNA of male origin if mixed with vaginal secretions. In the case of stains a larger sample is required than in conventional tests but this need is continuously being reduced. It is important that stains are dry, as moist conditions promote bacterial growth which degrades DNA. The test loses sensitivity but not specificity. Spurious bands do not appear, although useful (large) bands can disappear. Suitably stored samples can be studied years later. Hair roots can be examined, but at least 20 are required.

7. The enzyme MstII recognises the following pattern on a double strand and cuts as shown




The pattern is symmetric about the centre and so both strands are recognised. The enzyme consists of two identical proteins linked together to form an active site (cutting edge) and both strands are cut at an equivalent point between the same bases.

8. In order to sort out the fragments on the basis of size, a jelly like material with holes about the size of the DNA fragments is used in which the fragments run an obstacle race. The soup of DNA fragments is placed in a slot at a starting position and the race is run by applying a modest electric voltage (between the start and the finishing line). As the DNA fragments are negatively charged the finishing line is made the positive end of the electric field. The fragments start their race but their individual speed depends on the size of the charge and the mass of each fragment. Thus the fragments spread out as they run in proportion to their mass with the smallest `horse' winning and the largest hardly leaving the gate. A dye is added which moves faster than the smallest fragment and tells the operator when to stop the race. This procedure is called electrophoresis. The `race' at the finish has so many horses that there is a continuous spread of material. To find out if there are any fragments of complementary sequence to the probe and to see where they occur in the gel, a technique called `Southern' Blot Hybridisation is carried out. As the DNA in the gel is double stranded and thus unreactive, it is first denatured with alkali to produce single strands. The pattern of single stranded DNA is then transferred (by a technique called blotting) on to a thin nylon membrane, rather like preparing an inked printing block. The DNA in the pattern is bound covalently (strongly) to the membrane in a pattern identical to that in the original gel. The membrane is now brought into contact with the radioactively labelled probe and the probe pairs with any corresponding sequences (hybridisation) on the membrane. After a while all the unbound probe is washed away and the membrane is dried and exposed to X-ray sensitive film. The original pattern of DNA banding remains but only those bands that have bound to the probe will be visualised. The visual pattern is thus much simpler.

9. See paper delivered at this Conference by Eric Magnusson. This research is supported by the Law Foundation of NSW.

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# What is Involved in Producing Sound DNA Evidence?

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**DNA doesn't lie!? - How to examine the Laboratory conducting the Test**

**DNA** results have appeared in court. Fantastic odds have been quoted - ten billion to one! What does it all mean? Do the tests follow reliable scientific premises? Are the results reliable? What safeguards did the laboratory performing the test use to ensure its validity?

This paper will show how to examine the reliability of DNA results performed by a testing laboratory. To do this an understanding of how the laboratory functions, both at managerial and at operator level, is required. By examining a variety of areas an overview can be constructed and the functioning of the laboratory can be assessed. The overall aim is to establish the continuity and consistency of the process.

The areas which need to be evaluated are as follows:

*Critical Records*

These contain formulations of solutions and also Lot and Batch Numbers including expiration dates. They are used to track certain batches of chemical reagents used in the tests which may not perform to specification. If these records are absent or improperly maintained it indicates that any inconsistencies in the results may be difficult to trace and any problems which do occur may be compounded.

*Equipment Calibration and Maintenance Logs*

The equipment and maintenance logs are a record of the care and attention paid to maintaining the equipment used to perform the test. They indicate the application of the principles of good laboratory practice and their presence and proper maintenance is consistent with a well managed and maintained laboratory. Many chemical reactions are temperature and time dependent. The temperatures and times which are used for chemical reactions are only as reliable as the equipment that is used to measure these parameters. Another important measured parameter is pH - certain enzymes only perform within a specified pH range. Incorrect, or non-measurement of pH may result in an incomplete reaction preventing the production of usable results.

*Planning and Laboratory Organisation Manuals*

The work flow of the laboratory needs to be well planned in order to produce results in the appropriate time frame and to avoid the possibility of errors, for example sample switching.

> Sample question: What measures are taken (in your laboratory) to separate different areas of testing, for example criminal and paternity tests?

*Personnel Structure*

The laboratory should demonstrate a management philosophy that enables all those who are employed to understand the importance of their position and responsibility, for example all employees are responsible for the quality of their workmanship. Job descriptions and decision trees provide indications that management has broached the difficult problem of defining responsibilities. However, these charts only serve to indicate certain guidelines and it is important to assess whether functional lines of communication and consultation exist. A decision tree should be available for the direction and assistance of new staff members. If the laboratory lacks any clear management philosophy and documents do not exist it will often be that the lines of communication and consultation do not exist and no-one is responsible or accountable.

*Documentation of Specimen Handling*

This should cover the receipt and treatment of each specimen from the time it enters the premises to the time the results are despatched and the specimen is returned to the client.

## Further Investigation of the Laboratory

Critical records should contain the following information:

*DNA Isolation*

The first step in working with DNA is to extract the DNA molecule from the biological material in which it is found. To do this a series of chemical reactions is performed. The following items are used to assess the outcome of these manipulations and to direct the next steps in the process of identification.

1. Yield gel (quantity of DNA).
2. Quality of DNA (degraded or undergraded).
3. Lot numbers - calibration standards (these provide a means of measuring the amount of DNA present by using known amounts as a comparison).
4. Per cent of human DNA present (as distinct from bacterial DNA).
5. Pictorial representations of the above manipulations.

### *DNA Digestion*

Restriction enzymes are used as a form of chemical scissors to cut the DNA into pieces small enough to manipulate and identify. The following information should be recorded for restriction enzymes:

1. Lot numbers and expiration dates.
2. Quality control - level of restriction enzyme required to completely digest the amount of DNA present.
3. Digestion control lot numbers.
4. Star activity - if present.
5. Test gel photographs.
6. Methylation (PstI vs. HaeIII).

### *Electrophoresis*

Electrophoresis is the separation of the DNA fragments according to size. The DNA molecule is negatively charged and when placed in an electric field will migrate toward the positive electrode. The smaller fragments move further away from the point of origin than the larger fragments giving rise to the `banded' appearance of the gel when seen at autoradiography or after staining with ethidium bromide.

For all gels the information listed below should be documented:

1. Run Time.
2. Gel length.
3. Gel thickness and formulation.
4. Running voltage.
5. Calculation of end point (pre-set time).

The record of electrophoresis should also include:

1. Date on.
2. Date off.
3. Gel loading patterns.
4. Loading amount of sample.
5. Lot numbers of all reagents used.
6. Lane position of each sample.
7. Expiration dates of all reagents.
8. Pictorial record of ethidium bromide stain.
9. Time logs.

### *Southern Transfer*

Following electrophoresis, the separated bands of DNA must be transferred from the agarose gel to a medium which is more easily handled, for example a nylon `membrane'. This is achieved by placing the nylon on top of the gel and using paper towels to draw a solution through the gel and the `membrane'. The DNA is carried out of the gel and onto the nylon membrane and `binds' irreversibly to the `membrane'.

Ensure that complete transfer of all the alleles has taken place - including all the alleles at the bottom of the gel. If alleles at the bottom of the gel are not transferred, a heterozygote, that is a two band pattern, may appear as a homozygote, that is one band will appear.

Controls required: Hybridisation strip (for probe)

Sensitivity control.

*Hybridisation/Probing*

Hybridisation is the process by which the known piece of DNA or probe is attached to its complementary sequence or `mate' in the unknown sample. The eventual picture depends on a number of factors, for example stringency, type of probe (single or multi-locus). The overall issues are probe sensitivity and hybridisation conditions. The quality of the probe affects the outcome of the test. Factors affecting the quality of the probe are:

1. Is the probe cloned or synthetic?
2. If cloned then the nature of the insert must be examined.
3. How is the insert separated and recovered from the vector (the bacteria in which it multiplies)?
4. Is there any cross-reactivity with:
    a. Bacterial DNA.
    b. The vector itself.
    c. Other human DNAs.
5. What is the published data for the probe?
6. Is the sequence of the probe characterised and reported and has its frequency in the population been determined and published?

The hybridisation conditions affect the outcome of the test. The degree of stringency determines what the probe attaches to and how much information is retrieved. Too much information can be as useless as too little information. The type of probe used determines the amount of information yielded, for example single or multi-locus probes. In forensic work the probes used are labelled with a radioactive isotope, for example $^{32}P$.

*Autoradiography*

Autoradiography is the process whereby we visualise where the probe landed during the hybridisation process. It involves the use of a radioactive isotope whose emission particles collide with the silver particles on a special type of X-Ray film leaving a black residue in a position corresponding to where the probe landed on its complementary sequence in the unknown sample. With any autoradiograph the testing laboratory must be able to document all the bands that appear. All extra bands must be accounted for. All autoradiographs should be clear, that is have no background. All band shifts require an explanation.

*Data Analysis and Reporting*

The original raw population data should be available. Other information which should be reviewed is:

- molecular size
- probes
- frequencies
- computer algorithms
- bin size
- visual
- quantifiable
- are there any changes to molecular weight standards?

_ is there a data base comparison of whole blood vs. forensic samples?

# A View from the Bench

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The proffering of DNA profiling evidence in the courtroom occasions the judicial mind to travel along a well defined path. The first question the judge must answer in the particular proceeding is - `Is this evidence relevant?' If the answer is yes, then, subject to various discretions, the exercise of which might occasion the judge to exclude it, the evidence is admissible. The next question is, `What weight is to be attached to it?' If the judge is sitting with a jury he or she can immediately divest themselves of this problem because the law says that the weight to be attached to admissible evidence in a jury trial is entirely a matter for the jury, subject to the judge being permitted in law to make such comments about the weight of the evidence as appear to be proper, and providing the jury is told that those comments in no way bind them. If the judge is sitting without a jury he or she is the tribunal of fact in the proceeding and must consider the weight to be attached to the evidence.

## DNA and Expert Evidence

DNA profiling is, of course, upon its face, evidence which falls within the category of expert evidence. The law of evidence has since developed so as to establish the following propositions. Firstly, the general rule of evidence at common law is that witnesses may only testify to that which they personally observed or encountered through their five senses, and the expert witness is set apart from the lay witness in that the expert witness is permitted by the court to express an opinion in evidence. Secondly, the qualification of an expert witness as such is a matter for the trial judge or magistrate. Thirdly, the boundaries of expert evidence extend to take in all those instances where a tribunal of fact needs assistance in resolving technical or complex questions, because the task involved is beyond the realm of experience of lay members of the community.

The well known legal writer Professor Thayer declared in his *Preliminary Treatise on Evidence at Common Law* (1898)

> . . . any rule excluding opinion evidence is limited to cases where, in the judgment of the court, it will not be helpful to the jury. Whether accepted . . . or not, this view largely governs the administration of the rule.

Generally speaking, the courts in this country have applied the practical test suggested by Professor Thayer to the problem `Will the evidence be helpful to the court?' Of course, expert evidence of opinion must be relevant to some issue which

arises in a trial, must also be reliable and the person expressing the opinion must be sufficiently qualified as an expert in the relevant area of expertise. Two major qualifications touching the admission of expert evidence are that a witness in giving an opinion cannot give an opinion as to the ultimate issue of fact in the case, and the opinion cannot be based on hearsay. But even these exceptions have their qualifications. For example, it is common when a defence of insanity is advanced by an accused person in criminal proceedings, for medical practitioners to be asked to give opinions about the very matters the court has to find - `Did the accused know the nature and quality of the act he was doing?' and `Or, if he did know it, did he know that it was wrong?'

There is no point in dealing here with the principle that expert evidence must be relevant to an issue in the proceeding before it can be admitted. The range of issues that can be thrown up in proceedings is infinitely variable and the application of this step is a factual decision for the judge. The issue which will usually make DNA profiling relevant is, of course, the issue of identity.

It should be emphasised that relevance, in Australian law, does not necessarily mean admissibility - for once relevance is established the exercise of any one or combination of various judicial discretions to exclude otherwise admissible evidence may come into play. The first of these discretions is generally known in Australia as the *Ireland* discretion or the *Bunning v. Cross* discretion. The High Court has formulated this discretion in relatively recent years. The judge in a criminal trial has a discretion to reject evidence sought to be tendered by the prosecution by reason of it having been obtained by unlawful and unfair means. The leading decisions in this matter are those of the High Court in *Ireland v. The Queen* [1970] ALR 727; *Bunning v. Cross* [1977] 141 CLR 54 and *Cleland v. The Queen* [1983] 57 ALJR 15. The philosophic (or policy) basis for the *Ireland* or *Bunning v. Cross* discretion is that it functions as a sanction against less than proper conduct on the part of the police or other law enforcement officers in criminal investigation and obtaining of evidence. In the exercise of the discretion the judge weighs and balances two competing public interests.

In criminal cases, there are other judicial discretions which may be exercised to bring about exclusion of evidence nevertheless. The first has been described by members of the High Court as,

> that familiar discretion, applicable in all criminal trials, to disallow evidence if the strict rules of admissibility would operate unfairly against an accused (Stephen and Aickin JJ in *Bunning v. Cross* [1977] 141 CLR 54).

The same discretion has been described by Lord Widgery CJ in *Jeffrey v. Black* [1978] QB 490, as a general discretion `which every judge has all the time in respect of all the evidence which is tendered by the prosecution'. His Lordship went on to say that it was a discretion to be exercised when it would be `unfair or oppressive' to allow particular evidence to be called by the prosecution. Secondly, in the case of *R v. Christie* [1914] AC 545, Lord Moulton referred to another judicial discretion (which is thought by some to merely be a variety of the first already referred to) in these terms:

> The law is so much on its guard against the accused being prejudiced by evidence which, though admissible, would probably have a prejudicial influence on the minds of the jury which would be out of proportion to its true evidential value, that there has grown up a practice of a very salutary nature, under which the judge intimates to the counsel for the prosecution that he should not press for the admission of evidence which would be open to this objection, and such an intimation from the tribunal trying the case is usually sufficient to prevent the evidence being pressed in

> all cases where the scruples of the tribunal in this respect are reasonable. Under the influence of this practice, which is based on an anxiety to secure for everyone a fair trial, there has grown up a custom of not admitting certain kinds of evidence which is so constantly followed that it amounts almost to a lieu of procedure.

This discretion, or variety of discretion, is commonly utilised to exclude admissible evidence where the judge is nevertheless satisfied that its probative value in terms of the prosecution case is slight and its prejudicial value against the accused person is substantial and out of proportion to its probative value.

The essential rationale which underpins the *Ireland* and *Bunning v. Cross* discretions is revealed in the following statements from some of the judges in those cases. In *Ireland*, Barwick CJ referred to the competing factors in the exercise of the discretion thus:

> On the one hand there is the public need to bring to conviction those who commit criminal offences. On the other hand there is the public interest in the protection of the individual from unlawful and unfair treatment. Convictions obtained by the aid of unlawful or unfair acts may be obtained at too high a price. Hence the discretion.

Stephen and Aickin JJ describe this statement in *Bunning v. Cross* as follows:

> That statement represents the law in Australia; it was concurred in by all other members of the Court in *R v. Ireland* and has since been applied in a number of Australian cases.

Their Honours went on:

> What *Ireland* involves is no simple exercise of ensuring fairness to an accused but instead the weighing against each other of two competing requirements of public policy, thereby seeking to resolve the apparent conflict between the desirable goal of bringing to conviction the wrongdoer and the undesirable effect of curial approval, or even encouragement, being given to the unlawful conduct of those whose task it is to enforce the law. This being the aim of the discretionary process called for by *Ireland* it follows that it by no means takes as its central point the question of unfairness to the accused. It is on the contrary concerned with broader questions of high public policy, unfairness to the accused being only one factor which, if present, will play its part in the whole process of consideration.

Their Honours added, referring to the *Ireland* discretion,

> It applied only when the evidence is the product of unfair or unlawful conduct on the part of the authorities . . . Its principal area of operation will be in relation to what might loosely be called `real evidence' such as articles found by search, recordings of conversations, the result of breathalyser tests, fingerprint evidence and so on.

Their Honours concluded by making some observations particularly appropriate to DNA profiling evidence:

> The relevance of the competing policy considerations to which we have referred becomes of a special importance in an age of sophisticated crime and crime detection when law enforcement increasingly depends upon . . . scientific methods, whether of identification, by fingerprints or voice prints or of ascertainment of bodily states as by blood alcohol tests and the like. In many such cases the question

> of fairness does not play any part. `Fair' or `unfair' is largely meaningless when considering fingerprint evidence obtained by force or a trick or even the evidence of possession of say explosives or weapons obtained by force or a trick or even the evidence of possession of say explosives or weapons obtained by an unlawful search of body or baggage aided by electronic scanners.

The following cases will indicate the practical application of the *Ireland* or *Bunning v. Cross* discretions. In *Ireland* it was held that evidence of photographs of the accused's hand, taken without his consent, and evidence based on such photographs should have been excluded pursuant to this discretion. In *Bunning v. Cross* the court considered evidence of the results of a breathalyser test in circumstances where it was said that the statutory procedure for the obtaining of the test was not followed. It held that such evidence was admissible in circumstances where the unlawful conduct of a police officer had resulted from a mistake and not from deliberate or reckless disregard of the law and that the nature of the illegal conduct had not affected the cogency of the evidence, cogency being a factor in determining the admissibility of evidence obtained illegally where the illegality arises only by mistake. In the case of *R v. Dugan* [1984] 2 NSWLR 554 which involved the well known identity Darcy Dugan, it was held that the conduct of police officers who received information of plans to hold up a service station and who then took such steps as were considered necessary to render safe the pistol proposed to be used; proceeded to bug a car so as to be kept accurately informed of the movements of the accused and then staged a successful ambush, had not indulged in such conduct as would warrant the exercise of excluding evidence pursuant to the *Bunning v. Cross* discretion.

In Australia, cases of completely unqualified witnesses are fortunately rare. In 1960 the members of the High Court exposed a charlatan in the case of *Clark v. Ryan* [1960] 103 CLR 486. This man posed as an expert in the causes of motor accidents but was really nothing more than an expert in the art of giving evidence. Once turned loose in the witness box he rapidly became an advocate for the side calling him.

In a more serious vein, in the New South Wales case of *The Queen v. Palmer* (Unreported decision 29 November 1976), the case for the prosecution was that the accused person, a practising hypnotist, had committed offences upon young women in whom he had induced a state of hypnotic trance. The prosecution at the trial called a Dr Cunningham and plainly enough put him forward to the jury as a man of stature and learning. He gave evidence damaging to the accused after describing himself as holding a degree of Doctor of Philosophy from the International Episcopal University in London and of having been in professional practice as a properly qualified expert in hypnosis. Subsequent to the trial, enquiries disclosed that while there was no institution described precisely in the terms of the University named by Dr Cunningham in his evidence, there is an International Free Protestant Episcopal University in London. The Court of Criminal Appeal said of that institution:

> This can fairly be described as of low, or indeed, no, academic standard and of corresponding poor repute.

The judgment of the court continued:

> Other material shows that Dr Cunningham obtained his Doctorate of Philosophy from this institution by correspondence when he was in New Zealand. In brief, this material shows that, so far as Dr Cunningham ranking with equal stature to the other four experts, he could well, had this information been available, have been cross-examined with some vigour in pursuit of the proposition that he is a charlatan. In addition to this matter discrediting his academic qualification, he was twice before Magistrates' Courts in Brisbane in 1965, on the first of which occasions he was

> convicted after pleading not guilty to a charge of having adopted a medical title by inference, and, on the second of which, he was convicted on two similar, but not precisely identical, charges. In each instance he was fined. In addition, he apparently has a criminal record, the most recent entry on which discloses that in 1975 he was fined $200 for smuggling, $500 for possession of Indian hemp and was sentenced to twelve months hard labour for importing prohibited imports. He was released from this twelve months imprisonment after serving one month and placed on a two year good behaviour bond as well as being fined $1,000.

The court added:

> The inescapable fact is that one of the two experts called by the Crown and relied upon by the Crown as a witness of stature and integrity is now shown to be vulnerable to strong attack. If the appellant's counsel had been able to discredit either or both of Dr Cunningham's professional stature or personal credibility the jury might well have been reluctant to convict upon a Crown case in which it had been necessary to place such a marked degree of reliance upon such a witness.

The court further added that it was:

> satisfied that it is not consistent with the due and proper administration of the criminal law to permit this verdict to stand against the present appellant following as it does a trial in which one of the principal Crown witnesses was, quite innocently and properly on the then state of knowledge, put before the jury as a witness of professional and personal stature and repute.

It is much more common in Australia for disputes to occur as to whether a witness with established proper formal qualifications has been shown to be qualified on a particular topic. The Victorian case of *R v. Darrington and McGauley* [1980] VR 362 is a good illustration. In that case, evidence of expert opinion that certain drugs taken by an accused person had probably deprived him of the capacity of forming a murderous intent was excluded by the trial judge. The Court of Criminal Appeal upheld this decision. Young CJ and Jenkinson J held that, although the qualifications of the expert established that he could in law express opinions concerning the influence of the drugs on mental processes and behaviour, the evidence did not show that he was able to form a better opinion than a person unversed in that science. Anderson J. held that the question upon which the expert's opinion was tendered was within ordinary human experience.

## DNA and Statutory Provisions

The truly effective starting point in the process of DNA profiling must, of course, be the direct obtaining of relevant bodily samples, although samples from relatives may be of assistance. There are a large range of statutory provisions in Australia which relate to the taking of such samples and the English legislation will also be referred to.

In the United Kingdom the provisions of the Police and Criminal Evidence Act 1984 permit a request to a suspect for samples to be accompanied by a form of caution under the Code of Practice for Identification of Persons by Police Officers. The suspect is told, in terms, that, if refusal occurs, the request and refusal will be given in evidence at his or her trial and of the possible consequences. Statistics show that very few suspects in the United Kingdom refuse.

The Police and Criminal Evidence Act 1984 (UK), section 62, provides inter alia for the taking of what is termed `an intimate sample' from a person in police detention with the authorisation of an officer of at least the rank of superintendent and with the consent of the suspect. Reasonable grounds must exist for the authorisation and both it and the relevant consent must be furnished in writing. `Intimate sample' is defined in the Act and includes blood, semen and saliva. Such samples other than urine or saliva may only be taken by a registered medical practitioner.

Relevant statutory provisions in Australia are as follows:

Section 353A of the *Crimes Act 1900* (NSW) permits a police officer to search any person in lawful custody upon a charge of committing an offence and `take from the person anything found upon that search'. The section further provides where there are reasonable grounds for believing an examination of the person of the suspect will afford evidence as to the commission of an offence, for a legally qualified medical practitioner acting at the request of a police officer of at least the rank of sergeant or his nominee, to make a reasonable examination of the person of the suspect.

Section 81 of the *Police Offences Act 1953* (SA) permits examination by a medical practitioner of a person in custody and gives the suspect an option to nominate his choice of medical practitioner. The examination is predicated upon there being `reasonable grounds for believing that an examination of the (suspect's) person will afford evidence as to the commission of an offence'.

Sections 6 and 7 of the *Criminal Process (Identification and Search Procedures) Act 1976* (Tas.) permit examination by a medical practitioner of a person in lawful custody where `there are reasonable grounds for believing an examination of his body may afford evidence of the commission of the offence'. The examination is lawful insofar as it is confined to that which `is reasonably necessary to discover any facts which may afford such evidence, and to take possession of and retain any matter or thing that may afford such evidence . . .' Specific authorisation is given for the sampling of blood, saliva and hair and, indeed, such force as is reasonably necessary may be employed. Where a person has actually been charged with an offence, application can be made to a magistrate for an order authorising the search and examination.

Section 259 of the *Criminal Code Act 1899* (Qld) authorises a legally qualified medical practitioner to make such an examination of the person of the suspect as is reasonably necessary to ascertain facts which may afford evidence of the commission of an offence. Section 236 of the *Criminal Code Act 1913* (WA) is in like terms.

Section 145 of the *Police Administration Act 1984* (NT) permits a registered medical practitioner to examine the body of a person in lawful custody if a police officer has reasonable grounds for believing that the examination may provide evidence relating to specified offences. Either the consent in writing of the suspect or an order by a magistrate is necessary. The Act permits the doctor to take specimens from the suspect and reasonable force may be employed therein. The Act makes provision for the presence of a doctor chosen by the suspect.

Finally, in Victoria, no bodily samples can presently be taken without the consent of the suspect. An Act introduced by the Government Opposition touching this matter is presently before Parliament but its future is uncertain. A Committee headed by the Director of Public Prosecutions is due to make a report in the near future on this area of the law.

## United States Judicial Procedures Relating to DNA

Because so much of `the action' in relation to DNA profiling has taken place in the United States it is helpful and interesting to detail the contrast between United States judicial procedures which have been used to test and evaluate in this area of forensic

science and those which would be utilised in this country. From late 1987 the FBI Academy in Quantico, Virginia, investigated methods of DNA analysis in its own laboratory. The consideration for validation of the techniques included an assessment of the typing methods from fresh body tissues and liquids kept under control conditions; the determination of whether, in dried stains, polymorphic patterns change during storage and an examination of the effect on DNA profiles of a number of substances which might be commonly encountered prior to or during testing. Underlying all this activity was the necessity for the testing to attain what is known in the United States as `The *Frye* Standard' (*Frye v. United States* 293 F. 1013 (DC Cir.) [1923], or, alternatively, compliance with the Federal Rules of Evidence).

The *Frye* case concerned the admissibility of the evidence as to the use of a primitive polygraph and the results obtained. The relevant portion of the court's judgment is unsupported by reference to legal principle but, nevertheless, has been consistently applied in many courts in the United States. The court said:

> Just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential force of the principle must be recognised, and while the courts will go a long way in admitting expert testimony deducted from a well-recognised scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs.

Applying this test, the court found against the admissibility of the impugned evidence, in that the polygraph in question `had not yet gained such standing and scientific recognition among physiological and psychological authorities'. Thus the proffered evidence must pass over two hurdles. Firstly, it must be given by a qualified expert who is prepared to endorse the technique and methodology personally. Secondly, it must be shown that the technique and methodology is widely accepted by the scientific community within whose expertise it falls.

Many branches of scientific activity including electron microscopy, voice-prints and so-called `truth drug' administration have been considered in terms of the Federal Rules or the *Frye* standard.

The process is well illustrated by events in *The State of Florida v. Tommie Lee Andrews* case (Unreported decision 2 March 1988) which was one of the earlier instances of a conviction in the United States utilising DNA probes. In that case the result of conventional testing of semen on a vaginal swab was inconclusive. A blood group substance was identified but was that of a very substantial proportion of the population. However, DNA testing was able to establish a pattern which would occur in one of 840 million persons. Two trials of the defendant were held - the first jury being unable to reach a verdict.

At the first trial the prosecution called an expert in molecular biology to give evidence that the DNA test procedures, in effect, satisfied the *Frye* Test. The witness examined both the results and the laboratory where the tests were performed. The trial judge admitted the evidence of the tests but would not permit complementary statistical evidence. In the second trial both the test results and the relevant statistical material were admitted into evidence and the jury convicted. In the given circumstances of this case - there being no visual identification of the defendant - it is fair to say that the DNA tests provided the essential basis for the conviction.

*Frye* has been recently considered in Australia in *R v. Lewis* [1987] 29 A Crim R 267, a decision of the court of Criminal Appeal of the Northern Territory. Part of the headnote to that case reads:

> In determining the admissibility of opinion evidence, the court must consider the body of knowledge from which inferences are drawn and decide whether it is sufficiently established to have gained general acceptance with the scientific community from which it originated.

The court was obliged to consider the evidence of two dentists called by the Crown in a prosecution which evidence sought to link the accused with bruise marks on the skin of a witness. Maurice J. referred to *R v. Carroll* [1985] 19 A Crim R 410, an earlier decision of the Court of Criminal Appeal of Queensland - as `unanimously denouncing expert evidence purporting to connect marks of this kind to a suspect as unsafe'. His Honour believed that a principle similar to that of *Frye* could be extracted from the decision in *Carroll* and continued,

> It could not be asserted that the *Frye* test has become law in Australia; nonetheless it provides a useful guideline in determining whether novel forensic evidence should go before a jury, and it cannot be argued that the underlying concerns it was formulated to meet are not as important today as they were in 1923.

The admissibility of DNA identification tests was recently considered in *The People v. Joseph Castro*, a decision of Justice Gerald Sheindlin of the Supreme Court of the State of New York. The relevant facts are succinctly set out in His Honour's judgment:

> The defendant stands accused of two counts of murder in the second degree, it being alleged that on February 5 1987 he stabbed to death twenty year old Vilma Ponce, who was seven months pregnant at the time, and her two year old daughter. A wrist watch worn by the defendant at the time of his arrest was seized. What appeared to be bloodstains on the watch were noted by the detectives. The defendant stated that the blood was his own.
>
> The People, intending to prove at trial that the origin of the bloodstains on the defendant's wristwatch was the blood of the adult victim, and not the blood of the defendant, seek to introduce evidence of DNA identification tests (Unreported decision 14 August 1989).

The admissibility hearing in this case took over twelve weeks and was held pre-trial. His Honour adopted the *Frye* standard as explained in a later case of *The People v. Middleton* 54 NY 42 [1981], a decision of the Court of Appeals, wherein it was said of *Frye* `the test is not whether a particular procedure is unanimously endorsed by the scientific community but whether it is generally accepted as reliable'.

His Honour posed three questions for himself:

- Is there a theory, which is generally accepted in the scientific community, which supports the conclusion that DNA forensic testing can produce reliable results?
- Are there techniques or experiments that currently exist that are capable of producing reliable results in DNA identification and which are generally accepted in the scientific community?
- Did the testing laboratory perform the accepted scientific techniques in analysing the forensic samples in this particular case?

He ultimately answered the first two questions in the affirmative but, as to the third, held that the DNA identification evidence of inclusion was inadmissible as a matter of law stating:

> The testing laboratory failed in several major respects to use the generally accepted scientific techniques and experiments for obtaining reliable results within a reasonable degree of scientific certainty.

In this connection, His Honour applied the 1985 decision of *The People v. Mountain* 66 NY 197 [1985] holding that in the particular case the laboratory testing results were not sufficiently reliable to be presented to a jury.

There is an interesting postscript to the *Castro* case. Quite soon after the admissibility hearing, Castro changed his plea to guilty in return for a stipulated minimum sentence of twenty years imprisonment. In the course of his arraignment he admitted to Justice Sheindlin that the watch produced by the police was indeed his watch and that the blood on it was that of his victims.

## Admissibility in Australian Courts

It is suggested that an Australian court would approach the admissibility question in the *Castro* case - had it occurred here - somewhat differently. This does not imply that the Australian approach would be more efficient. But, as seen above, although it has been acknowledged as a useful guideline, the *Frye* test is not the law in Australia.

In this country it would certainly be possible to hold an admissibility hearing pre-trial. There is specific provision made for this in Victoria, for example, by recent amendments to the *Crimes Act*. But the primary emphasis in such a hearing would be on an enquiry to see if two requisites could be met. They are:

1. Is the relevant witness qualified as an expert by training and/or experience? and

2. Does the relevant expertise relate to a matter which the court accepts involves expertise beyond ordinary human experience?

It may well be that during this enquiry, which would involve the taking of *viva voce* evidence, doubts could arise as to the reliability of the methodology involved. Indeed, it might be positively shown that some mistakes were made and the judge may find that this is the case. But it is suggested that an Australian judge, in these circumstances, would proceed to exclude from the trial proper before the jury the successfully impugned evidence by using the discretion to exclude where the reception of such evidence would be unfair to the accused. Or, the discretion to exclude prosecution evidence of low probative but high prejudicial value might be alternatively exercised. It is possible, too, that the Crown may decide - in the absence of such an exclusionary order - that as a matter of fairness it would not lead the tainted evidence. If it were simply a case of mere doubts arising as to reliability, the likelihood is that the Crown would go on to lead the evidence at the trial and its weight would be a matter for the jury. The defence could, of course, introduce relevant material from the pre-trial hearing into the trial proper and thus acquaint the jury with it.

## Conclusion

Thus, one can anticipate with a degree of confidence that it is in the areas of the obtaining of bodily samples for DNA profiling and laboratory methodology that considerable disputation will arise in the Australian courts. It may be suggested, for example, that a particular statutory procedure has not been followed - as in *Bunning v. Cross* itself. It may be suggested that a relevant consent was obtained by duress or deceit and was therefore not a real consent. Or, it may be argued - as it has been argued in several recent cases in the United States - that the samples have been contaminated by substances commonly found in laboratories thus producing erroneous test results. In Victoria, it is all still a matter of predicting the future. The first case involving DNA profiling in the superior courts has, in the words of CJ Dennis' Ginger Mick `came and went'. It was the case of *The Queen v. Kaufman*. Like Mr Castro in the Bronx, Mr Kaufman pleaded guilty.

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# DNA Profiling: Forensic Science under the Microscope[1]

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Forensic scientists and crime investigators have long dreamed of being able to identify the origin of blood and other body fluids with certainty. The currently developing techniques of DNA profiling promise a degree of accuracy greater even than current methods of fingerprinting suspects.

DNA profiling allows examination of human biological material at its most fundamental level - the deoxyribonucleic acid (DNA) molecule. This molecule, which is found in every living cell within the body, carries the genetic information that makes one individual separate and distinct from every other individual. The DNA profiling process involves extracting the DNA from a specimen such as semen, blood or tissue and chemically dividing the DNA into fragments. Because of the naturally occurring variations in the DNA molecule from one person to another, the sequence of fragments forms a pattern, similar to a bar code found on items in supermarkets, that is to all intents and purposes unique to the individual (Phillips 1988, p. 551; Thompson & Ford 1989). The different forms of DNA testing use different numbers of various sensitive probes and differ in their percentage of result certainty. DNA testing, however, is distinguished by its particularly low false positive rate. So long as stringently developed laboratory procedures are painstakingly adhered to, DNA profiling evidence should be as reliable as any form of scientific evidence that is brought before the courts.

Many claims have been made by the competing scientific houses marketing their versions of DNA profiling for the accuracy rates of their form of DNA testing. It is clear, though, that DNA profiling represents a dramatic step forward in the capacity of science to tell us whether two body samples obtained at different times come from the same individual. This paper identifies the relevance of the techniques for the legal system, especially in light of the *Castro* (Unreported. Supreme Court of the State of New York County of Bronx, 14 August 1989) decision in the United States, focusses on matters likely to be contentious for lawyers, and queries the civil liberties issues that accompany the development of DNA profiling techniques.

## Relevance for the Law

DNA profiling has particular application to the criminal law because of the possibility that it offers of determining whether blood or semen deposits located at the scene of a

crime come from a person suspected of having committed the crime. This is especially useful in investigations into sexual offences. The critical advantage of DNA profiling over more traditional serological tests is that the DNA profile obtained from a vaginal swab or a sample of blood can be matched with the sample of a suspect tested with near certainty. It is much more specific than traditional tests for typing blood such as ABO typing, HLA (human leucocyte antigen) typing or typing of red cell enzymes and serum proteins. If a combination of vaginal and seminal material is present, this can usually be seen and is susceptible of interpretation as a mixture in the vast majority of cases (Thompson & Ford 1988, p. 64). This was not so with traditional methods where the danger of contamination was significant.

Whenever an assailant is injured or leaves any form of body sample at the scene of a crime (such as hair roots, saliva, blood, semen, etc.), the DNA profile has the potential to identify the person from his or her own deposit of biological evidence. It can also exculpate the suspect. DNA profiling provides the possibility of identifying victims of `anonymous crimes' such as hit-run accidents. As well, it can give a clear indication whether the same person is responsible for a series of crimes, such as serial rapes.

DNA profiling has been used extensively overseas for paternity testing because of its capacity to determine to a high degree of certainty whether claims to be a child's parents or grandparents are valid. The application of such information is wide, varying from intrafamilial disputes, to resolving the fatherhood of a child for rape victims, to rights to social security benefits, to issues of inheritance. It is also of major significance to governments seeking to confine immigration to family reunion categories (Jeffreys, Brookfield & Semeneoff 1986; Freckelton forthcoming(a)) and is being used extensively in Britain for this purpose. This context also provided the first encounter for the Australian legal system with DNA technology during 1988 when DNA testing showed conclusively that a young man seeking to migrate to Australia was in fact the son of a refugee already living in Victoria.

## No Panacea

### *Limited application*

Although DNA profiling has been claimed to be likely to have a very significant impact on the legal system should authorities have power to take intimate body samples without subjects' consent, these claims have been significantly exaggerated. The greatest application of the new techniques is in relation to paternity determination - in the family dispute and immigration areas. Division 8 of the *Family Law Act 1975* (*Cwlth*) and *Family Law Regulations 21A - 21G* already deal with the uncooperative test subject, allowing the magistrate or judge to draw appropriate conclusions about obligations to pay maintenance, etc. should the subject refuse to comply with parentage tests ordered by the court.

In the criminal arena, DNA profiling will have a more limited impact. It is rare that the perpetrator of a serious crime leaves body samples amenable even to this new form of testing at the scene of the crime. In the area where DNA testing has its greatest criminal investigative application - allegations of sexual assault - its real utility can be overstated. Most victims do not, and are unlikely to begin, reporting to police. Many of these who do report do so too late for DNA profiling to be an option. Of those who do report in time, the occurrence of sexual intercourse is often not an issue in dispute. The existence or non-existence of consent will be the critical matter. DNA profiling can offer nothing to resolve this problem. However, in the case of serial rapes or where there is a real doubt about the identity of the assailant, DNA profiling potentially has a great deal to offer.

*Cost and time*

However, DNA profiling is neither quick nor cheap. The testing process takes, on current estimates, between 9 and 18 days per sample. Depending on the system used, the cost will vary but Dr Gutowski of the Victorian State Forensic Science Laboratory has publicly estimated employment of the Lifecodes System at between $100 and $150 per test for materials alone. Some estimates are higher. This factor will tend to limit the employment of DNA technology to those occasions when it is genuinely thought that testing is likely to yield useful results. DNA extravaganzas such as that seen in the `Pitchfork Rapist' case in England, where an entire township was tested, are unlikely to be repeated in times of economic restraint.

*Quantity of samples*

DNA technology also requires significant amounts of samples, unless polymerase chain reaction procedures are employed to amplify quantities of testable body tissue. Such techniques reduce the accuracy of DNA profiling significantly. While this is likely in due course to change, current technology requires at least 50 _L of blood, 10 _L of semen or at least 10 hair roots for testing. This is a significant limiting factor for the feasibility of DNA testing.

*Fallibility*

Moreover, DNA profiling is not infallible. It has recently been the subject of serious challenge in a United States court (*The People v. Castro*, Unreported decision 14 August 1989; *see also* Lander 1989; Thompson & Ford 1988; Thompson & Ford 1989; Beeler & Wiebe 1988) and has to be acknowledged as carrying with it some risk on the basis of subjectivity of interpretation. This is not to maintain that it is an unreliable testing procedure - quite the contrary - but rather to acknowledge that it is not as infallible as early claims by its commercial entrepreneurs suggested. The 1989 *Castro* decision, which is discussed in detail below, usefully details a series of laboratory procedures that have the potential to render unreliable the results of DNA profiling tests.

*Limited police powers*

A significant impediment in many jurisdictions to the employment of DNA technology is the absence of police powers to compel suspects to provide a body tissue sample (usually blood) for analysis.

In England, where the technology was first developed, `intimate body samples' can only be taken from a person in police detention if the person acquiesces and if a police officer of at least the rank of superintendent authorises it. The officer can only do so if he or she has `reasonable grounds' for suspecting the involvement of the person in a serious arrestable offence and for believing that the sample will tend to confirm or disprove the suspect's involvement. Where consent is adjudged by a court to have been refused `without good cause' (undefined), a judge or jury may draw adverse inference from the refusal if they wish (s. 62, *Police and Criminal Evidence Act* (UK)).

In Australia the Victoria Police Force is alone in having no power to compel the provision of body samples. New South Wales, Queensland, Western Australian and South Australian legislation allows a police member of or above the rank of sergeant to compel a medical examination of a person in custody for an offence if the member has reasonable grounds for believing that such an examination will afford evidence as to

the commission of the offence (s. 353A(2) *Crimes Act 1900 (*NSW*)*; s. 259 *Criminal Code 1899 (*Qld; s. 236 *Criminal Code 1899 (*WA*)*; s. 81(2) *Police Offences Act 1953* (SA)). The Tasmanian legislation differentiates between intimate body sampling in relation to the offence with which the person in custody has been charged and in relation to other offences with which the police believe him or her to have been involved (ss. 6 and 7 *Criminal Process (Identification and Search Procedures) Act 1976 (*Tas.)). With respect to the former, the legislation is similar to that of most states, but with respect to the latter, the consent of a magistrate is required (s. 145, Northern Territory*h respect to the latter, the co*h resh respect to the latter, the consent of a magistrate is required (s. 145, Northern Territory Police Administration Act 1979 giveh respect to the latter, the consent of a magistrate is required (s. 145, Northern Territory Police Administration Act 1979 giveh respect to the latter, the consent of a magistrate is required (s. 145, Northern Territory Police Administration Act 1979 giveh respect to the latter, the conseh respect to the latter, the consent of a magistrate is required (s. 145, Northern Territory Pprofiling. The 1966 case of *Schmerber v. California* 384 United States 757 [1966] ruled on the constitutionality of a doctor's extraction of blood, at police direction, from a person suspected of having been intoxicated at the time of an accident, contrary to his expressed wishes. The Supreme Court rejected the driver's Fourth Amendment claim that extraction of blood against his will was an unreasonable search and seizure. It also denied that it represented a violation of the driver's Fifth Amendment privilege against self-incrimination basing its decision on the distinction between testimonial or communicative evidence, and acts which it classified as noncommunicative in nature. Finally, the court denied that such a procedure was a violation of the Fourteenth Amendment right to due process.

It has, however, been argued that: `Permitting the government to force suspects to give blood samples raises a spectre of coercion which should not be tolerated under the Constitution' (*The People v. Castro* p. 28ff). It has been suggested that the likely frequent use of DNA profiling against the will of suspects may infringe against the Fifth and Fourteenth Amendments' due process clauses (*The People v. Castro* p. 28 ff).

The court may make an order directing a relevant suspect to give a sample of his or her blood if the court is satisfied that:

a) there are reasonable grounds to believe that the relevant suspect has committed the offence in respect of which the application is made; and

b) material reasonably believed to be from the body of a person who committed the offence has been found -

   i) at the scene of the offence; or

   ii) on the victim of the offence or on anything reasonably believed to have been worn or carried by the victim when the offence was committed; or

   iii) on an object reasonably believed to have been used in or in connection with the commission of the offence; and

c) there are reasonable grounds to believe that the taking of the sample of blood from the relevant suspect would tend to confirm or disprove his or her involvement in the commission of the offence; and

d) in all the circumstances, the making of the order is justified.

Notably, the court is not obliged to make the order. However, its grounds for failing to do so are not usefully explicated. Moreover, the meaning of subsection (d) is entirely unclear and the section can only be described as the provision of the widest possible, but unguided discretion to the court. It is not possible to predict at this stage what arguments could be properly put to address the issue of when the order would not be `justified'. The amendments, however, do provide for a system for destruction of samples if a person is not charged within 6 months of the time of the taking of the sample or if the person is acquitted of the charges. There are criminal penalties for breach of this regime. At the time of writing, the draft bill had received strong support from police and related organisations but had been criticised by legal and medical groups. The government is expected to introduce the bill, or a variant on it, into State Parliament by early 1990.

## Civil Liberties Issues

Those with a concern about civil liberties (Cuthbert 1989; Freckelton 1989b) will be uneasy on a number of grounds with the granting of powers to the police to take intimate body samples without the subject's consent.

### *Self-incrimination*

Our legal system has traditionally eschewed forcing people to incriminate themselves by becoming the instruments of their own downfall. That principle has suffered a number of encroachments in recent years. Thus, drivers pulled over by police or involved in accidents can be forced to provide a breath or blood sample and defendants intending to claim an alibi as a defence are now required to provide advance notice to the prosecution. In some jurisdictions medical reports have to be exchanged. Other than that, our legal system has insisted upon the primacy of the privilege against self-incrimination and has orchestrated a balance between defence and prosecution with this as its basis. The prosecution has to prove its case without assistance from the accused person.

As a matter of principle, if we are to provide police with the power to insist upon the provision of intimate body samples for DNA analysis under any circumstances, even utilising recourse of some kind to a magistrate's order, we must recognise the significance of our decision and re-assess the balance of the criminal justice system accordingly. DNA profiling will inevitably be a boon primarily to the prosecuting authorities. The issue in the self-incrimination context is whether the advantage given to the prosecution by being able to force the suspect to self-incriminate in this way will operate unfairly.

The accuracy and reliability of DNA profiling properly conducted in well-functioning, independent laboratories is of a very high order. Thus, if such conditions existed, there would be little danger of false-positive or incorrect findings were intimate body samples to be extracted from suspected persons under compulsion. So far as the privilege against self-incrimination is concerned, the issue for Australians is whether a meaningful distinction exists between evidence that is compelled in the form of words and evidence that is actually tangible.

### *Accountability of police*

Many important issues revolve around the means by which samples are obtained from suspects and how the information derived from those samples is placed before tribunals of fact. A prerequisite for giving the police the extra powers that they seek to take intimate body samples should be our preparedness to trust them to adhere to proper

procedures and to police their own abuses adequately. There is ample and recently documented reason for believing that the present system for investigating complaints against police, depending as it does on an unsatisfactorily performing Internal Investigation Department (IID) of the Victoria Police, is an inadequate check on police abuses (Selby 1988; Freckelton & Selby 1989). Until that is remedied by the proven success of Ombudsmen, Police Complaints Authorities or by governments in implementing independent Criminal Justice Commissions (as recommended by Commissioner Fitzgerald) with sufficient powers to investigate police impropriety, further powers should not be given to police.

*A no-win situation*

Although police do not presently have the powers that they would wish to compel provision of intimate body samples, it is significant that they have been singularly successful in obtaining fingerprints, photographs and intimate samples from suspects when they want them. In England the police were able to prevail on approximately 5,000 individuals to cooperate with their inquiries by providing such samples in one case. Concerns have already been raised about the processes by which some of those samples were obtained. Recently in New Zealand, in the Teresa Cormack case media pressure was extreme in `encouraging' the few suspects who had not `cooperated' to provide the samples sought by the prosecuting authorities. This process left a great deal to be desired with a form of media involvement in the criminal investigation process that should not be repeated. The imposition of pressure on individuals to cooperate with police wishes that they have their photographs taken or their fingerprints recorded has been and continues to be a fertile source of complaints against police conduct. Unsuccessful bids by defence counsel have not infrequently been made to have such improperly obtained information declared inadmissible on public policy grounds. Considerable distress has also been caused to people when they have not been charged, or have been acquitted or not convicted of criminal charges, but have met with no satisfaction in having such records stricken from police data banks.

The problem arises in two ways. First, if legislation does not empower police under certain circumstances to take intimate body samples without the suspect's consent, there is an incentive for police to exert unacceptable pressure on suspects to agree to provide such samples. There is every reason to believe that similar problems or perhaps more serious ones will be experienced if the present situation continues in which police have to rely on their powers of `persuasion' to obtain evidence which could be highly probative in the courtroom.

Secondly, the alternative mooted by the draft Victorian legislation and that existing in many other jurisdictions, permits police, through the aegis of a cooperating medical practitioner, to use all reasonable force to subdue an objecting individual and by intrusive means take from him or her an intimate body sample. The use of any form of coercion or force in police stations should be denounced by all concerned with civil liberties in our community. There are also real difficulties with the notion that medical practitioners be suborned into, or in any way involved in such a process.

*Informed consent*

The taking of intimate body samples from an unwilling subject, usually by blood sample, is inevitably an intrusive procedure. Doctors, nurses and dentists in Victoria and elsewhere have all expressed concern about such a procedure without the informed consent of the patient. The Victorian Council for Civil Liberties and the Victorian Criminal Bar Association (in 1989 submissions to the Attorney-General's Department) have argued that such a procedure represents an act of violence that is incompatible

with proper behaviour in police stations and with modern notions of defensible medical practice.

*Targeting of the suspect*

A problem with the Victorian legislation is the fact that it is the rights of `the suspect' which are under threat. It is not necessarily the person who has been arrested and charged with the offence, in respect of which it is desired by investigating authorities to extract an intimate body sample, who is the target of the mooted legislation. It is merely the `relevant suspect' of a serious crime.

It is this writer's view that because of the legitimate concerns about:

- the abrogation of the privilege against self-incrimination;
- the continuing lack of accountability for abuse of existing police powers;
- the accountability of many forensic laboratories to police commands;
- the inappropriateness of the use of force in police stations;
- the inappropriateness of coercive, medically intrusive measures being performed without informed consent;
- the inappropriateness of targeting of `suspected persons'; and
- the fact that DNA profiling has limited application to the overall quality of criminal justice

the approach proposed by the Victorian Attorney-General should not be accepted by government. Although the English legislation has the conceptual and not insignificant problem that it allows adverse inferences to be drawn from the legitimate exercise of a right to refuse to undergo a testing procedure, in this writer's view it is preferable to the approach being proposed by the McCutcheon Bill.

**Admissibility of DNA Evidence**

British and Australian courts have not had occasion as yet to determine definitively what should be the criteria for receiving evidence of new scientific techniques or theories. The approach has generally been a laissez-faire one, allowing the evidence to be put before the tribunal of fact, so long as it is relevant and does not offend one of the long cherished rules of evidence.[2] This has left the responsibility with the judge or jury to decide how much weight should be given to the expert testimony to which they have been exposed.

However, there are increasingly strong indications that the 1923 United States decision of *Frye v. United States* 293 F1013, 1014 [1923] is at the least being absorbed into the law of Australia. It was held in that case that:

> Just when a principle crosses the line between the experimental and the demonstrable stages is difficult to define. Somewhere in this twilight zone, the evidential force of the principle must be recognised, and while the courts will go a long way in admitting expert testimony deduced from a well recognised scientific principle or discovery, the thing from which the deduction is made must be

> sufficiently established to have gained general acceptance in the particular field in which it belongs.

In 1977 the New South Wales Court of Appeal in *R v. Gilmore* [1977] 2 NSWLR 935 went some way towards introducing into Australia the *Frye* test, citing the United States case, adopting its language of `field of expertise' and using it in the context of voice identification evidence. *Gilmore* was followed in 1983 by the same court in *R v. McHardie and Danielson* [1983] 2 NSWLR 733. A hint of a similar approach had come in 1976 in Queensland when the Full Court of the Queensland Supreme Court had confronted difficulties in relation to the expertness of a witness called to testify as an expert upon the effect of wearing seat-belts. Justice Dunn focussed on the need for the judge to find as a fact that `there exists relevant technical or scientific knowledge' not possessed by the fact-finder and a need for that knowledge. He held that:

> The state of the evidence was such that, in my opinion, whilst there may be some room for difference of opinion upon the matter, it has not been shown that the learned trial judge was wrong in the relevant sense in his conclusion that `the study of seat-belts' has become a recognised field of specialist knowledge (*R v. McHardie and Danielson*).

In November 1985 the Queensland Court of Criminal Appeal rejected expert odontology evidence upon the identity of bite marks found on the body of a victim. Justice Kneipp held that there was:

> A body of eminent opinion which holds that valid identifications cannot be made by reference only to bruise marks or they should be referred to only for the purpose of excluding suspects and not from (sic) positive identification (*Carroll* [1985] 19 A Crim R 410).

Similar evidence relating to bite marks upon a victim and their similarity to the dentition of the accused person fell to be considered by the Northern Territory Court of Criminal Appeal in 1987 in the case of *Lewis* [1987] 29 A Crim R 267. Justice Maurice specifically referred to the previous Queensland odontology case and the passage cited above. Neither he nor his brother judges dissented from it. However, they stopped short of explicitly adopting the *Frye* test. Justice Maurice held that

> the jury should (not) have been permitted to place any reliance on the dentists' opinions. It really matters not whether that conclusion is supported by saying the evidence was strictly inadmissible, or its prejudicial effect far outweighed any probative value it may have had, or simply that it would be unwise to place any reliance on it (*Lewis* [1987] 29 A Crim R 267, p. 274).

Justice Muirhead noted pointedly that there was `no established universal view' as to the reliability of the technique in identifying, as opposed to excluding, a suspect.[3]

It is unclear, therefore, whether a superior court in Britain or Australia, if pressed, will unequivocally adopt the *Frye* criteria for determining whether a technique such as DNA profiling should be admitted into the courtroom. It does appear likely, though, at the very least that in formulating such criteria, judges may well borrow *Frye* language and focus upon the degree of dissension about any new technique within the scientific community.

Within the United States, the *Frye* test has been subjected to considerable and at times stringent criticism. It has been said that it is unduly difficult to determine what

constitutes `general acceptance' within the scientific community, what the relevant scientific community should be regarded as being at any one time (Williams 1987-88; p. 14), and how one determines the `scienticity' of a theory or technique in the first place. Some judges have departed absolutely from the *Frye* test, the court in *United States v. Williams* 583 F 2d 1194,1198 (1978), for example, asserting that

> the established considerations applicable to admissibility of evidence come into play and the probativeness, materiality, and reliability of the evidence on the one side, and any tendency to mislead, prejudice, or confuse the jury on the other, must be the focal points of inquiry.

The *Kelly* court (*The People v. Kelly* 17 Cal 3d 24; 546 P 2d 1240; 130 Cal Rptr 144 (1976)), by contrast, adopted what has become the majority stance and maintained that the essentially conservative standard of the *Frye* test shielded the jury from the unwarranted impact that a new scientific discovery could exert upon their otherwise reasoned considerations.

Generally speaking, the critics of the *Frye* test have maintained that it lacks clarity, is unduly rigid, sweeps too broadly, and is inconsistent with the traditional judicial prerogative to decide the accuracy and reliability of expert testimony (McCormick 1981; Saltzburg 1975; Gianelli 1980). The cases have evidenced patchy, but apparently increasing application of the *Frye* test in the United States to determine whether, pursuant to Rule 702 of the Federal Rules of Evidence, expert scientific evidence would be `helpful' to the tribunal of fact (659 F 2d 750 1981; Freckelton 1987b). The most recent criticism of the *Frye* test has come from a consensus statement issued by prosecution and defence employed scientists in the leading case on DNA evidence:

> All experts have agreed that the *Frye* test and the setting of the adversary system may not be the most appropriate method for reaching scientific consensus . . . The *Frye* hearing is not the appropriate time to begin the process of peer review of the data. Initiating peer review at this time wastes a great deal of the courts' and experts' time. The setting also discourages many experts from agreeing to participate in the careful scientific review of the data (Lewin 1989a; Freckelton 1987a).

During the 1980s United States courts strove to resolve some of the uncertainty surrounding the implementation of the *Frye* test. Thus in 1984 it was stressed that the *Frye* test only applied to novel scientific techniques and methodologies, as against opinion testimony which, while controversial in its conclusions, is based on `well-founded methodologies' (*Ferebee v. Chevron Chemical Co* 736 F 2d 1529, 1535 (1984)). Even in the case of novel scientific evidence, the opinion expressed need not be generally accepted but the methods by which it was reached must be methods upon which other scientists in the field would reasonably rely to reach their own conclusions, even though those may possibly be different (*Osburn v. Anohor Laboratories* 825 F 2d 908, 915 (1987)). The party offering the novel scientific evidence has the burden of demonstrating that it has been accepted as reliable among impartial and disinterested experts within the scientific community (*Kluck v. Borland* 413 NW 2d 90, 91 (1984) re thermography evidence). Such impartial experts' livelihood must not be intimately connected with the new technique (*The People v. Young* 418 Mich 1; 340 NW 2d 805 (1983) re electrophoresis of evidentiary bloodstains). Courts will look at papers written by scientists in the field or like fields (*United States v. Kozminski* 821 F 2d 1186), whether any trade organisation has recognised a test (*United States v. Kozminski; United States v. Distler* 671 F 2d p. 962) and the statistical reliability of a test (*United States v. Distler; United States v. Williams* 443 F Supp p. 272).

## Implications of *Frye* for DNA Evidence

As a result of traditional judicial mistrust of scientific evidence (Best 1911; *Whitehouse v. Jordan* (1981) 1 All ER 267; *Lord Arbinger v. Ashton* (1873) 17 LR Eq 358; *Thorn v. Worthing Skating Rink Co* (1877) 6 Ch D 415), concern within the legal fraternity about the reliability of novel scientific evidence in the wake of the concerning cases, and the moves toward greater use of the *Frye* test in Australia, it is inevitable that DNA evidence will be scrutinised particularly carefully at first by the courts. These factors take on added weight after the problems exposed with the Lifecodes testing systems by the August 1989 decision of *The People v. Castro* (infra). Judges are likely to focus upon the parameters of DNA profiling's claimed accuracy, the possibility of human error during its testing processes, subjectivity in interpretation of results and any likelihood of bias in its reliability factors as a result of degradation or particular racial provenance of samples. Because of its probative value, the techniques have high prejudical potential should any of the claims made on their behalf be flawed. Thus, the onus is on the prosecution to satisfy the courts that the likelihood of error in employing DNA technology is so minimal as not to represent any significant danger of false correlation of samples.

An early question that must be answered is whether the courts will classify DNA profiling techniques as `novel'. In this they have little guidance by way of precedent outside the United States. However, because of DNA profiling's reliance upon techniques with which courts are for the most part as yet unfamiliar, it is likely that DNA profiling evidence will be regarded as novel. This established, it is probable that the *Frye* test will be applied as this so far has been the approach in the United States (*The People v. Castro;* Beeler & Wiebe 1988; Thompson & Ford 1989). However, it should be recognised that many constituent parts of some of the techniques are known and recognised by the courts. HLA testing and electrophoresis, for example, are not novel. As well, bar code-like patterns have been employed for decades in analysis of electrophoretic patterns of serum protein and enzyme polymorphisms. Where these `classical' approaches employed protein stains, DNA profiling employs enzyme-substrate colour change systems or autoradiography if radiolabelled probes are utilised. It can cogently be contended that the practice of pattern analysis of step-ladder like fragments is little different to longstanding protein polymorphism analysis.

Assuming the *Frye* test to apply, attention will focus on determining whether scientists within the relevant scientific community regard the methodology by which the DNA experts express their opinions as reliable. Experts will need to be sought whose livelihood does not depend intimately upon DNA technology. They will need to be prepared to depose that the methodology is well-known and regarded as dependable, accurate and mainstream by most scientists with acquaintance of it.

Evidence will then need to be given by experts concerned with the testing procedure about its different stages, the possibilities of contamination of samples and the likelihood of false results being reached. They will need to be thoroughly conversant with the laboratory's procedures and able to explain and defend them. Particular scientists with expertise, in the calculation of statistics may need to be called. Attention will need to be paid to explanation of any parts of the interpretation of results that could be regarded as subjective, to analysis of the procedures for exclusion of false positives and to the application of quality control mechanisms. Because the testing process is a long and complex one, trial judges will wish to be satisfied that the scope for human error has been minimised by adherence to appropriate protocols and adherence to standard laboratory procedures to safeguard against confusion of samples. Finally, the continuity of the evidence in the particular

case must be able to be guaranteed by reference to careful logging of the processes undergone by samples at all relevant times.

**New Developments**

The admissibility of DNA profiling evidence is not an open and shut issue. The August 16 decision of *The People v. Castro* (Unreported. New York Supreme Court, County of Bronx, Indictment 1508/87 1989) is the leading authority on the admissibility of DNA profiling evidence.

A woman and her two-year-old daughter were stabbed to death in their Bronx apartment. Acting on information, detectives interrogated a neighbourhood handyman, Joseph Castro. They noticed on his watch a small bloodstain, which was sent for analysis to Lifecodes scientists who extracted about 0.5 _g of DNA. This was compared to DNA from the two victims:

> The DNA was digested with the restriction enzyme Pst1, a size-fractionated on an agarose gel, and transferred onto a Southern blot. The blot was then hybridised with probes for three RFLP loci; DXYS14, D2S44 and D17S79, as well as a probe for a Y-chromosone locus to identify sex . . . On 22 July 1987, Lifecodes issued a formal report to the district attorney stating that the DNA patterns on the watch and the mother matched, and reporting the frequency of the pattern to be about 1 in 100,000,000 in the Hispanic population. The report indicated no difficulties or ambiguities (Lander 1989).

The case of *The People v. Castro* provided the first opportunity on which the DNA profiling techniques have been put under the forensic microscope.[4] It has been described by New York attorney, Peter Neufeld, as `unprecedented in the annals of the law' (Lewin 1989b). So concerned were the scientists for the prosecution and the defence about the possibility that the court might be misled by the evidence that had been put before it, that they convened a mini-scientific conference to thrash out some of the difficulties that had emerged in the application of the Lifecodes system of DNA profiling during evidence given in the case. This all occurred during the currency of the case! The result of the gathering was a `consensus statement' that:

> Overall, the DNA data in this case are not scientifically reliable enough (to reach a reliable conclusion) . . . If this data were submitted to a peer review journal in support of a conclusion, it would not be accepted (Lewin 1989b).

Acting Justice Sheindlin of the New York Supreme Court chose to approach the admissibility questions by a three-pronged analysis:

- Is there a theory, which is generally accepted in the scientific community, which supports the conclusion that DNA forensic testing can produce reliable results?
- Are there techniques or experiments that currently exist that are capable of producing reliable results in DNA identification and which are generally accepted in the scientific community?
- Did the laboratory perform the accepted scientific techniques in analysing the forensic samples of this particular case?

It was held that there was `unanimity amongst all the scientists and lawyers as well as that DNA identification is capable of procuring reliable results' (Unreported judgment, p. 8). Acting Justice Sheindlin noted that it was the areas of interpretation of autoradiograph results that presented `special problems' but held that DNA forensic identification tests to determine inclusion and exclusion are reliable and meet the *Frye* standard of admissibility (Unreported judgment, pp. 26, 28). So far as the performance of the Lifecodes laboratory was concerned, `the defence was successful in demonstrating . . . that the testing laboratory failed in its responsibility to perform the accepted scientific techniques and experiments in several major respects.' It was found that the DNA tests could be used to show that blood found on the suspect's wristwatch was not his, but could not be used to show the blood was that of the victim. Thus, the testing laboratory performed sufficiently reliable tests of exclusion, but not inclusion, within a reasonable degree of scientific certainty.

The *Castro* case has raised a number of disturbing problems, not so much with DNA technology but more with its application and with laboratory standards, procedures and safeguards. Dr Lander, subpoenaed by the defence, has taken the lead in expressing concerns. (Lifecodes tendered to the court a formal reply to Dr Lander's criticisms). Some were the subject of explicit ruling by the *Castro* judgment. They may be reduced to the following broad areas:

*Discrepancies between forensic report and laboratory findings*

The only autoradiogram involving the probe DXYS14 showed five bands in one of the lanes examined and only three in the other. Lander has pointed out that this was contrary to the formal Lifecodes' report to the district attorney. The explanation given in court by Dr Baird, Lifecodes' director of paternity and forensics, was that the two non-matching bands could be discounted as being contaminants `of a non-human origin that we have not been able to identify' (Lander 1989, p. 502). At the least, there should have been some account of this situation in the formal report. Acting Justice Shiendlin went further, holding that the existence of the extra two bands was of critical importance `in determining whether the forensic DNA testing performed in this case demonstrates these bands to be human DNA or non-human DNA . . . Further testing was required' (Unreported judgment, p. 32). The result of this finding was the ruling that:

> the credible testimony having clearly established that the testing laboratory failed to conduct the necessary and scientifically accepted tests, the evidence demonstrating an inclusion is inadmissible as a matter of law.

*Deficient laboratory records*

Dr Baird was called upon to give evidence of controls employed in relation to a finding of sex. Initially, evidence was given that the control DNA came from the female-derived HeLa cell line. Subsequently, it was suggested by the same witness that the control came from a male scientist with a short Y chromosome. After evidence was given by the defence about the likelihood of this being the case, Dr Baird told the court that no precise record had been kept of which DNA preparation had been used but it was apparent that the control DNA came from a female technician (cf. *Morling Report* pp. 103, 105). Such inconsistency and absence of record-keeping does not conduce to confidence in the professionalism of a forensic laboratory.

*The use of controls*

The confusion over the identity of the donor of the control DNA highlighted the issue of whether a sex test should be considered reliable without a demonstrable control on the autoradiogram to prove that the experiment had worked correctly. On this scientific opinions expressed during the course of the *Castro* case differed (Lander 1989 p. 503; *see also Morling Report* pp. 76, 86; *Shannon Report*, p. 51). The judge's response was to declare that in the absence of both male and female controls, `it is difficult to determine whether the probe hybridised correctly. The failure to include both controls renders the experiment uninterpretable' (Unreported judgment, p. 31).

*Identification and matching of bands*

There is room for believing that the Lifecodes' report in *Castro* fell into the approach so vigorously condemned by the South Australian *Shannon Report* (1984) in that the approach adopted by the investigating scientists was one of looking for similarities in samples rather than focussing on dissimilarities. Lander is particularly critical of Lifecodes' preparedness to make direct comparisons between lanes containing different DNA samples, rather than considering each lane in its own right:

> Personally, I do not understand how the presence of matches at D17S79 and DXYS14 has any bearing on the determination of a match at D2S44: each test must be evaluated independently, especially as the individual probabilities of a match for each locus are multiplied together at the end (Lander 1989, p. 503).

He also points out that the stated Lifecodes' matching rule, that two fragments are said to match when their positions differ by less than three standard deviations, was breached in the *Castro* results. That should have led to an adjudication of no match. His view is that the subjective process of visual matching may have taken over to the detriment of the integrity of the matching process (Lander 1989, pp. 502-3). Lifecodes in its reply to Dr Lander's evidence acknowledged the rule but sought to confine its operation to `comparison of like samples', maintaining that it could not be extended to the analysis of forensic samples `because contamination and degradation effects on evidence must be taken to account when the gels are evaluated' (Reply p. 3).

*The impact of degradation of DNA samples*

The small quantity of DNA present on the watch which was examined by the Lifecodes' scientists was to a degree degraded (Macalister 1989), a problem which was compounded by the fact that the suspect was a member of the Hispanic population. The danger was that the sample on the watch was a heterozygote with a relatively high band (above 10.25 kb) undetected because of degradation. Opinions differed as to the possibility of obtaining the necessary high reading, but it is clear that the problem existed to some degree.

*The impact of probe contamination*

Various artefacts were discovered in the results. Lifecodes sought to explain these by the unsatisfactory occurrence of contamination of probes. Dr Lander reports that Dr Baird testified that Lifecodes `continued to use probes even after learning that they were contaminated, while apparently keeping no precise records of when such probes had been used' (Lander 1989, p. 503). He has pointed out that this would make calculation of the likelihood of false matchings impossible because samples may also

be contaminated. Presumably, however, this could be cured by appropriate use of controls in the testing process. Acting Judge Shiendlin held that `the use of a contaminated probe is unscientific and unacceptable. Immediately upon discovering a contaminated probe its use should have been discontinued' (Unreported judgment, p. 29).

*Calculation of matching probabilities*

Lander colourfully maintained that the Lifecodes process of calculation of matching probabilities is `like catching a match with a 10 foot wide butterfly net' (Lander 1989, p. 504) as it failed to take account of the actual threshhold used for declaring matches. He also criticised the account taken by Lifecodes of heterogeneity of particular populations, maintaining that this led the company seriously to miscalculate its statistics. Acting Justice Shiendlin held that:

> The rule for declaring a measured match must be the same rule which is used for declaring a match between the measurements and the data pool. This was not done in this case. Because of this error, the population frequencies reported by Lifecodes in this case are not generally accepted by the scientific community' (Unreported judgment, p. 34).

Acting Justice Shiendlin's solution to the complex problems presented by the *Castro* case was to suggest the holding of a routine pre-trial conference in relation to DNA evidence and a practice whereby the proponent of the evidence would be obliged to give discovery of a variety of matters:

- Copies of the autoradiographs, with the opportunity to examine the originals;
- Copies of laboratory books;
- Copies of reports by the testing laboratory;
- A written report by the testing laboratory setting forth the method used to declare a match or non-match, with all relevant criteria;
- A statement by the laboratory setting out the method used to calculate the allele frequency in the relevant population;
- A copy of the data pool for each locus examined;
- A certification by the testing laboratory that the same rule used to declare a match was used to determine the allele frequency in the population;
- A statement setting forth observed contaminants, the reasons for them, and tests performed to determine their origin and the results of the tests;
- If the sample is degraded, a statement of tests performed and the reasons for them;
- A statement setting forth any other observed defects or laboratory errors, the reasons for them and their results; and
- A chain of custody of the documents.

Such a procedure has a great deal of merit. Although such a procedure is unlikely to be insisted upon by Australian courts, such a list provides an excellent checklist of steps for forensic scientists in the area. For trial lawyers, the list provides a most useful set of criteria to ensure scientific accountability.

## Concluding Remarks

DNA techniques have made their first foray into the courts and tribunals of the United States, Britain and Australia. Until the *Castro* case they had not been the subject of substantial objection by either defence or prosecution counsel. As a result of that case, and particularly as a result of the concerns expressed by experts involved in the case, it can confidently be forecast that DNA profiling will henceforth receive a baptism of fire in the courts.

*Castro* notwithstanding, the techniques are likely in due course to be regarded as satisfying the criteria of the *Frye* test. However, for this to be so, a number of preconditions will need to be met. The fact is that novel scientific evidence has always been met with mistrust by judges. After 17 August 1989, this mistrust will be amplified. Judges are concerned that techniques will make their way before juries before the reliability of those techniques can be adequately assured. They are worried that judges and juries may be duped by very prejudicial material that may be deceptive in its quality. The *Castro* experience has called into question the professionalism of some of the laboratories employing forensic DNA technology in the same way that the Shannon and Morling Royal Commissions in Australia caused concern about forensic scientists' professionalism.

For DNA techniques to be admissible in the courtroom, the procedures adhered to by laboratories using them need to be standardised and scientifically stringent. The following are necessary:

- Professional laboratory practices need to be adhered to;
- Uniformity in testing and reporting procedures, so far as possible, needs to be secured (*see* Atchison & Cordner paper in this volume);
- Unimpugnable records must be kept;
- Cross-checks should be done on testing materials;
- Control tests on samples should be conducted, with particular emphasis on samples that may in any way be denatured or degraded;
- Measurements should be as objective and verifiable as available technology permits;
- Possibilities of contamination should be excluded to the highest possible degree;
- Opportunities for human error should be procedurally guarded against;
- Procedures should always be directed toward discovery of non-matches rather than matches of samples;
- Results of findings should never be overstated;

_ Supervision of laboratory personnel should be stringent; and

_ Routine checks of results and procedures should be done on traditional quality assurance principles.

Further, the acceptability of the technology will depend upon the articulateness of those testifying about it and their effectiveness as communicators. This entails preparation for presentation of its steps in the courtroom by overheads, graphics and diagrams, where appropriate, and preparedness on the part of the experts to make only those claims for DNA technology of which they can be completely confident (Malone 1988, p. 49; also inference chart concept, of Magnusson in E. Magnusson and B. Selinger, `DNA Profile Evidence and the Inference Chart Concept', this Proceedings).

However, there are enough aspects of DNA profiling that remain controversial and subject to human error and misinterpretation for trial lawyers to have a responsibility to test DNA evidence in the courtroom. It is likely that they will focus upon laboratory conditions and procedures and upon the propriety of each of the investigative steps followed in each particular case. Chiefly, this will mean examination of the extraction of the DNA, restriction digestion, gel electrophoresis, the process of hybridation and especially the interpretation of the results of the autoradiography (Thompson & Ford 1989). The well prepared forensic scientist from a well functioning laboratory should be able to meet such questions more than adequately with straightforward explanations.

The problems raised by *Castro* will be resolved. The stakes are too high for them not to be. DNA technology promises to provide a form of evidence to judges and juries upon whose accuracy and reliability they will be able to rely with a confidence that they can rarely experience. It has an already demonstrated potential to result in increased conviction of the innocent. At present, though, the utility of DNA profiling in the criminal context is dependent in many jurisdictions upon the success of the police in `persuading' suspects to provide intimate body samples. The challenge facing our legislators, assisted by those whose responsibility it is to draft proposals for law reform, is to determine the powers that should be given to our investigative agencies to demand intimate body samples.

**Footnotes**

1. For a fuller discussion of the issue canvassed in this paper, see I. Freckelton, `DNA Profiling - A Legal Perspective' in A. Ross, J. Robertson & J. Burgoyne (eds), *DNA Technology*, Ellis and Horwood (forthcoming).

2. In *R v. Murray* (1982) 7 A Crim R 48, for example, expert evidence in relation to polygraph examinations was ruled inadmissible by a District Court Judge because the evidence was hearsay and self-serving.

3. This was the substance also of the approach adopted by Acting Judge Shiendlin in *Castro*.

4. Of the few reported cases on the issue of DNA identification, none prior to *Castro* had held such evidence inadmissible. It had scarcely been challenged at all. In New York 3 cases had dealt with it: *The People v. Wesley*, 140 Misc 2d 306 (Sup Ct. Albany County 1988); *The People v. Lopez*, NYLJ (Jan 6 1989) p. 29 col. 1 (Sup Ct Queens County 1988); *Baby Girl S*, 140 Misc 2d 299 (Sup Ct NY Co 1988), the first dealing with the issue on the basis of *Frye* and the other two deeming the evidence admissible. One appellate court had found the evidence admissible under the relevancy and *Frye* standards (*Andrews v. State*, 533 So 2d 841 (Fla App 5 Dist 1988)) while by August 1989 at least 9 states had admitted DNA evidence at trial.

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# Bridging the Legal-Science Gulf

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**DNA** matching evidence has become news. There have been a number of recent newspaper reports of its use in criminal trials both here and overseas. One commercial facility for conducting tests has already been established in Sydney and on 22 September 1989 the Attorney General for New South Wales announced that the Government Analyst was to establish a DNA testing facility.

Evidence based on DNA matching (also called DNA finger-printing or DNA typing) has been hailed as the most important development in scientific evidence for law enforcement this century. Undoubtedly it represents a new and powerful technique but recent experience in America suggests that in common with other scientific evidence, it needs to be treated with some caution and is clearly open to challenge.

For this reason legal advisers will be required to come to grips with this kind of evidence. The purpose of this paper is to explain the tests and highlight those parts of the testing procedures which can be used to a client's advantage in appropriate circumstances.

### What is DNA Matching?

DNA matching is a method of identifying biological samples by analysing the genetic material or DNA contained in the cells. Its promise lies in the fact that the blood, semen or other human tissue found at the scene of the crime can be matched using this technique to a sample taken from a suspect with near certainty. Similarly, blood or other tissue found on a suspect can be matched to that of the victim. Alternatively, the fact of a non-match is equally powerful evidence to exclude a suspect.

DNA matching can also be used to establish parentage with unprecedented precision. The test could conceivably become the arbitrator in contested paternity cases.

Its chief advantage over traditional testing, such as blood typing, is the fact that the probability of a coincidental `match' can be so remote that the `match' or `non-match' can be declared with near certainty.

Further, only a very small amount of biological material is necessary to conduct the test. The semen on a swab taken from a sexual assault victim is ample and about the equivalent of one drop of blood can provide accurate results.

Also, DNA unlike many biological materials, does not degrade quickly so that samples weeks, months or even years old can still provide accurate results. However, concerns have been raised by the finding in *The People v. Castro* (Unreported, Sheindlin J.S.C., 14 August 1989) a decision of the Supreme Court of the State of New

York, when in a pre-trial contest over the admissibility of DNA evidence it was found that while the tests themselves are capable of producing reliable results, the tests conducted in that particular case were not sufficiently reliable to go to a jury. Much of the information in this paper is drawn from the *Castro* experience but it must be pointed out that in Australia the legal procedures would probably be quite different.

In *Castro's* case, the court was applying the test set down in *Frye v. US* 293 F 1013 (DC AR 1923) (the *Frye* Test) to determine whether the scientific technique to be used in evidence had achieved sufficient recognition in the scientific community to become admissible as evidence before the jury. In Australia, the general rule is that the value and accuracy to be given to expert evidence is a question for the jury to determine. Although there have been movements towards adoption of a *Frye* test in Australia (*see Lewis* (1987) 29 A Crim R 267; *Carroll* (1985) 19 A Crim R 410). At the moment such a procedure has not gained sufficient foothold so that in the near future at least, it can be predicted that a jury will decide the question of any challenge to DNA matching techniques. The final question being of course whether the DNA evidence is sufficient to support an inference of guilt. A fact cannot be a basis for an inference of guilt unless the jury is satisfied beyond reasonable doubt of the existence of that fact *(Chamberlain v. The Queen* (1984) 153 CLR 521). Indeed, the *Chamberlain* case is a good example of the jury being the final decision-maker on the question of contested scientific evidence. To appreciate the weaknesses and strengths of DNA matching evidence it is necessary to learn a little about DNA.

## What is DNA?

All animals and plants are made of cells and inside each cell is a smaller sub-cell known as the nucleus. Inside the nucleus of each and every cell is a long linear chemical known as deoxyribonucleic acid or DNA. DNA is made up of two strands of alternating units of sugar and phosphate (*see* Figure 1 shown on page 5 of these Proceedings). At regular intervals the strands are joined by cross-members called bases. The strands are not straight but form into a shape something like a spiral stairway. Another analogy might be like a twisted ladder. It is very long indeed, a loose analogy would be to think of a railway line with tracks as the strands of DNA and the sleepers as the bases. On that scale, the railway line would be over a million miles long.

DNA is the fundamental natural material which determines the genetic characteristics of all life forms. Essentially, it codes for the make-up of the animal or plant in which it is contained, thus, while both a human and a dog have DNA in their cells, the reason why the dog is a dog and the human is a human is because the code or blueprint in the DNA is different between the two.

The code is made up by the bases which link the two strands of the DNA. There are only four different kinds of bases, Adenine (A), Thymine (T), Cytosine (C), and Guanine (G). Each cross-member is made up of two bases joined together but Adenine can only join the Thymine and Cytosine can only join the Guanine.

An example of a sequence of DNA is shown in Figure 1 below. The bases are arranged along the length of the strands and it is the particular sequence of the bases which is the code for making all the different types of cells and proteins in the body and which is responsible for all their different functions.

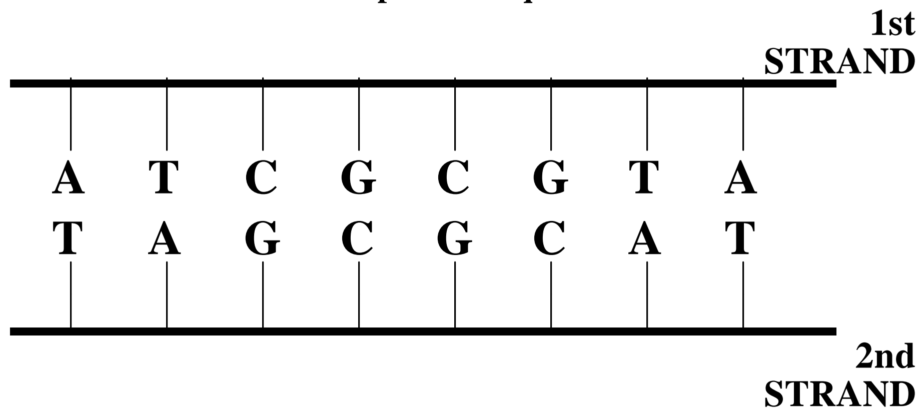
The sequence or code is all important in understanding the functions of DNA. Thus, one particular length of DNA might be responsible for making the haemoglobin molecule which is part of the blood. This one section of DNA can be referred to as a gene. Along the length of the DNA are many thousands of genes for all bodily cells and functions.

Chromosomes are larger groups of genes. Each person has 46 chromosomes, 23 received from one parent and 23 from the other. The DNA in each cell of an individual is identical so the DNA in one's big toe is identical to the DNA in the cells of one's ear or brain cells. The DNA in one human is about 99 per cent identical to the DNA in any other human and thus is of no use for telling the difference between people. However, the other 1 per cent is the part of the DNA which can vary between people and it is this part which is important for present purposes. The DNA matching tests do not focus on that part of the DNA which causes people to have a different appearance but what the technique does focus upon are parts of the DNA which do not appear to have any function at all and which are `spare'. Current theory is that between the operative part of the DNA (that is genes for specific functions) there are long stretches of base sequences which are of no identifiable use. Quite often these sequences are made up of repeating shorter sequences. The same sequence of say 12 base pairs might be repeated many hundreds or thousands of times in the `spare' or `anonymous' part of DNA. These areas of DNA are called polymorphisms.

It is these sections of repeating short sequences which are the key to the new technology of DNA matching because the number of repeats is characteristic of the individual. There are a large number of these repeat sequences in the whole human DNA.

*Figure 1*

**Example of a Sequence of DNA**




**DNA Matching**

There are currently three different tests available. All have some features in common, one of which is that they have been developed by private corporations and are patented. As far as Australia and New Zealand are concerned there is only one test commercially available, namely that offered by the Lifecodes Corporation, whose head office is in New York. Its local laboratory is in Artarmon in Sydney. The New South Wales Government Analyst is presently testing the Lifecodes System for possible future use under licence. A second system was developed by Cellmark Diagnostics and in the recent case of the murder of Janine Balding, evidence was given in Sydney by a scientist brought from England for that purpose (*Sydney Morning Herald*, 22 September 1989). The third test is offered by Cetus Corporation. As the Lifecodes' technique is available in Australia the discussion will focus on that test as being the most likely to arise in practice.

There are a number of steps involved and it is simplest to list them:

_ The starting point is the collection of biological material from the scene of the crime or the clothes or possessions of the suspect. The specimen is treated in a test tube to release the DNA contained in the cells and to remove unwanted cellular material so as to purify the DNA.

_ Next an enzyme, called a restriction enzyme, is added. Its function is to cut the DNA at specific points along its length which the enzyme recognises by the base code. It is known where the enzyme cuts the DNA along its length. This step then leaves a `soup' of DNA pieces of different lengths. For the purpose of visualisation it might be said that the restriction enzyme effectively is able to recognise the start and finish of the `spare' sections of the DNA.

_ This `soup' is then added to a flat agarose gel, which is something like the consistency of gelatine. A mild electric current is applied, positive at one end and negative at the other. The DNA tends to move towards the positive and the DNA particles of varying size migrate down the gel. The gel is like a molecular obstacle course and the shorter pieces of DNA are able to move through the dense gel much faster than the longer pieces. This effectively separates the DNA along the gel according to the size of the lengths of DNA (*see* Figure 2). At this stage the gel is commonly stained with a fluorescent dye to check that the DNA has `run' down the gel properly. Thus down each lane of the gel there should be a fairly even spread of DNA of different lengths, largest pieces towards the starting point and smallest at the furthest point. At this point the stained gel should look like Figure 3.

*Figure 2*

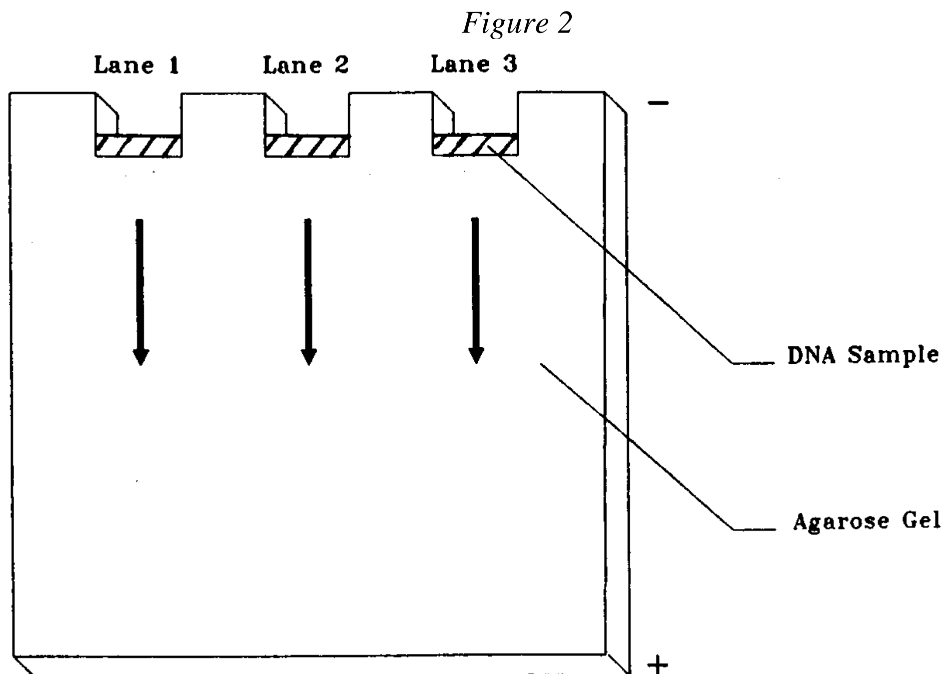
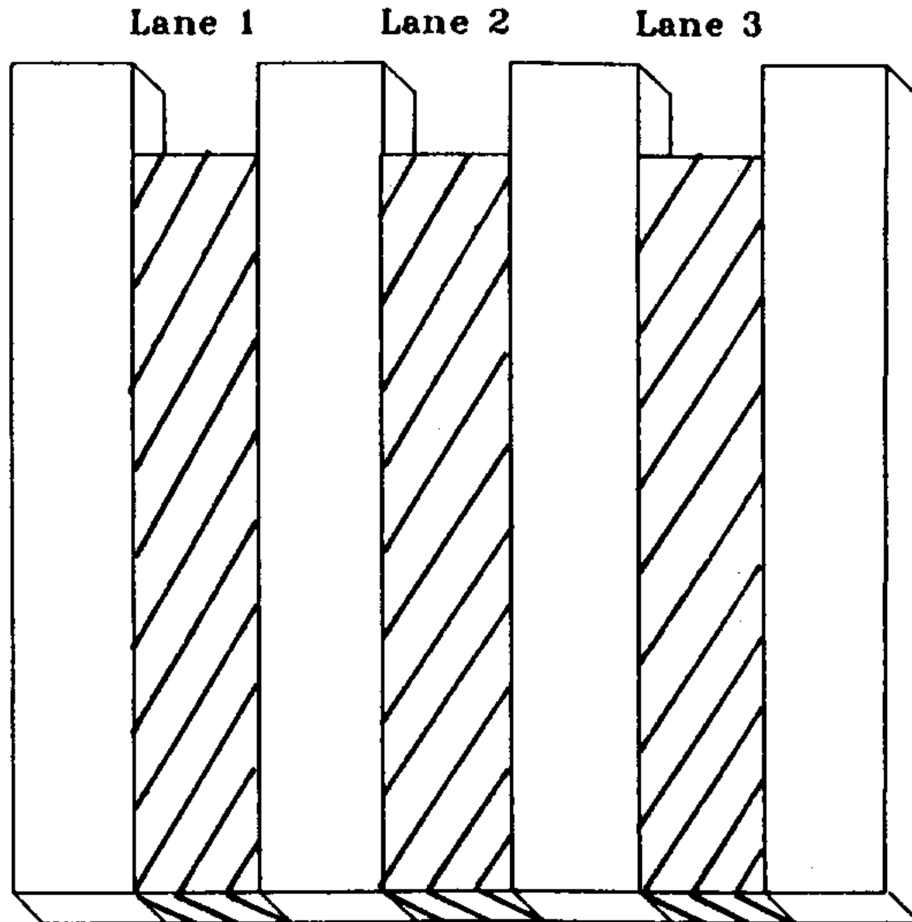


*Figure 3*




- The DNA is then de-natured, that is the double strands are broken into two single strands and the DNA is transferred on to a nylon membrane where it becomes fixed.
- The key step in the process is the addition to the membrane of a `probe'. The probe is a special type of molecule which can recognise a particular known spare section of DNA and lock onto the short repeat sequence in that particular `spare' part of the DNA. The probe is made by using radioactive chemicals.
- The plastic membrane is then placed on an X-ray film which records the position on the membrane where the probe has attached

When developed, the X-ray film should show two bands in each lane. One band represents the `spare' section of DNA received from the mother and the other band represents that received from the father.

The gist of the system is that samples from different sources are run in different lanes so that if the bands are in the same place on the gel they are likely to have come

from the same source. The distance the band has moved down the gel is a measure of the length of the spare section of DNA. The length is measured against a known standard run on the same gel in a different channel.

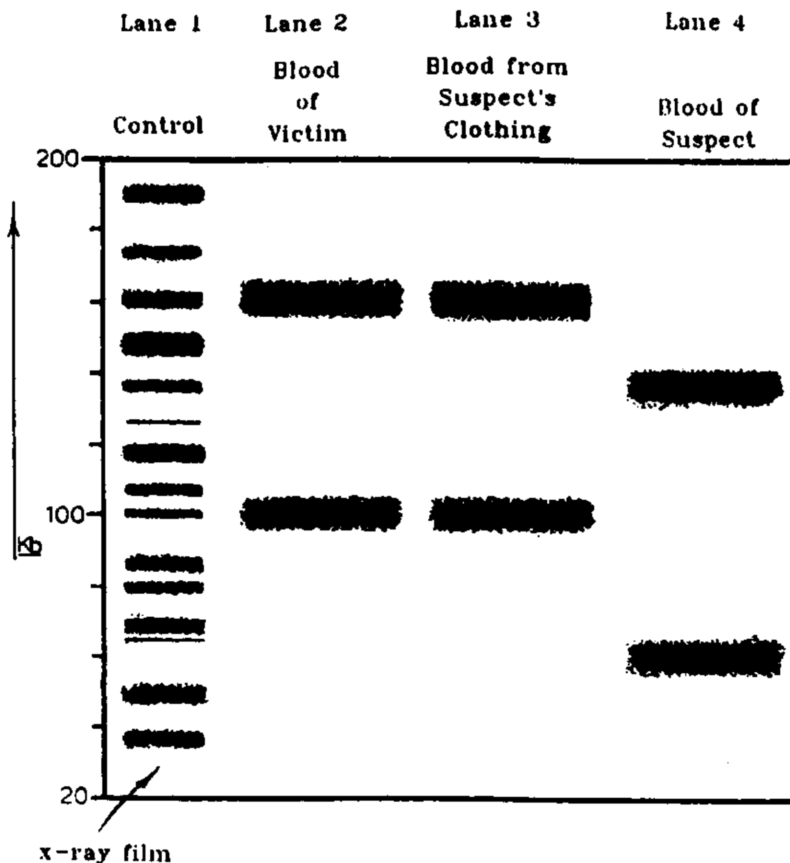
The bands are initially matched by sight. Figure 4 demonstrates what the X-ray film looks like in case of a match. Lane 1 represents a control of known length of DNA. Lane 2 is the blood of the deceased victim while Lane 3 is blood found on the clothes of the suspect. As the bands have travelled the same distance down the gel the polymorphic DNA is the same size in Lanes 2 and 3. On the far left of the diagram is a measurement obtained from the control in Lane 1 of the length of the polymorphic DNA in terms of the number of bases in the DNA fragment.

By reference to a data bank of results from individuals in the population the scientist is able to calculate the probability of a particular person having a polymorphic section of DNA of that length. This process is most clearly explained in a diagram; *see* Figure 5. The horizontal axis represents the length of the DNA fragment identified by the probe (as measured by the distance it travelled down the gel). The vertical axis is the frequency of that length of DNA in the population generally. Thus, if the samples from two different sources match then the scientist can conclude by use of a mathematical formula which takes an account of both bands, that the chances of such a match occurring by coincidence are a certain probability, say one in fifty.

Figure 6 indicates the situation where a non-match has been declared.

The real power in the system is that there are other probes available to test the same sample by merely washing the former probe from the nylon membrane and applying a new probe which recognises a different section of polymorphic DNA elsewhere on the gel.

*Figure 4*



*Figure*

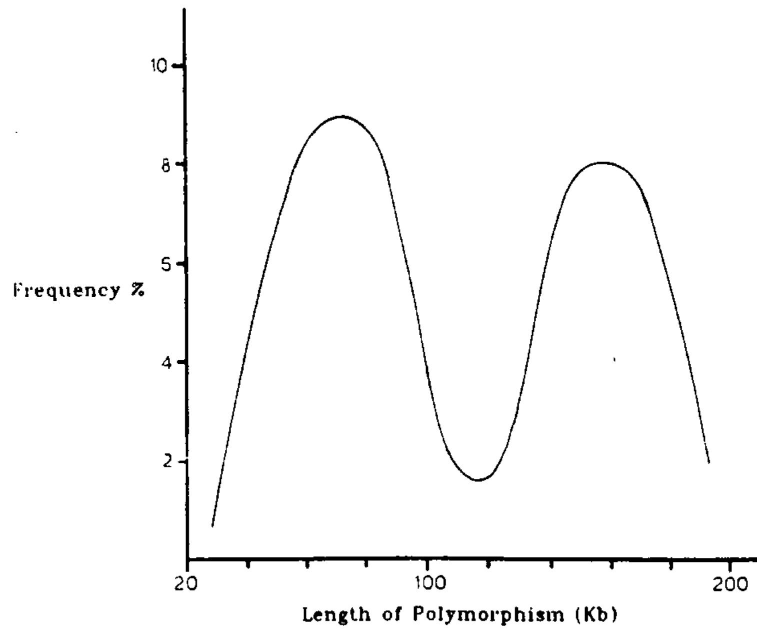
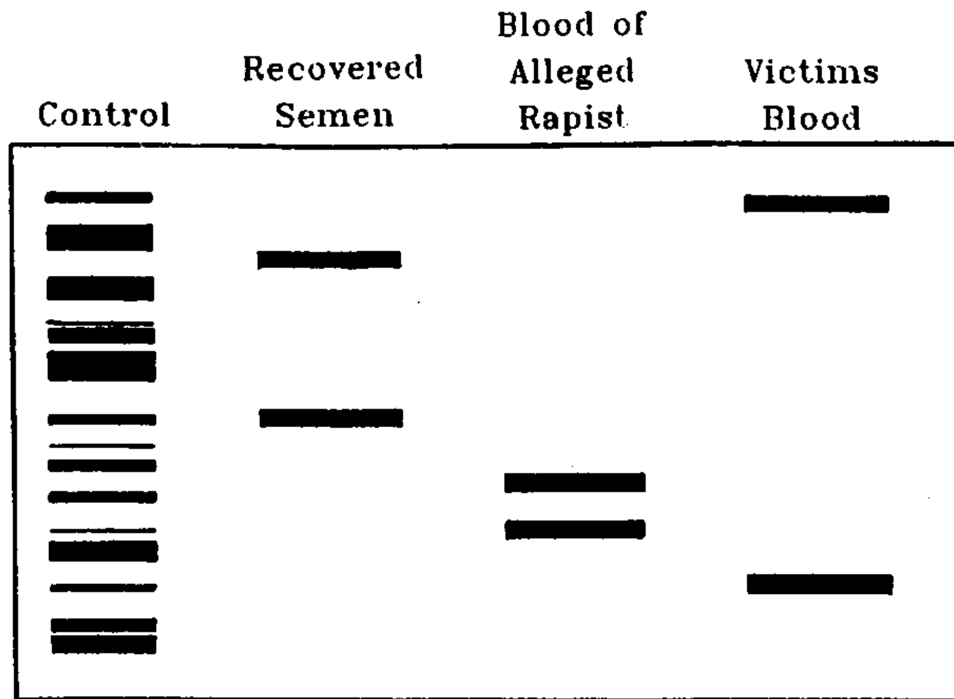



*Figure 6*



The same process is followed and if a match occurs again at a probability of, say, one in one hundred, then the combined effect of the two probes is multiplied so that the chance of a coincidental match using the two probes is one in five thousand.

If a third probe is applied with a probability of, say, one in seven hundred and fifty, then the combined effect of the matches using the three probes is that the chances of a coincidental match rise to one in 3.75 million. Fourth and subsequent probes can multiply the probability of a coincidental match even higher.

The advantages for the law enforcement agencies in identifying suspects are enormous. Similarly, for a person charged with a serious offence the effect of a non-match is equally powerful.

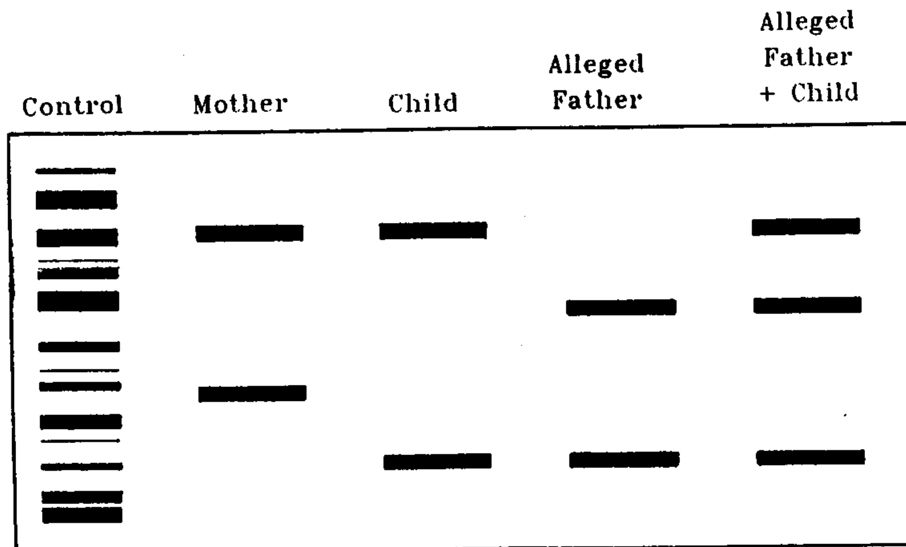
## Paternity Cases

Similar results can be obtained in cases of contested paternity and many of the difficulties of its use in criminal cases are avoided by having fresh whole samples available. For example, in Figure 7 the DNA pattern that one would expect is, to find one half of the child's DNA matching one of the bands of the mother and one of the bands of the father. The fourth lane is used as a control. A conclusion with an extremely high degree of certainty can be reached, again using multiple probes.

## Problems with the Test

The test is clearly complex and it is this complexity which provides the greatest source of challenge for the lawyer and the greatest difficulty for the scientist. Some problems are as follows:

*Figure 7*



*Quality of the samples*

The alarm bells should start ringing in a lawyer's ear when there is any question about the freshness, age or quantity of the sample taken from the scene of the crime. While DNA typing is possible on old samples, because the samples are themselves biological material, they can be contaminated by bacteria whose DNA might be falsely recognised by the probe as human DNA on the results. Further, although DNA does not degrade quickly under ideal conditions, with exposure to heat, light or liquids, the DNA can degrade and produce unreliable results. There are certain tests which can be conducted to overcome these problems and the question in each case will be whether these tests were done at all and if they were done, whether they were done properly. This is where some difficulty can arise. In paternity cases if there is an error in the procedures more blood can be obtained and the test repeated. However, in cases where there is only a small amount of material to start with (such as the blood spot in *Castro's* case), the experimenter gets only one chance to perform the test and may even have insufficient material on which to perform the necessary checks and counter-checks.

There are methods to check and resolve for these problems. First, to ascertain whether the DNA has degraded prior to the experiment a special probe can be applied which checks that there are still large pieces of DNA and the DNA is not made up entirely of small fragments thus likely to give aberrant results (as would be the case where the DNA is degraded). These are commonly known as non-polymorphic probes as they recognise a part of the DNA which is common to all individuals.

Similarly, in the case of degraded or contaminated samples (and any sample which has been exposed to light, heat or air for anything longer than a day or two might fall into this category) it is necessary to conduct a test utilising a probe which identifies bacterial DNA to exclude this possibility. Alternatively the probe used may be specific to human DNA and will not bond with bacterial DNA. It is important to ascertain if these protective measures have been taken.

*Matching the bands on the gel*

Difficulties can arise when making a match of the bands on the gel. In practice, the outline of the bands is fuzzy and of unequal width. A match can sometimes be a subjective decision. For this reason it may be important to view the autorads themselves to ascertain their integrity in this regard. There are machines available to analyse by a computer whether a match is made or not, but from a legal sense this might be an even more dangerous way to test for a match because the experimenter would have to convince the court that the machine was operating properly and explain all its workings. When making the match the scientist measures the distance the sample has moved down the gel and by comparison to a sample of known length run in a control lane (*see* Figure 4) the length of the DNA in the band is measured. There is some inaccuracy in this measuring process and the resultant error tends to be a constant applied by different laboratories. Commonly it is something of the order of 2 per cent. This error is calculated by using a statistical model based on the reproducibility of results from the technique using prior dummy runs employing the same sample over and over. Then an accepted statistical measure is applied (two standard deviations) to calculate the anticipated error when measuring the length of the DNA in the bands of the test under examination. It would be most surprising if a reputable laboratory had not applied the correct procedures at this stage, but the witness should be aware of such matters and be prepared to justify the error rate applied. It may also be necessary for this error to be checked from time to time because it can change with new batches of reagents, etc.

Particular problems of identifying the bands apply when the Cellmark test is employed. Unlike the Lifecodes' test which commonly only gives two bands per lane,

the Cell Mark test has anything up to 15 bands of varying intensity. On this test it is necessary only to have one auto-radiograph but the problems in ascertaining whether the bands match or not can be enormous, and a fruitful field for the lawyer.

Another problem with reading the bands is that there can be too much or too little DNA in the band to admit of accurate measurement. In the case of too much DNA in the band, the band might be enlarged or the outline of the band might become very fuzzy. Once again, this problem can be overcome in a number of ways. If the amount of DNA in the sample being loaded on the gel is unknown then the sample can be diluted, say, one in ten and again one in one hundred, and those dilutions run on adjoining lanes will give a variety of intensities in the bands on the autorad. Another method is to ascertain the amount of DNA in the sample prior to loading it on the gel by using a spectrophotometer which employs light to measure the quantity of DNA in the sample.

In the case of too little DNA the problems are obvious where the band is very faint. Where there is very little biological sample available for testing the Cetus test can be employed. What this test relies on is isolating the DNA from the biological sample and then employing an enzyme to create multiple copies of the DNA obtained from the sample. In this way the original DNA is amplified using a method called polymerase chain reaction. The amplified DNA from the sample is spotted on to a membrane and a radioactive genetic probe added. The probe locks on to any DNA spot which contains the allele the probe is designed to detect. A radiograph is produced and rather than testing for the length of polymorphic DNA segments, the Cetus test determines whether certain DNA segments are present in the sample or not. That is, the test depends upon the rarity of specific alleles the two samples might have in common. This test has not yet been as widely accepted as the Lifecodes' or Cellmark tests.

Again, simple measures are available to overcome the obvious problem of too little or too much DNA being loaded on the gel. But the absence of such control and checks can be critical in the appropriate case, particularly where there is only one opportunity to conduct the test and if the test goes wrong, the benefit of hindsight will not cure the potentially unreliable result.

Another problem with reading the gels is that a phenomenon called `Lane Shift' can occur. For example, although the two bands shown in each lane are equally distant from one another, they in fact do not match in terms of their distance travelled down the lane. This problem arose in the *Castro* case where such a result was declared to be a match because lane shift had occurred from time to time and been recognised by the testing laboratory. Lane shift occurs for a variety of reasons, one of which is that when the gel is made it is heated into a liquid and then poured on to a flat surface, just like making jelly. But it is still quite viscous and the gel may not be of uniform thickness throughout. Thus the DNA might travel through different thicknesses of gel causing a variation in its rate of travel.

Once again, there are simple procedures to check and resolve lane shift. First, a mixing experiment is performed where a 50:50 mixture of DNA from, say, the semen found at the scene of the crime and DNA from the suspect are run on a separate lane; if only two relatively narrow bands appear then the samples can be safely pronounced and matched. Obviously, if four bands appear then the samples do not match.

Another method to avoid lane shift is to have regular lanes of control DNA across the gel. By comparing the controls in lane 1 and lane 4 it can be determined whether the gel is operating correctly or whether lane shift is occurring.

*Contamination*

Another area for caution is in the danger of cross-contamination of samples, where for instance, the sample from the scene of the crime is contaminated by the suspect's sample. Samples should be analysed separately but if there is cross-contamination, or even worse, contamination of reagents common to both analyses, the whole test

becomes doubtful. The problem of contamination is of particular importance in the Cetus test where a small amount of contaminant in the original sample can subsequently be amplified by the polymerase chain reaction. Contamination can be controlled through the use of careful laboratory procedures. Contamination has proved to be a vexing problem to molecular biologists in research laboratories.

*Population genetics*

The next major group of problems relates to the mathematical calculations of the frequency in the population of a particular polymorphic length of DNA. The problem can only be explained with some background information. As stated earlier, the whole human DNA can be divided up into genes which code for the various functions of the cell. The genes are located at specific and identifiable places amongst the 23 chromosomes. There are two copies of each gene, one received from the mother and one from the father. The different copies or versions of the genes are called alleles. One copy of the allele becomes `turned off' so that only one of the two copies becomes operative to produce the characteristics of the person. But there are in fact two copies of each which is why two bands appear on the gel for each gene probed. The probes employed in the DNA matching techniques recognise both alleles. The length of that allele is the critical measurement for the test.

The tests rely upon one large assumption, namely that alleles of that particular length occur in the population randomly and with a frequency determined by the screening tests performed on a large number of persons - something like a market research study. This randomness only occurs when two conditions are satisfied, first the alleles examined must not be `linked' and secondly, the population must be in what is known as Hardy-Weinberg equilibrium.

*Linkage*

Studies have shown that the randomness of an allele is disturbed because some alleles are linked. An obvious example is the allele for olive skin and the allele for dark eyes in the Caucasian population. It might be that these two genes are situated on the same chromosome and so are not truly independent. That is, there is an increased likelihood that these two genes will be passed on together to the child's DNA. This is less likely if the alleles are on separate chromosomes, but even on separate chromosomes alleles can still be linked. The question then becomes whether the alleles examined by the different probes are linked because the tests assume that particular alleles are not linked. Such a fact can only be established from research based on the two alleles. The witness giving evidence ought to be able to justify the use of that particular probe by reference to the facts known about those particular alleles which establish that they are not linked. There are still big gaps in the knowledge about the mechanism of linkage. The important thing to note is that an assumption is made which might be open to challenge.

*Hardy-Weinberg Equilibrium*

A specific allele in a population will be in Hardy-Weinberg equilibrium when the allele frequencies remain constant within the population from generation to generation. The tests assume this to be the case. The assumption is based on the screening data in the data base which should conform to the Hardy-Weinberg mathematical prediction ($P^2 + 2PQ + Q^2 = 1$, where P and Q are the fractions of the population having two different alleles).

There are some areas of doubt about this assumption and the resultant conclusions about frequencies. First, the screening data obtained so far must by necessity, be from a limited sample size. Because it relies on an assumption of random mating there must be separate data bases and screenings for different population groups. It is assumed that different population groups do not interbreed freely - an obvious example would indicate that it would be unsafe to determine the allele frequency of a particular gene from an Australian Aboriginal with an allele frequency data base obtained from North American Indians because the two populations are quite independent.

Thus, problems can arise when there is no data base to examine the particular population background of the donor of the sample. This will not be a problem for the larger racial groups but will be a major problem for smaller population groups such as Australian Aboriginals or Pacific Islanders. It is suggested that it would be unsafe to use Caucasian allele frequencies on a donor from a different racial group.

The validity of the frequency data is dependent upon the assumption of Hardy-Weinberg equilibrium. Even if the screening data (of admittedly limited scope) conforms to the Hardy-Weinberg formula it cannot therefore be concluded that the alleles are in equilibrium. The correct conclusion is that there is no evidence that the alleles are not in equilibrium. It might well be that for the particular racial background of the donor in question the data base information is inaccurate.

Such problems are magnified in persons of cross-racial origins and this might particularly apply to Australia where there is a sizeable proportion of recent migrants from different gene pools. In the case where the client falls into the category of coming from one of the smaller population groups, a recent migrant or first or second cross-cultural progeny, special care needs to be taken. Perhaps details of racial history need to be obtained from the client. An expert may need to be consulted in cases of doubt.

Special problems occur with persons with Islander racial backgrounds because, from a genetic point of view, if the gene pool has been isolated for many generations, the allele frequencies can be markedly different to those from a Continental gene pool.

This casts some doubt on the assumption that allele frequencies remain constant in the population and that the relative frequencies of the different alleles found can be safely multiplied together to form the probability of a random match. While a case involving a white Australian suspect may not provide any difficulties, in cases where the client is of recent migrant stock, Aboriginal, Islander, or some other relatively isolated population group, it may well pay to seek expert advice from a population geneticist to cast doubt on the DNA matching evidence.

From a legal point of view, it should be remembered that the expert giving evidence in favour of a DNA match has to rely on screening tests performed by others. It is quite likely that the particular witness has no personal knowledge of whether or not those screening tests were properly carried out and is quite unable by his or her own evidence to prove the validity of such tests.

This leads to the final problem namely, that referred to as the free enterprise element. The tests are available from companies that have a financial interest in the acceptance of the tests by the courts. Further, the published studies supporting statistics have been conducted by those who are employed by the companies which have a financial stake in the technique.

## Conclusion

DNA matching is certainly a powerful new technique for eliminating or incriminating suspects in criminal offences. There should not be bland and uncritical acceptance of this new evidence. The *Castro* case demonstrates that one can argue with science and

win. In a courtroom the stakes are high and scientists should expect their opinions and techniques to be closely scrutinised.

The *Castro* case is an indication that the technique can produce reliable results so that the likely area of challenge to DNA evidence is whether the technique was correctly performed in the particular case. This paper has highlighted some parts of the testing procedure which might prove fruitful to the lawyer in challenging DNA evidence or areas where the scientist might take extra care in expectation of a challenge.

The important thing to remember is that the technique is not infallible and, depending upon the circumstances of the particular case in hand, the evidence may be able to be challenged with some prospect of success.

# DNA Profile Evidence and the Inference Chart Concept

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The Inference Chart concept has been proposed as a way to make forensic science intelligible to courts and to restore to the jury its prerogative to decide matters of fact. The Inference Chart is a jargon-free outline of the logical pathway followed by forensic scientists or other experts in reporting laboratory tests and drawing conclusions from them. It is expected to be of particular value when courts are confronted with the jargon and complexity of DNA technology. Although Inference Charts were originally designed to remove the confusion caused by technical language or by conflict between experts, they provide safeguards against the possible incompetence of the expert in laboratory technique or choice of strategy, against logical errors in reasoning from test results to conclusions, against inadequacies in statistical data, and against misleading forms of reporting scientific results. The Inference Chart procedure, with its attendant safeguards, provides courts with a practical way to assess DNA evidence during its initial testing in Australian courts.

Even without DNA identification evidence, the complexity of the methods used by modern forensic science has imposed a heavy burden on courts which try to comprehend the evidence. In Australia, as in other countries which use the adversarial system, spectacular conflict has arisen when the burden has become too great. Examples accumulate of juries being misled by experts whose competence they were unable to judge, or confused by those whose language they were unable to understand.

With the advent of DNA technology, courts are threatened with an even greater risk that the evidence will not be understood. This situation is exacerbated by the problems of reliability that have now begun to appear overseas when courts rely on DNA identification evidence (Lander 1989; Scheindlin 1989). It is clear that a radical approach is necessary if courts are to discriminate between good science and bad science. A method of presentation must be found which will make the expert's argument intelligible to an inexpert court.

The Inference Chart (Magnusson & Selinger 1986) provides just such a method. In cases where its introduction as an exhibit is necessary, the availability of the Inference Chart and its associated documents to counsel at briefing is of obvious value.

Although the primary function of the Inference Chart is as an aid to comprehension, the use of such an exhibit clearly makes it possible to provide some of the other

safeguards against the misuse of science which the Australian justice system currently lacks. When the expert's conclusions are reflected in court against the sequence of logical steps which must be taken to make the conclusion valid, it is unlikely that the conclusions will be pressed unless those steps have already been taken. The attention of the court is returned to its proper object - the nature of the evidence - and away from extraneous factors.

The Inference Chart introduces standard forms of words for reporting conclusions, particularly when statistical data are involved. It requires that any conclusion based on population statistics be accompanied by a probability estimate, thus avoiding the variable interpretations that follow such phrases as `consistent with . . .', `probably the same as . . .', and `unlikely to be the same as . . .'.

## Inference Chart Principles

The concept of the Inference Chart is based on a set of principles which must be adhered to if science and the law can be safely mixed. These principles are:

- Decisions about matters of fact are the prerogative of the jury, the function of the expert being to provide the court with the information necessary to allow it to reach its own independent assessment of the conclusions drawn from the results of laboratory tests.

- Claims as to accuracy of testing methods require substantiation, normally by independent, professional investigation of the proficiency of the scientist and the laboratory.

- Since faults in laboratory procedures may at any time lead to invalid conclusions in a scientist's report, checks (`controls') must always be included to establish that the laboratory procedures were operating reliably at the time that the actual forensic test results were obtained.

- The onus of establishing the validity of a conclusion rests with the expert. Since no conclusion can be valid until alternative explanations are eliminated, no conclusion should be stated until evidence to that effect has been presented and its reliability corroborated.

- The `presumption of innocence' rule is hollow unless the court is informed of the level of protection afforded by scientific testing to a falsely accused person. It is the function of the court, not of the scientist, to decide what level of protection it requires. Consequently, the scientist's estimate of the probability of an erroneous conclusion, arising from technical faults and statistical uncertainties, must always be included as part of the conclusion.

## Inference Chart Safeguards

Although a report from a fingerprint expert will necessarily be different in detail from that of a scientist investigating arson or ballistics, the overall logical structure of a report which pays proper attention to reliability is always the same. Consequently, an Inference Chart may be constructed on a template of the safeguards necessary to protect the court from unsafe conclusions (Scheindlin 1989). It is very important that the safeguards incorporated in the concept of the chart appear in logical sequence.

The Inference Chart reduces the expert's report to a series of steps to be taken one-at-a-time. It concentrates attention on the logical validity of each step and assumes no technical knowledge not possessed by the ordinary man. The report is presented as a series of questions each capable of a YES/NO answer. The primary principle of the Inference Chart method of presenting evidence to a non-technical jury is that an unbroken chain of YES responses is required before a final positive conclusion can be stated. By presenting the evidence as a logical chain, a single NO declares the evidence INVALID. By this principle it is asserted that the onus of proof rests with the expert witness to establish the validity of his reasoning before stating the conclusion. Because of the potential of complex scientific evidence for prejudicing a non-technical jury, the need for scientists to fully disclose the results of the checks and safeguards listed above cannot be overestimated.

The major safeguards incorporated in the chart provide protection against the following dangers, in order:

- General technical incompetence - by including proficiency testing of the expert himself as the primary means of appraising his expertise.

- Errors in the reported test results - by requiring that satisfactory results in `controls', carried out in parallel with the tests on crime samples, be obtained before test results are reported. The expert must also demonstrate the reproducibility of his methods by duplicate testing.

- Errors in logic - by requiring an unbroken sequence of YES responses in the logical pathway before an incriminatory conclusion can be stated. Alternative explanations must be identified and satisfactorily eliminated before a preferred explanation is stated.

- Inadequate or unrepresentative statistical data - by requiring the expert to establish the adequacy of the statistical data on which his calculation of the relative probability of correct identification depends.

- Misleading forms of reporting - by requiring that conclusions always be accompanied by relative reliability/risk information. Inclusionary evidence is stated in a standardised form, chosen to enable a non-technical court to reach an informed opinion of the weight of the evidence.

The Inference Chart does not deprive the expert of his right to state his preferred conclusion, but it does provide the court with the information necessary to decide whether or not it can be safely accepted. Where the evidence of one expert is challenged by another, the Chart makes it possible for the jury to follow the argument. If the Chart is properly drawn up, every possible point of disagreement must relate to one or other of the questions in the logical chain.

## Elements of an Inference Chart for DNA Profile Evidence

As implemented at present, the analysis of DNA fragment polymorphisms to provide evidence in civil and criminal proceedings is problematical in several ways (Lander 1989). When first tested in courts in the USA and the UK, the potential weaknesses of DNA identification evidence were either not apparent or were not put under scrutiny. Inevitably, the issues which were not dealt with initially were raised later in circumstances which cast serious doubts about the reliability of DNA profile evidence (Scheindlin 1989). These issues have not yet been fully resolved.

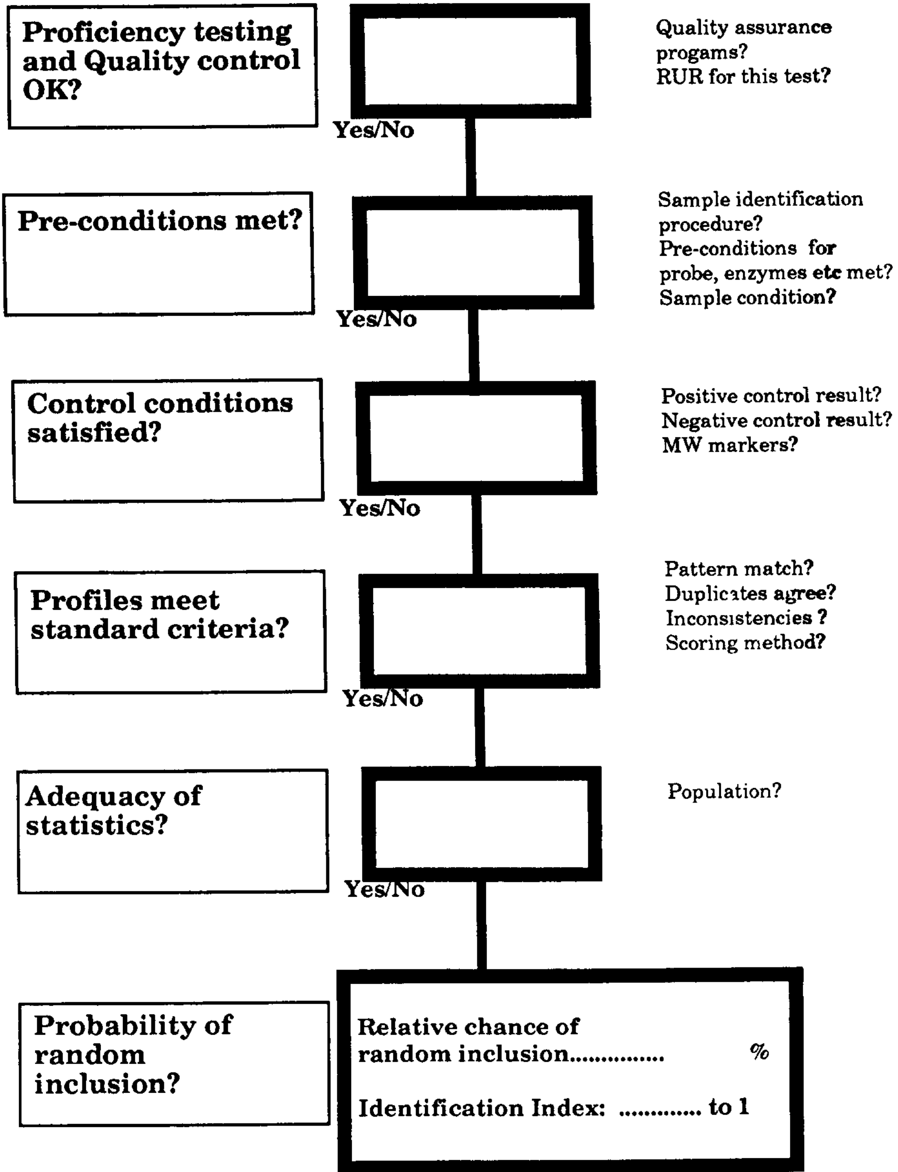
The problems now hampering DNA profile analysis can only be properly solved by having the forensic science profession reach agreement, on a fairly wide scale, on methodology, including the types of probes to be used and the types of controls, duplicate analyses, and other checks to be incorporated; test strategy, especially the aims of each type of analysis, viz. identification v. exclusion; band-scoring methods, including the tolerances allowed for variation in the position of individual bands and groups of bands; statistical methods for determining inclusion and exclusion probabilities; and the selection of population data on which probability estimates are to be based. As indicated in Scheme I, uncertainties in these areas impinge on every one of the danger categories listed above.

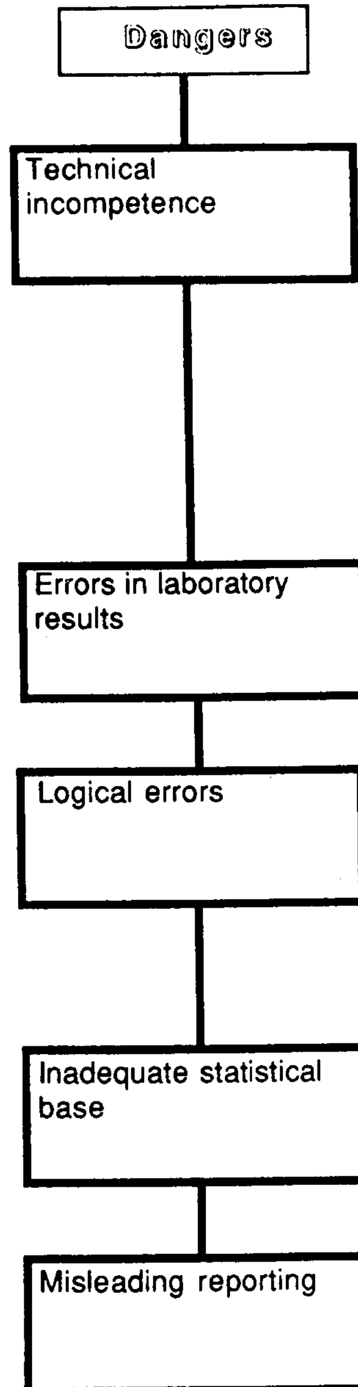
## Quality Assurance

Quality assurance by independent, external authorities is not the traditional way for courts to validate the expertise which an expert witness claims for himself. Rather than inquire about the performance of scientists on tasks of known origin, courts have preferred to judge their expertise by assessing the witness' educational qualifications and subsequent experience. This procedure has little to recommend it today, firstly, because courts are not qualified to assess scientific qualifications and, secondly, because so few scientists who have risen to the level of testifying in court actually received training in the techniques which they now use. It is now being recognised that quality assurance by independent checking is just as important in forensic science as in other branches of science and major schemes for inter-laboratory cooperation are now being put into operation, both in Australia and overseas. Whether or not the courts are concerned with objective methods of establishing an expert's competence, it is certain that the public is concerned. It is important that the full resources of the forensic science profession be utilised in monitoring the competence of forensic scientists in Australian laboratories, especially when they use a technology as new as DNA profiling.

## Conclusion

As identification evidence derived from DNA profiling begins to be tested in Australia, it is important that the courts apply proper safeguards against the errors in methodology or logic to which the method is prone. The reasons for adopting a systematic approach to forensic science safeguards in this case include the unwarranted reputation for infallibility that the new technique may have achieved, the news that serious errors have been made in its use in the USA, and the new and complex technology which it requires.


DNA Fingerprinting Inference Chart
Proficiency testing and Quality control OK?
Yes/No
Quality assurance progams?
RUR for this test?
Pre-conditions met?
Yes/No
Sample identification procedure?
Pre-conditions for probe, enzymes etc met?
Sample condition?
Control conditions satisfied?
Yes/No
Positive control result?
Negative control result?
MW markers?
Profiles meet standard criteria?
Yes/No
Pattern match?
Duplicates agree?
Inconsistencies ?
Scoring method?
Adequacy of statistics?
Yes/No
Population?
Probability of random inclusion?
Relative chance of random inclusion............... %
Identification Index: ............. to 1




**Quality assurance schemes not operating**

**Lack of agreement on methodology**

**Inadequately tested strategies - probe behaviour**

**Unknown behaviour of DNA in exposed or degraded tissue samples**

**Inadequate controls**

**Disputed band scoring**

**Dissimilarity attributed to "Missing band"**

**Dissimilarity attributed to "Band shifts"**

**Paucity of data on general population and on minority groups**

**Misunderstood probability estimates**

**Scheme: Potential dangers in DNA profile tests**

## References

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# DNA Profiling: Standardising the Report

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It is to be expected that courts will want to have the clearest possible understanding of the results of DNA profiling in a particular case. The forensic science community has a responsibility to assist in this understanding by grasping the opportunity, prior to the introduction of this technique into evidence in courts, to present the results of DNA profiling in a standardised manner. This will not only make it easier for the legal system, as the material will be presented in the same format by each institution in each court, but it will also enable other scientists to readily assess the validity of the results. Standardised formats are not easily applied to every branch of forensic science, but DNA profiling is an example of a technique providing data which not only can be, but should be standardised.

## The Structure of the Standardised Report

The report is divided into three sections: summary and conclusions; specific data; and availability of additional information.

### *Summary and Conclusions*

This section is specifically for use by the court in cases where no issue is taken with the results. As such, the summary is brief and contains as little jargon as is consistent with accuracy.

### *Specific Data*

This section contains specific data recorded by the scientist which can be independently assessed without recourse to the scientist's personal notes. It is not meant to preclude the examination of the original notes but acts as a summary of the

data which an independent scientist would be able to use to assess the accuracy of the conclusions drawn in the first section.

Variable number tandem repeat (VNTR) probes hybridised under high stringency (single locus) appears to be the technique most favoured in DNA profiling (Budowle et al. 1988). However, strict definition of an allele is not possible with these probes as an assumption that alleles differ by one consensus sequence (Baird et al. 1986) is probably not valid (Jeffreys 1987). This contrasts with the type of probes which detect alleles of defined size but do not have the high discrimination power of VNTR probes. The two problems facing a scientist when using VNTR probes are determining allele size and determining the frequency with which the allele occurs in the population.

**ALLELE SIZE** The size of a DNA fragment is not measured directly as would occur with DNA sequencing but is an apparent size based on comparison with standard size markers electrophoresed at the same time. The rate of movement of the DNA fragments during electrophoresis is then mathematically converted to obtain a value for the apparent size of the human DNA fragment (Elder & Southern 1983). The basic assumption of this comparative method of size determination is that the standard DNA and the human DNA fragments behave in the same way during electrophoresis. However, it is known that DNA molecules of the same size do not necessarily move at the same rate under electrophoresis (Elder & Southern 1983; Lalande et al. 1988) this being strikingly apparent in dealing with the problem of band shifts (Evett et al. 1989).

Measurement of an absolute size for a human DNA fragment is therefore dependent on some statistical variation inherent in the experimental procedure. A standard deviation of 0.6 per cent of the fragment size has been cited by Baird et al. (1988) and two fragments are considered to be of different size if their values differ by more than 1.8 per cent (Balazs et al. 1989). Whether or not this value has been strictly adhered to in practice is in dispute (Lander 1989).

In forensic biology cases a more critical factor often not considered is that the estimation of standard deviation is usually based on data using DNA from fresh blood samples. When DNA is isolated from non-ideal stains a much larger source of variation due to band shifts (also called offset (Evett et al. 1989)) may occur. Clearly, the method used to estimate statistical variation of fragment size should be carried out with samples prepared under the same conditions. However, given the large range of exhibits encountered in forensic science cases, estimation of the variation due to offset may be difficult. In addition, in accumulating such statistical data it is usually assumed that the stain and the control blood sample originate from the same person: this may not be so and would invalidate any statistical analysis. Thus, a method of internal control which accounts for the component of variation due to offset, would be a better approach to this problem.

When an allele size is stated in a report the statistical variation of the technique should be given. Two fragments which differ in size by more than two standard deviations (to be more precise 1.96 standard deviations) are usually considered to be different (Armitage & Berry 1987). It should be emphasised, however, that this is at a 5 per cent level of significance, which is an arbitrary level. Therefore, sufficient data should be presented to enable a standard statistical test to be performed by an independent scientist to assess the degree of the `match' between the control blood and the suspect stain.

**ALLELE FREQUENCY** Although there appears to be some disagreement as to how much statistical variation can be allowed in assessing the size of a DNA fragment, there is much greater controversy over the methods used to calculate the population frequency of an allele. There appear to be three types of methods in use.

(i) Binning: An allele can be defined by this procedure as the combination of all fragments within the range of plus or minus two standard deviations. Thus, the frequency of an allele is calculated by combining (binning) all frequencies within this range of sizes. This procedure has been complicated by inconsistencies in the size of the `bin' taken even to the extent of there being variation within one laboratory (1.8 per

cent has been taken by Balazs et al. (1989)) compared with 0.8 per cent in a later publication (Lander 1989). Whatever the size of the bin used to determine the frequency of an allele it should be the same as the size used to determine band match (see above), that is the bin would be four standard deviations. This relatively large bin would result in higher frequencies for an `allele' with a corresponding reduction in discriminating power. In fact, because of this reduction in discriminating power, the whole question of using VNTR probes may need to be reviewed. However, the major objection to the binning procedure is that it can produce artificially low allele frequencies, and may, in fact, produce non-existent alleles. This has been demonstrated by Evett et al. (1989) using fragments of standard DNA of known size.

(ii) Standard Comparison: A recent approach to this problem has been outlined by Lander in his `Expert's report in *The People v Castro*' in the USA. This requires the electrophoresis of standard DNA markers adjacent to the human DNA. The frequencies of all `alleles' within two adjacent markers are then combined. This procedure is similar to `binning' but does not rely on an estimate of standard deviation. However, the size of the `bins' are variable along the length of the gel and would be determined by the markers used. Contrasting frequencies between different laboratories would therefore occur, but one company (Promega) sell DNA `binning markers' for using in DNA profiling.

(iii) Frequency Probability: Rather than quoting an absolute frequency for an allele and, recognising that the population frequency of a VNTR probe is a continuum, Evett et al. (1989) have suggested a procedure for calculating the probability of an allele frequency. By this procedure a probability curve for frequency data is calculated and this is used rather than the raw frequency data. This has the advantage of not requiring large numbers of population figures and allows for the fact that an `allele' is imprecise. To allow for statistical variation in fragment size the maximum probability which occurs within plus or minus two standard deviations is used. Although this method offers a solution to the imprecise nature of a VNTR allele, its final acceptance will depend on further assessment by statisticians.

Each of the three procedures for estimating allele frequency has deficiencies and any deficiency in the method chosen should be detailed in the report so that the court is able to assess the accuracy of the allele frequency estimate. Of the three, the technique of binning is the more widely used and it is suggested that this method be used at least in the interim period until a more satisfactory technique is developed. A bin of four standard deviations width is suggested as the most appropriate.

### *Availability of Additional Information*

The third section details the availability of additional information and it is believed that this availability should be considered before the results are accepted. The additional information required is as follows.

PROBES It is important that the probes used be readily available for independent testing. Guidelines relating to the availability of the probes should be similar to those proposed by the Society for Forensic Haemogenetics (1987) viz., results of testing with DNA probes should not be accepted unless the probes are available without restriction. The use of probes not readily available because of patent restrictions or prohibitive costs should not be accepted because the tests may not be able to be repeated by independent laboratories. The Standardised Report requires disclosure of any restriction on the availability of probes.

EXPERIMENTAL DETAILS Full details of experimental conditions should be available for critical assessment of the accuracy of the results. The Standardised Report requires disclosure of the availability of the notes of the individual experiment and the laboratory's manual of the technical procedures.

PUBLISHED SUPPORT DATA Details of the probes should have been published in reputable scientific journals, which have a wide distribution in the scientific community, and include population statistics, sequence data on the probes when available and their genetic characterisation. This requirement is to ensure that the techniques used are acceptable to the wider scientific community, thus tapping the whole range of experience in the specialised areas of DNA technology. The Standardised Report requires disclosure of the name of the probe, its repeat sequence length (when applicable) and literature references attesting their use.

QUALITY ASSURANCE Quality assurance programs should be in operation to assess the accuracy of the techniques and the technical competence of the particular scientist carrying out the tests. These programs should test the ability to discriminate alleles of similar size and the accuracy of determining the size of the alleles. The standardised report requires the disclosure of the presence or absence of particular quality assurance programs in the testing laboratory.

**STANDARDISED DNA PROFILE REPORT**

**SECTION I**

**SUMMARY AND CONCLUSIONS**

A: Item Listing

This report compares the DNA profile of blood from a tube labelled "SMITH, John" with DNA isolated from blood stains on the following items:

Item No.1 A T-shirt

Item No.2 A pair of trousers

Item No.3 A pair of shoes

B: DNA Profiling results

DNA profiles were obtained with DNA isolated from blood stains on the T-shirt and the pair of trousers. The DNA profile of these stains statistically matched the profile of John SMITH which is found in approximately one person in 16,000 in Victoria.

DNA profiles were obtained with DNA isolated from blood stains on the pair of shoes. The DNA profile of these stains did not statistically match the profile of John SMITH.

Name/Qualifications/Address of Scientist in Charge of Testing Procedures:

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Signature/Date: ..........................................................................................

## SECTION II

## SPECIFIC DATA

A: Allele Size

Fragments found (kilobases)

| | Probe ( pABC ) | | | | Probe ( pDEF ) | | | | Probe ( pGHI ) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | kb | (P) | kb | (P) | kb | (P) | kb | (P) | kb | (P) | kb | (P) |
| Control: | 7.12 | - | 6.51 | - | 3.25 | - | 4.75 | - | 2.15 | - | 3.65 | - |
| Item 1 | 7.15 | (0.48) | 6.49 | (0.62) | 3.24 | (0.62) | 4.78 | (0.32) | 2.15 | (1.00) | 3.63 | (0.36) |
| Item 2 | 7.13 | (0.82) | 6.54 | (0.46) | 3.28 | (0.13) | 4.72 | (0.32) | 2.17 | (0.12) | 3.67 | (0.36) |
| Item 3 | 7.25 | (0.0003) | 6.51 | (1.00) | 3.25 | (1.00) | 4.75 | (1.00) | 2.12 | (0.02) | 3.70 | (0.02) |

Statistics: Standard deviation (average) = 0.6% of control fragment size.

P = Probability of obtaining, by chance alone, the difference between the control (fragment size) and the item (fragment size).

Explanation and interpretation of the value of P

When the size of a DNA fragment is repeatedly measured a variation, dependent on the standard deviation of the system, will be obtained. The fragment size of the control sample (see above table) is taken to be the mean value (m) of such a series of size determinations. A subsequent size determination may give a different size (x) and the chance of obtaining such a difference (x-m) can be expressed as a probability (P) e.g., when P = 0.5 the difference (x-m) would occur in 50% of size determinations.

A probability of less than 0.05 indicates that the two fragments are different (i.e., do not statistically match).

B: Allele Frequency (Control Sample)

| Allele (size range in kilobases) | Allele Frequency (total for size range) |
|---|---|
| a. 7.03 - 7.21 | 0.12 |
| b. 6.43 - 6.59 | 0.17 |
| c. 3.22 - 3.28 | 0.17 |
| d. 3.99 - 4.81 | 0.10 |
| e. 2.12 - 2.18 | 0.15 |
| f. 3.62 - 3.69 | 0.15 |

The limits of the range represent plus/minus two standard deviations from the fragment size.

Population frequency calcuation (control sample) = 2ab x 2cd x 2ef = $6 \times 10^{-5}$

These data are based on tests on 520 people taken at random from Victorian Blood Bank donor population.

## SECTION III

## GENERAL INFORMATION

A. Availability of Probes:

Probes are available without restriction: YES/NO

If YES, what is the source of probes?
.............................................................................................................................................

If NO, what restrictions apply? ....................................................................................................

B: Experimental Procedures:

i. The full notes of experimental procedures used in this case are available without restriction: YES/NO

If NO, what details are restricted? .....................................................................................................
.....................................................................................................................................
.....................................................................................................................................

ii. The full details of the experimental procedures as shown in a procedure manual are available without restriction: YES/NO

IF NO, what details are restricted? ....................................................................................................
....................................................................................................................................
....................................................................................................................................

C: Probes used in the analysis:

| Probe | Repeat Sequence Length | References |
|---|---|---|
| pABC | 25 bases | 1, 2 |
| pDEF | 15 bases | 1 |
| pGHI | 20 bases | 1 |

1: Smith, A.B. (1987) Nucleic Acids Res. 123, 1234
2: Jones, C.D. Genetics 23, 123

D: Quality Assurance:

Quality assurance tests are regularly conducted to determine the following:

(i) The accuracy of allele size determination YES/NO

(ii) The reproducibility of allele size determination YES/NO

(iii) The technical competence of the scientist conducting the tests YES/NO

9/10/89
D.DNA.1.BA/CP

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# What Authority should Police have to detain Suspects to take Samples?[1]

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It is an understatement to say that DNA profiling represents a revolution in forensic technology. It is probably the single most significant forensic development since fingerprinting became a generally accepted means of identification. At this time the process provides access to information that is individual specific at one level and species specific at another. In the future, given the nature of the information accessed by DNA technology, it is probable that a great deal more information about individuals will become available.

It is obvious that DNA information can be of immense value in inculpation of persons charged with offences. Not as obvious to many, is that DNA-derived data can also be of equal value in exculpation. Indeed, given the current difficulties of capacity of the criminal justice system to deal with expert evidence and recent controversial cases involving such evidence, it appears that exculpation is of greater actual and potential value than inculpation.

Comparison of the authority to obtain DNA evidence available in New South Wales, with that applicable in England, reveals a vastly different philosophy behind the provisions. The *New South Wales Crimes Act* 1900, provides police with access to body samples only **after** charge, whereas the British Police and Criminal Evidence Act 1984, makes provision for access **before** charge[2] for the dual purpose of inculpation and exculpation.

A further issue is the question of force. Legal policy makers have in the past chosen to adopt a `force' perspective to the taking of forensic samples. This view is erroneous and reflects a period when the police were seen only in terms of force. The Police and Criminal Evidence Act 1984 (UK), offers an alternative based upon an interplay between consent and adverse inference.

The British Police and Criminal Evidence Act 1984, has a lot to offer Australia, in that it represents an attempt to balance, in a very realistic way, the rights of an accused person against the need for police to have adequate powers for law enforcement. The author does not support the view held by many, in New South Wales at least, that any increase in police authority and powers necessarily represents a loss of individual rights.

Authorities presented by the need for police to address particular problems requiring provision of body samples for investigative purposes, should be dealt with in rational terms, rather than being simplistic rhetorical appeals suggesting loss of individual rights. It is necessary in dealing with such proposals that the dangers that

flow from not granting adequate authority are appreciated. For example, the encouragement of unlawful behaviour by law enforcement officers and the possibility of exclusion of evidence so gained, can only punish the innocent victims of crime and fail to convict the guilty.

There are certain dangers to the justice system from wrongful conviction of the innocent, but there are great dangers faced by the justice system in allowing obviously guilty persons to escape justice.

Presentation of expert evidence before Australian courts has been surrounded by substantial recent controversy. Adoption of relatively recent developments in Britain requiring advance notice of expert evidence, access to exhibits and results, has the potential to bring about significant improvement to this situation.

An additional issue is that of media treatment of DNA profiling. It is already apparent that the media are constructing a view in the minds of the public that the DNA profiling technique is a panacea to the high levels of reported crime. Nothing could be further from the truth. DNA profiling will have relevance to a very small number of cases. The danger in media treatment to date is that expectations will be created that cannot be fulfilled. These expectations represent dangers for police, scientists and for the criminal justice system itself.

## Current Police Powers in New South Wales

In New South Wales police power to take fingerprints, photographs and to make medical examinations of persons in custody upon a charge are provided by section 353A of the *Crimes Act* 1900. Subsection 2, reproduced below, provides authority for police to use reasonable force to allow a medical practitioner to make an examination and take samples.

> s.353A(2) When a person is in lawful custody upon a charge of committing any crime or offence which is of such a nature and is alleged to have been committed under such circumstances that there are reasonable grounds for believing that an examination of his person will afford evidence as to the commission of the crime or offence, any legally qualified medical practitioner acting at the request of any officer of police of or above the rank of sergeant, and any person acting in good faith in his aid and under his direction, may make such an examination of the person so in custody as is reasonable in order to ascertain the facts which may afford such evidence.

Two aspects of the section are particularly relevant to the present discussion, first, is that of force, and second, is the requirement that the person to be medically examined be in custody upon a charge.

### *Force*

Dealing firstly with the question of force, it has been held that the section provides authority for police to use such force as is reasonably necessary to enable a medical practitioner to carry out the examination (*McAneny v. Kearney* [1966] Qd R 306). While it may be convenient for legal policy makers to provide for the exercise of *force* to facilitate, through a medical examination, the taking of samples, there are obviously finite limits to the application of force. There is a point beyond which force cannot be exercised in response to a person who is intent upon resistance. Is a resisting person to be treated with continuing reciprocal force on an increasing scale until unconsciousness and then a medical examination be conducted? This approach is not regarded as a satisfactory solution to the situation but it seems to be the fashion.

Exercise of force by peace officers in Canada is proposed by the Law Reform Commission of Canada. There are no current provisions in the Canadian Criminal Code for the taking of forensic samples. The Law Reform Commission in their Report on Obtaining Forensic Evidence (1985) has proposed that samples be obtained generally by consent, but from an unwilling suspect on judicial order. However, in the case of more serious offences (an offence punishable by penal servitude for 5 years or more), where there is danger of loss or destruction of evidence, if the time is taken to obtain a judicial order and there is no less invasive procedure, available samples may be taken by force by a peace officer (exercise of force by the peace officer is to facilitate the taking of required samples by a suitably qualified person).

The British experience is that force is unnecessary. Persons suspected of crimes, when given the alternative of consent or adverse inference, almost always opt for consent. It is true that it is possible to argue at length about the reality of the consent and whether it is truly freely given. However at the end of the day, most suspects conform and provide the samples as requested.

Additionally, it can be expected in the not too distant future, that at least some members of the medical profession will refuse to conduct examinations under such circumstances on ethical grounds.

The British approach, in response to what are termed intimate samples, requiring consent of the person in police detention (detention is distinguished from charge) before an examination can take place, offers a far more realistic approach than either Canada or New South Wales.

*In custody upon a charge*

New South Wales provisions require that a person, from whom samples are to be taken without consent, is to be in custody upon a charge. This necessarily requires arrest and charge before samples can be taken from a non-consenting person. This contrasts with the English provisions that allow samples to be taken from persons in police detention where there is a reasonable belief that the sample will tend to confirm or disprove involvement. It is clear that this is intended to facilitate inculpation. It is also clear that the taking of samples is intended to assist exculpation.

## Police and Criminal Evidence Act 1984 UK

The Police and Criminal Evidence Act, 1984 (UK), unlike the *Crimes Act*, 1900 in New South Wales makes expansive provision for detention of persons suspected of committing arrestable offences. Arrestable offences are those: for which the sentence is fixed by law; and offences for which a person over the age of 21 years may be sentenced to a term of five years. Detention must be to secure or preserve evidence of the commission of an offence.

*Detention in police custody*

Before discussing authority for the taking of body samples it is necessary to understand the general detention provisions of the Police and Criminal Evidence Act.

Detention of suspects is permitted for periods up to 96 hours for investigation of offences. A suspect may be detained on reasonable grounds for up to 24 hours on the authority of a custody officer (Sergeant), up to 36 hours on the authority of a Superintendent, and up to the maximum of 96 hours with judicial authority. A review officer (Inspector or Superintendent) conducts a review of the justification for continued detention at six hours initially and subsequently at nine hour intervals.

On the completion of a period of detention the suspect must be either released, without charge or on bail, or be charged. Where the person is charged and there are other offences that require investigation, an application may be made under the *Magistrates Courts Act* for a further period of detention in police custody for up to three days.

*Taking of body samples*

The British provisions allow for exercise of force in some cases and consent in others. Body samples are classified into two groups by the Act, intimate and non-intimate. Section 65 of the Act defines an intimate sample as: a sample of blood, semen or any other tissue fluid, urine, saliva or pubic hair, or a swab taken from a person's body orifice; and a non-intimate sample: a sample of hair other than pubic hair; a sample taken from a nail or from under a nail; a swab taken from any part of a person's body other than a body orifice.

Body samples may only be taken in cases where involvement of the suspect in a `serious arrestable offence' is suspected on reasonable grounds. A serious arrestable offence is defined by the Act and relates to more serious offences. Section 116: offences include treason, murder, manslaughter, rape, kidnapping, incest, buggery, and indecent assault. Also included are arrestable offences which involve the security of state, interference with justice or investigation of offences, death, serious injury, substantial financial gain and serious financial loss.

*Non-intimate samples s. 63*

Non-intimate samples may be taken from persons in police detention without consent with the authority of a police officer of superintendent rank.

The officer giving consent must have reasonable grounds for suspecting that the person from whom the sample is to be taken is involved in a serious arrestable offence and have reasonable grounds for believing that the taking of the sample will tend to confirm or disprove that person's involvement s. 63 (1)-(4).

The detained person must be given advice that the authorisation has been given and the grounds for giving it. These same details must be recorded in the detained person's custody records s. 63 (6)-(9).

*Intimate samples s. 62*

Intimate samples may only be taken from a person in police custody with the authority of a police officer of Superintendent rank and the written consent of the detained person s. 62 (1), (4).

Authorisation by the Superintendent is based upon reasonable grounds for suspecting that the detained person was involved in a serious arrestable offence and reasonable belief that taking of the sample will tend to confirm or disprove involvement of the detained person s. 62 (2).

Where the consent by the detained person is refused without `good cause', the court, and the court and jury, may draw inferences that may amount to corroboration of any evidence against the person in relation to the refusal s. 62 (10).

*Caution upon refusal*

Persons who refuse to comply with requests for the provision of samples are cautioned in the following form of words:

> You do not have to (provide this sample) (allow this swab to be taken), but I must warn you that if you do not do so, a court may treat such a refusal as supporting any relevant evidence against you.

*Adverse inference from non-consent to intimate samples*

The Police and Criminal Evidence Act provisions for inference to be drawn from `a refusal without good cause' is an ideal approach to the problems presented by exercise of force on an unwilling suspect. Few innocent persons will refuse to give blood or other samples if the situation is fully explained to them. A different situation of course applies in cases where the suspect is guilty: he or she may not wish to give consent to the taking of intimate samples; the suspect must, however, weigh the possible cost.

Do they consent and give the sample and have forensic evidence provide valuable support to the prosecution case, or do they refuse to cooperate, and risk the court drawing adverse inference from the refusal to give consent?

This seems to be a far more reasoned manner of approaching the question of taking samples from suspects, rather than giving authority to police who, with the aid of a medical practitioner, are empowered to forcibly take the sample, but realistically cannot exercise that authority.

## Comparison of New South Wales and British Provisions

While consent is not a factor in the decision to seek similar samples from a person in custody in New South Wales, the *Crimes Act* permits `medical examination' on the authority of a member of the police force of or above the rank of sergeant.

Realistically, however, regardless of the authority provided to police, acting in this case through a medical practitioner, where a person refuses to submit to, for example, the taking of blood or other `intimate samples' what are police to do? Are they to use force and hold the person down while the sample is taken from the person in custody? The scenario is compounded by the need for police to find a cooperative medical practitioner who will agree to take part in the forceful taking of blood or other samples.

The philosophy behind the Police and Criminal Evidence Act sections in relation to intimate and non-intimate samples is clearly intended as a mechanism to confirm or disprove involvement, whereas the New South Wales *Crimes Act* provisions, due to the need to `be in custody upon a charge', seem to be based only upon proving involvement. It seems desirable to have a provision that will allow early elimination of suspects by the taking of intimate samples, with appropriate safeguards such as consent, than be required to charge before such samples can be taken. The capacity for a court to draw inference from a refusal without good cause seems to protect the innocent suspect in detention and also to aid investigation of the crime.

## Advance Notice of Expert Evidence

A further feature of relevant British legislation is the requirement that advance notice be given by both parties of intention to lead expert evidence.

The Police and Criminal Evidence Act 1984 makes provision for Crown Court Rules, amended in 1987, to require both prosecution and defence to give notice of intention to call expert evidence (Rule 3 (1)).

The rules require that upon committal for trial, or the making of an order for retrial, that any party intending to call expert evidence give notice to the other party of this intention. The required notice must be in writing.

A statement in writing indicating the result of any examination must be conveyed to the other party where it is intended to use the results of any examination in evidence. The other party must be afforded a reasonable opportunity to record any observation, test, or calculation that it is intended will be offered in evidence.

Where a party believes on reasonable grounds that release of details of the expert evidence to be called at the trial will lead to intimidation or attempted intimidation of a witness intended to be called, then that party does not have to comply with the rule requiring advance notice (Rule 4). Where this situation exists, the party withholding details of the evidence must give notice as to the refusal and the grounds relied upon. Evidence withheld in these circumstances will only be accepted at the discretion of the court.

*Chamberlain Royal Commission*

Commenting on presentation of evidence before the Royal Commission of Inquiry into the Chamberlain Convictions, Justice Morling (1987) praised the manner in which arrangements had been made to ensure that work and tests conducted by the Victorian State Forensic Science Laboratory could be observed by experts nominated by both parties (p. 311).

On the testing of `blood' on the Chamberlain's vehicle by experts for the Crown, Justice Morling commented adversely:

> Apparently the various experts did not consult together to decide precisely what was established by the tests. Before the Commission, no witness would take responsibility for what was put to the jury. The error appears to have been the result of lack of expertise by some experts, lack of proper equipment and lack of consultation between all the experts involved in this important part of the Crown case (p. 314).

Any matters in dispute related to the tests, examinations, results, and conclusions could be discussed by the experts during the examination and then again before the case commences in court. Agreement would be reached about many matters and the areas of continuing dispute would be known by both parties and if necessary an expert with competence, accepted by both parties, could be obtained to conduct the examination or examinations required.

There would, of course, be situations where agreement could not be reached. In these cases it would be necessary for the Director of Public Prosecutions to consider the matter in the light of the disputed examinations, procedures or experts and revise any decision to proceed in light of the dispute. The important difference being that the Director of Public Prosecutions would be making such a decision with greatly improved information available on which to base that decision.

In an Australian context, introduction of similar rules would have two major potential benefits, the first, relating to court time, and the second, relating to dispute before the courts and to the possibility of later upset of any findings of the court.

**COURT TIME** With current proposals in New South Wales to do away with the committal process, there will be no opportunity to test the quality, or indeed the competence, of expert witnesses prior to the matter coming before the court for trial. Advance notice of expert evidence has the potential to improve the management of cases before the higher courts considerably. Disputes and their extent will be known to both parties prior to commencement of the trial.

**DISPUTE AND UPSET OF COURT FINDINGS** There have been a number of recent upsets in Australian cases involving expert evidence, these include *Splatt* in South Australia and *Chamberlain* in the Northern Territory/Commonwealth. It is possible to adopt the attitude to these cases that they are the problem of the state or territory involved; however, it is more realistic to interpret these difficulties as those of the Australian legal system and its capacity to deal with difficult cases, particularly those involving forensic or expert evidence.

The entire criminal justice system is discredited where there is a tendency of the system to either release too many persons perceived as guilty by most of the population, or not convict persons perceived as guilty. Similar judgments are made by the population about cases where persons are convicted and later released after an inquiry or Royal Commission.

The system would be better served in the context of the present discussion, particularly on disputed expert or scientific evidence if the number of instances where persons are convicted and later released due to doubt, were reduced. Introduction of provisions similar to those in operation in England requiring advance notice of expert evidence would probably go a long way to addressing the concerns expressed by Justice Morling.

## Conclusions

New South Wales *Crimes Act* provisions are based upon the person, from whom samples are to be taken, being in custody charged with an offence before samples can be taken without consent. They are also based upon the exercise of force by members of the police force upon a non-consenting accused. While a request could be made to a person before charge, the provisions are focussed only upon exculpation.

Situations where force is exercised by members of a police force upon citizens should be minimised. Force should only be resorted to if there are no other practical alternatives to gain the samples required. The English experience provides a clear indication that there are alternatives to the exercise of force to obtain body samples.

The English Police and Criminal Evidence Act arrangements for detention and obtaining body samples, support exculpation as well as inculpation. They are based upon consent by the detained person. Where consent is not forthcoming, in the case of non-intimate samples force is not available, and the detained person is cautioned about the implications of not giving consent. Where consent is not given, inference may be drawn by the court and the jury at any subsequent hearing. The inference may amount to corroboration of any evidence against the person that relates to the refusal.

Introduction of advance notice of expert evidence has the potential to reduce dispute over matters involving forensic evidence. It also has the capacity to reduce court backlogs by simplifying hearings and has particular application to New South Wales where there is active discussion on removal of committal hearings.

**Footnotes**

1. This paper is based on research supported by the Law Foundation of New South Wales.

2. The Police and Criminal Evidence Act 1984 (UK) provides for detention for various periods after arrest for the purpose of investigation. A suspect may be released without charge if there is insufficient evidence available upon which to base a charge.

## References

Law Reform Commission of Canada 1985, *Obtaining Forensic Evidence: Investigative Procedures in Respect to the Person*, Ottawa.

Report of the Commissioner, the Hon. Justice T.R. Morling 1987, *Royal Commission of Inquiry into Chamberlain Convictions*, Government Printer of the Northern Territory, Darwin.

The Police and Criminal Evidence Act 1984 (UK) and the Codes of Practice (s.66).

# Structure of the FBI Laboratory

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In order to fully understand the development of DNA technology within the FBI, it is necessary to first take a look at the structure of the FBI Laboratory. The FBI Laboratory consists of four separate sections, which are: the Document Section, the Special Projects Section, the Forensic Science Research and Training Section and the Scientific Analysis Section. The major portion of the development work of the FBI protocol for DNA profiling was developed at the Forensic Science Research and Training Centre. This particular centre is located at the FBI Academy in Quantico, Virginia. It was at the Forensic Science Research and Training Centre that the FBI undertook to develop the DNA protocol.

The Research Centre had an initial contract with the United States National Institutes of Health in 1984 to look into the possibility of determining the race of an individual from the mitochondrial DNA. With the advent of two commercial companies in the United States providing forensic DNA analysis, it was decided that further effort was needed on the part of the FBI. At that time, we undertook a multi-level research program to do the following things:

_ develop uniform nomenclature, standards and controls for forensic DNA testing in the forensic community;

_ provide technical training to implement this technology within the crime laboratory community;

_ continue research efforts for simpler, faster and less costly methods of DNA profiling; and

_ explore the establishment of a nationwide DNA profile data bank.

In January 1987, under these guidelines FBI asked its research scientists to visit the commercial laboratories with ongoing forensic work and to design a research program to meet the above standards. By July 1987, the FBI had organised its plan and had dedicated six scientists to devising a rapid, accurate restriction fragment length polymorphism (RFLP) protocol. Over the next 18 months, as many as 20 scientists were assigned to work on this particular problem.

The FBI laboratory included outstanding forensic scientists from around the United States as part of this research team. Under the visiting scientist program, scientists

from state and local forensic laboratories were invited to the FBI Academy for a four-month period, during which time they worked on this research project. By the middle of 1988, a new protocol was devised and was being validated both at the research centre in Quantico and in the new DNA laboratory in the scientific analysis section at the FBI headquarters. The validation process included studies concerning the effects of outside chemical agents on the analysis process, the development of population data banks, and the training of scientists who would be applying this technology in the FBI laboratory.

### Procedure

The protocol adopted by the FBI laboratory and introduced to case work analysis in December 1988 is an RFLP protocol that utilises the restriction enzyme HAE III, the separation of the DNA fragments using electrophoresis, Southern Blotting onto a nylon membrane, and then the consecutive probing of this membrane with four separate single-locus probes. At the end of the probing process, the examiner is able to reach one of three conclusions. The first conclusion would be that the profiles from the stain on the evidence matches the profile of the suspect or victim; the second is that the profile from the evidence does not match the profile from the suspect or victim; and the third is that no conclusion can be reached as to whether the profile matches or does not match the suspect blood stain. In those instances where the conclusion is that the profile matches the known DNA profile of the suspect or victim, the fragments are then sized utilising a computer program developed within the FBI laboratory. The DNA fragments on the question specimen must match the DNA fragments from the known blood in its size to a standard of plus or minus two per cent to be acceptable as a match. If the fragment falls outside the two and a half per cent criteria, it is considered either no match or inconclusive.

### Results

A survey of the first 100 cases that were completed by the FBI laboratory reflect that in only 18 per cent of those cases was there insufficient DNA available to perform the analysis protocol. In those samples where there was sufficient DNA to develop a profile, 75 per cent of the time the profiles matched the suspect or victim and in 25 per cent of those cases, the profile did not match the suspect who had been identified by the police. The FBI finds these statistics very satisfying in that they show that the technology not only points towards the perpetrator but also exonerates the innocent party.

### Costs

A survey of the costs associated with these first 100 cases reflected that beyond the initial outlay for equipment instrumentation, that approximately $30 was required per specimen run through our DNA protocol. The first 100 cases had an average of five specimens per case: this would be broken down into the known blood from the suspect, the known blood from the victim, and then three questioned stains which resulted in an overall cost of approximately $150 in reagents per case. These are preliminary figures on the first 100 cases and may go up or down as the volume of casework increases. It is important to point out that the initial start-up cost for a laboratory which is anticipating conducting DNA profiling on approximately 5000 samples per year is

$75,000 for the original equipment. This equipment should be useful for many years as most of it is transformers and electrophoresis equipment.

**Training**

In order to transfer this technology to state and local laboratories, the FBI has undertaken a very ambitious training program. We have devised a four-week course that is a hands-on application of our protocol to simulated evidentiary samples. This course is offered four times a year and to date, two classes have been offered, each class containing 30 scientists from state and local laboratories throughout the United States. Students from foreign countries who are going to be conducting RFLP analyses are welcomed into this particular course. However, it must be pointed out that the course is taught only in English.

**DNA Database**

The FBI intends to create a computer-based national data bank for DNA profiles. This data bank will consist of two major parts:

- The first part being population data wherein all laboratories conducting DNA profiling can enter more and more specimens into the population database thereby bringing it closer and closer to absolute unity with the world's population.

- The second databank is going to be investigative in nature. At the present time, it is designed to have three subparts:

    - The first subpart being the open case file. This file would consist of the profile data for unsolved homicides and rapes. By consulting this databank, scientists from different cities may find that the same profile of a rapist matches and therefore the investigators should pool their resources and their information in order to catch the individual. At the same time, a rapist may be identified and arrested in one city and upon the completion of the DNA profile, when searched against the unsolved case file, may find multiple rapes that can be attributed to this one suspect.

    - The second subpart under the investigative aspect consists of profiles from convicted sex offenders. In the United States, several states have already passed legislation whereby an individual convicted of a sex offence must provide a blood sample prior to being released from prison. These samples would be subjected to DNA profiling and those profiles stored in the computer. In those instances where the individual may be a suspect in a subsequent offence, the profiles can be electronically searched against the one on file.

    - The third investigative subpart will deal with missing persons and unidentified deceased. This particular file will be more along the lines of a paternal file with profiles from parents being kept on file to see if a profile from the unidentified individual

matches the profiles of the parent and therefore could be the child of those individuals.

## Ongoing Research

What does the future hold? At the present time, the Forensic Science Research and Training Centre is pursuing ways to improve the efficiency of the DNA profiling systems as well as seeing if there is new technology which can be applied to this area of forensic science. Current research is utilising polymerase chain reaction (PCR) technology looking towards the identification of variable number tandem repeat (VNTR) areas. By applying PCR technology to the specificity of VNTR areas, the possibility of a much more rapid identification of the individual becomes possible. By having primers for four or five different VNTR areas, a single stain can be extracted, amplified overnight utilising the five different primers, subjected to isoelectric focusing the next morning and then utilising a silver stain, the different bands identified and the probability of a profile match being calculated within a one-week period. We are working hard to achieve this goal.

## Conclusion

This paper has attempted to present an overview of the DNA technology as it is being applied in the United States at the present time. As of this meeting, the FBI has testified approximately ten times in either trials, or pre-trial evidentiary hearings in the United States. The technology is still new enough that we expect continued strong legal challenges to its admission into testimony. However, we have every confidence that the courts will find that this technology is accurate and a great addition to the arsenal of tools available to the forensic scientists for the solution of crimes.

# DNA Profiling: the Transition from Watching Brief to the Courts - a Victorian Perspective

## Watching Brief to DNA Profiling as a Service

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To date the Laboratory has carried out DNA profiling on 7 homicides and 9 sexual offence cases, that is, 16 cases in all. Five of those cases were actually started at the SFSL and completed at Lifecodes by Dr Gutowski prior to 1989. The remainder have been profiled in Melbourne this year. The SFSL has offered a full profiling service as of 1 July 1989, and consequently in addition to those cases, has a further 34 partially completed. Thus far, the Laboratory has been requested to carry out work for three other states and both territories. Obviously our rates are too competitive!

Although DNA profiling has been overplayed by the media, the public, and some scientists, there is no doubt that it is an exciting and significant step forward in the field of forensic biology. This paper will use four of the Laboratory's cases to highlight that point. Three of these cases are still sub judice, and consequently there will be partial restrictions on what can be said about them. In addition, they must be seen in the light of legislation which does not demand a blood sample from a suspect or defendant. A Bill which is likely to alter that situation significantly is currently before the House.

**Case study 1**

Over a period of more than 4 years, a series of rapes occurred in Melbourne's south eastern suburbs. These rapes (which number over 14) were linked by certain features such as head-covering, gloves, a kitchen knife, binding of the hands and feet, etc.

The police had no suspects and were baffled until footprint evidence linked a certain suspect to a string of flat burglaries. One of these burglaries was accompanied by what appeared to be preparations to rape the flat's occupant when she came home.

A suspect was then in view. As a result of this, all cases in the series of rapes were re-opened and, wherever possible, exhibits and samples taken in those cases were tracked down, re-submitted and re-examined at the SFSL. It was a tremendous

effort and the police officers and scientists involved in `Operation Shadow' and especially Mr John Scheffer, who is head of the Sexual Offences Section at the SFSL, and Dr Steve Gutowski, head of Research and Development, deserve great praise for their efforts.

Efforts were aided by SFSL policy on unknown offender rapes. Since 1982 virtually all rapes without a known, or at least suspected, offender have been screened. That is, seminal stains are located by acid phosphatase activity. One or a few acid phosphatase positive areas from each major exhibit are then analysed for the presence of spermatozoa, or in their absence, for $P_{30}$ and quantitative acid phosphatase. Then the remainder of the sample from each item is bagged, labelled and stored at -70$^{o}$C. Once an offender is found, the police informants re-submit their exhibits and the samples are taken out of the -70$^{o}$C for grouping, enzyme typing, and now DNA profiling.

In the end, exhibits and frozen samples from 11 out of the 14+ cases in the serial rape were collated at the SFSL for re-examination. From the 11 cases, the SFSL identified 46 items for DNA extraction and possible DNA profiling. Nine of these items had been stored at room temperature and 37 at -70$^{o}$C.

For comparison, there were samples of blood from six victims taken fresh in 1988, and bloodstains made from blood taken from three of the victims at the time of the offence and stored at -70$^{o}$C, in one case for three years, and in two other cases for two years.

The aim was to profile DNA from as many seminal stains as possible, eliminate any contributing DNA from the victims and match the remaining DNA with DNA from the suspect.

In Victoria, the police have no powers to obtain a blood sample other than with the voluntary consent of the suspect or defendant. The defendant steadfastly refused at this stage to give a blood sample so the police collected a number of possible comparison samples comprising two samples of chewing gum, underpants, sheets from the defendant's bed and panties from the defendant's girlfriend. Most importantly, the police also obtained the co-operation of the defendant's estranged wife and daughter. Most hope was held out for these samples since a partial profile from the child's father could be obtained by subtracting the maternal contribution to the child's genotype and this partial profile could be matched with profiles obtained from the victims, even in the absence of a full DNA profile from the defendant's blood, semen or saliva.

The first thing to do was to see if DNA could be extracted in sufficient quantity and of sufficiently high molecular weight for DNA profiling. A summary of yields is given in Table 1. At this stage the situation looked extremely promising. High molecular weight DNA had been extracted in sufficient quantities for DNA profiling from all control samples from the complainants, all control samples from the defendant, and 29 questioned samples from the re-opened cases. Indeed, there was at least one sample to be profiled from all 11 re-opened cases.

*Table 1*

**DNA Yields**

| Samples | Total Number | Number Successful | Yields (Âg) |
|---|---|---|---|
| Whole Blood | 9 | 8 | 1.35 - 15.0 per ml |
| Bloodstains | 3 | 3 | 0.75 - 4.25 in toto |
| Saliva | 2 | 2 | 1.50 - 5.50 in toto |
| Semen Stains (fresh) | 3 | 3 | 1.00 - 2.25 in toto |
| Semen Stains (-70$^{o}$c) | 37 | 23 * | 1.00 - 4.00 in toto |
| Semen Stains (RT/4$^{o}$C) | 9 | 6 | 1.25 - 8.00 in toto |

*One sample had part stored at -70$^{o}$C
Part stored at RT combined for analysis.

However it transpired that most of the DNA extracted from the stain material was bacterial and/or viral in origin and only 8 of the stains were shown to contain human DNA (Table 2).

*Table 2*

**Test Gel Results**

| Samples | Number | Number Human |
|---|---|---|
| Whole Blood | 8 | 8 |
| Bloodstains | 3 | 3 |
| Saliva | 2 | 0 |
| Semen Stains (fresh) | 3 | 1 |
| Semen Stains (-70$^{o}$C) | 23 | 6 * |
| Semen Stains (RT/4$^{o}$C) | 6 | 1 |

*One successful stain part stored at -70$^{o}$C at RT.

No result was obtained with the defendant's seminal stain but a good profile was obtained from the defendant's wife and the defendant's daughter. It was therefore possible to assign paternal bands as shown in Table 3.

*Table 3*

**Results**

| | Human | D14S13 (MLJ) | D17S79 (VI) | DXYS14 (CI) | D2S44 (YNH) |
|---|---|---|---|---|---|
| 1309/82/5 | + | + | + | NE | + |
| 1309/82/6 | + | + | + | NE | + |
| 1240/845/2E+2F | + | + | + | NE | |
| 1507/845/9aix, aiy | + | + | + | NE | |
| 1912/845/16(1) | + | NE | NE | | |
| 3281/845/9(8) | + | | | | |
| 434/867/1d | + | + | + | NE | |
| 517/889/20 | + | + | | | |

| Frequencies | | | Storage Conditions | | |
|---|---|---|---|---|---|
| | D2S44 | 2.8% | | 434/867/1d | RT |
| | D17S79 | 6.4% | | 517/889/20 | Fresh |
| | D14S13 | 0.3% | | Others | -70$^{o}$C |
| | DXYS14 | Not Applicable | | | |

As can be seen from Table 3, of the 7 samples from complainants, 5 samples gave readable patterns with probes D17S79, D14S13, and DXYS14. Two of the 5 also gave readable patterns with the probe D2S44.

For these two samples, it can be calculated that the bands from the deducted paternal profile present in the patterns from the sample are present in only 2.8 x 6.4 x 0.3 per cent of the population, or around 1 in 180,000 or so in an American Caucasian population. Unfortunately, due to the nature of the DXYS14 probe, no population statistics for the deduced paternal profile exist as yet.

In the end, that was sufficient for the police and the suspect is now in gaol. For the other three cases where DNA evidence was useful, matches were obtained with D17S79 and D14S13. The odds here are around 1 in 5000.

In a fifth case, there was a hint of a match with probes D17S79 and D14S13. However,the results were not clear enough for court.

Finally, it is of interest to note that of the samples which gave full or partial DNA profiles, one was fresh and only reacted with the human probe. Six samples were stored at -70$^{o}$C and reacted to varying degrees, including the two which gave successful patterns with all probes. On the other hand, one sample did give a successful result with all probes except D2S44, even though it had been stored for two years at room temperature. The police then charged Mr George Kaufmann on all counts. He immediately confessed to some of the rapes and gave a blood sample which immediately improved the best odds to better than 1 in 3 million. It is interesting that he would not admit (initially) to the rape of the very young or very old complainants and when it was put to him in interview said, `What do you think I am - an animal!' However he pleaded to all counts at the committal, and later at the Supreme Court trial. He is due to be sentenced in mid-November.

**Case study 2**

In mid-1988 an adult woman was brutally strangled in her home. It was a number of hours before the body was discovered and the police, the laboratory, and a pathologist called. Semen was very apparent in the vagina and there was sufficient trauma to the vagina to suggest a link between the seminal fluid and the homicide. To complicate the issue the dead woman's 18-month-old child had been left alive to wander around the house.

The police quickly produced six suspects (two with previous convictions) and in fact were very keen to charge one of the two stronger suspects. All 6 gave a blood sample voluntarily. Conventional Grouping and PGM IEF typing did not resolve the issue and indeed two of the suspects were included as possible sources of the fluid.

DNA profiling subsequently excluded all 6 suspects as the donor of the semen, much to the Homicide Squad's dismay. Subsequent investigations produced three further suspects, two of whom gave blood samples. The two were quickly eliminated and the focus of the investigation turned to the third, who as it transpired, had been courting the woman at the time the little girl had been conceived.

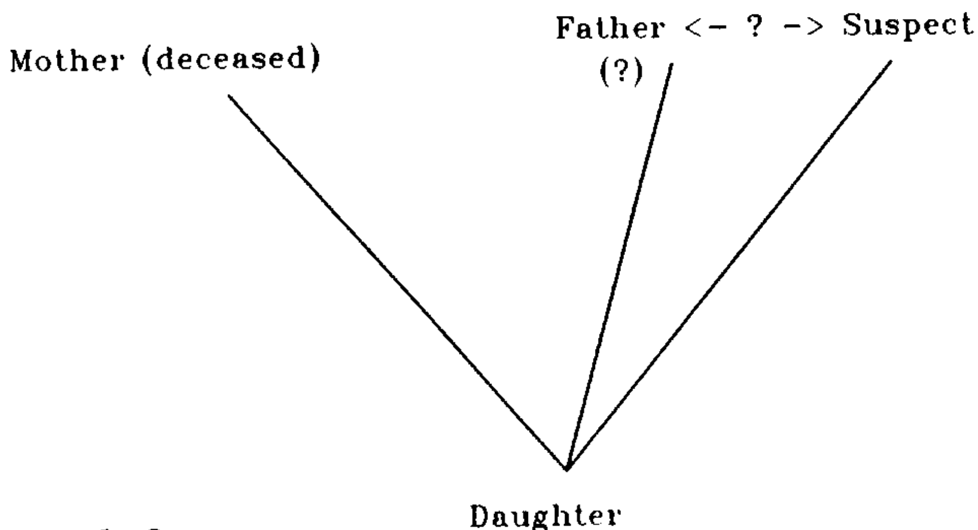
The child was profiled and it was ascertained that the father of the child was likely to be the source of the semen as the partial profile deduced was common to approximately 1 in 8000 of the American Caucasian population. It was put to the suspect that this was a double jeopardy situation - namely, that he could clear himself of the homicide and as the putative father of the child, with all attendant implications under the Commonwealth *Family Law Act*, simply by parting with a blood sample. He steadfastly refused.

A fight broke out at a pre-Christmas party at about that time, blood was spilt and the police called. The attending detail recognised one pugilist, namely the suspect for the homicide and took possession of a bloodstained shirt from both him and his boss, the second pugilist. A blood sample from the boss showed that the stains on both shirts in all likelihood originated from him.

*Table 4*

**9 Suspects**

**8 Blood Samples - All Excluded**

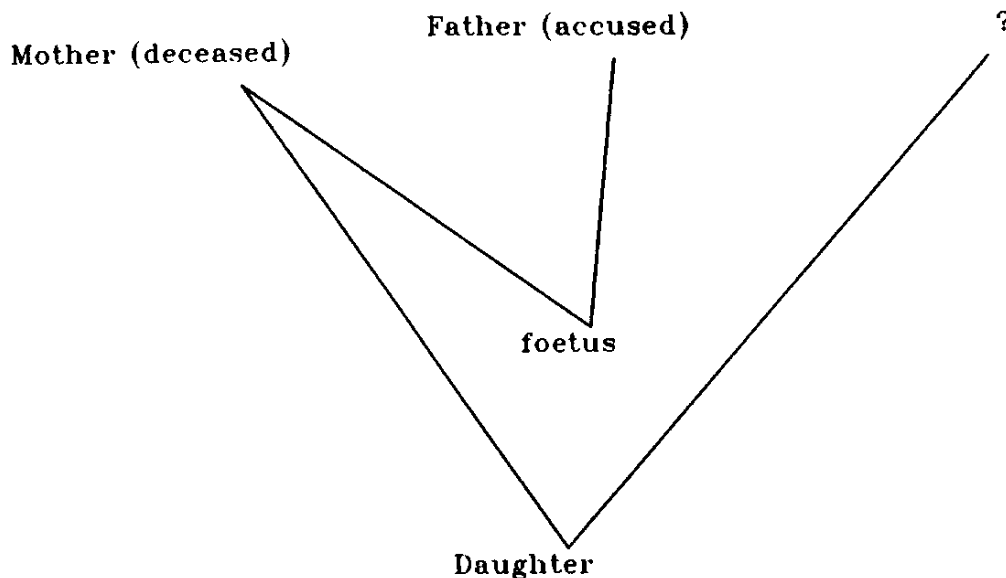


**Case study 3**

The third case study does not exclude or include an individual as the perpetrator of a crime, but rather poses an ethical question. A man in a country town was accused of murdering his wife late last year. The murder scenario painted by the suspect is complex, and forensic evidence tends to support his alibi, but does not exclude him as the murderer.

When the wife was killed she was pregnant. One scenario for the murder put up by the police was simply that the child was not the husband's. In case the accused would not volunteer a blood sample, blood was taken from his eighteen month old daughter. The accused did volunteer a blood sample and all samples were profiled, including that of the dead foetus.

*Table 5*




As it transpired, the foetus was in all probability fathered by the accused, but the child was not. SFSL was then left with the rather unpalatable situation that it might, if it made that information known to the police, be souring the relationship between the accused and the child, not to mention legal fiscal responsibility. After much deliberation the decision was made to inform the police who immediately saw yet another avenue for motive. It appears that it was fairly common knowledge that a certain member of the town had been friendly with the dead woman some years prior to her death and was uncommonly fond of the daughter. The accused has been committed for trial, probably to be held either later this year or early next year.

## Case study 4

A woman in Melbourne cried abduction and aggravated rape a few months ago. There was little circumstantial evidence to support aggravation other than a number of head hairs (54 - all of which carried some root material), nor was there any ejaculate in the vaginal area of the woman to link back to the defendant.

The rape allegedly took place at the defendant's flat to which the `victim' had supposedly been transported. The brown 20 centimetre hairs were examined microscopically, bulked and the DNA extracted. 0.25 Âg of DNA was obtained and subsequently profiled. Only two of the probes were sufficiently sensitive to give a result but did not match the DNA extracted from the woman's blood sample, at a frequency of approximately 1 in 370 Caucasians. Given that the defence argued that a number of women had been in the flat, the DNA evidence, together with the root material aspect lent credence to the prosecution case.

## Conclusion

It is rather unfortunate that the media, in all its forms, has overstated some aspects of DNA Profiling Technology. This has been achieved by both manipulation of the facts in search of a good story and indirect reinforcement of selective statements made by those scientists or commercial enterprises with a vested interest. This was fairly graphically illustrated by a recent `60 Minutes' segment which referred directly to the *Kaufmann* case (Case study 1).

The media have created a culture of belief, both in the technology and the population statistics associated with it. This may be the reason why DNA profiling was not adequately tested in the United States until recently. Mr Freckleton promised us a `baptism by fire' two years ago in Adelaide, but unfortunately, this has not happened. Of the cases mentioned, a number have reached committal stage and three have gone through the courts without being seriously challenged. It is hoped that there will be a few test cases to help iron out, early on, any misconceptions associated with the technology.

Perhaps most significant of all, is that, to date, the SFSL has excluded approximately twice as many suspects or defendants as it has included, in the association of, or with a crime.

# DNA Profiling: the Transition from Watching Brief to the Courts - a Victorian Perspective

## Watching Brief to DNA Profiling as a Service

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Awareness of DNA fingerprinting was focussed initially after attendance by Dr Stephen Gutowski at the 11th Congress of the Society for Haemogenetics in Copenhagen, August 1988. On his return to Australia interest in the possible potential of the technique was translated into a watching brief held by the SFSL Research and Development Branch. At this time Jefferys' (Dr A. Jefferys, Leicester University, United Kingdom) work, and issues of patents were attracting world-wide debate. Simultaneously, several other centres involved in molecular biology work turned their attention to forensic applications utilising various enzymes, probes, detection systems, and run conditions with mixed results.

In September 1986, Drs Gutowski and Atchison began assembling the basic items of equipment for DNA profiling and, through the contacts established overseas, gathered together a number of donated probes. The debate then growing in this field had, as one of its focal points, the issue of colour linked (that is non-radioactive) or radioactive detection with confusing and contradictory claims made by proponents and opponents of both.

As the genetic technology industry developed through the late 70s and early 80s, various licences and permits had been established to ensure adequate safeguards were in place for the industry. The SFSL had been accredited with a recombinant DNA licence and a $P^{32}$ radioactivity permit by April 1987. The strategy at this stage was to maintain up to date information for both radioactive and non-radioactive detection methods while becoming competent in the numerous manual steps the process entails.

During these earlier stages the SFSL, at the expense (unfortunately) of other parts of the forensic biology program, committed salaries, equipment and consumables to develop the technological capability for DNA profiling. By the end of 1987 it is estimated some $50-55,000 had been committed.

Because of the hype that had developed along with DNA technology itself, the SFSL was patently aware that a prudent and sound philosophical approach was mandatory to its introduction in Victoria. It was becoming clear as evaluation

progressed and the full picture unfolded, that DNA profiling was likely to realise the potential it promised and would thereby revolutionise forensic biology.

Consequently, a carefully formulated protocol for the introduction of this type of analysis was established. It provided for the founding of DNA analysis on very secure grounds by first using the technology in the relatively simple application of sexing of blood stains. This service was in place by June 1988 and concurrently the development of DNA profiling, using single locus probes was taking place. At this stage the smaller resource commitment had blossomed into a full research and development project and it was realised that without some additional special funding, progress would be relatively slow. (Some $160,000 had been invested in DNA development between 1986 and 1988).

Developments in the DNA profiling industry world-wide were occurring rapidly and in number. Several different approaches were being pursued as a result of the varied reasons for undertaking DNA development. In addition, the profit motives in some developmental laboratories were directing research into alternative systems, aimed at circumventing patents. This meant that the technology from some sources was only available as a service and not as a technique for application by SFSL scientists. Two major concerns with the `service only' approach were:

- difficulties with timely provision of investigative information; and
- doubts about standards, resources, and reputation of the service laboratory.

In the SFSL's view, only four viable options existed early in 1988 and they were as follows:

(i) **Cellmark (ICI)** This system was protected by patents and gave no autonomy whatsoever to the forensic laboratory using the system. All items for analysis had to be flown to the United Kingdom for profiling and court defence carried out by the Cellmark scientists.

(ii) **Cetus Corporation** This USA based system had, in our opinion, still to prove itself but did have potential - particularly in the area of trace amounts of material. Once again, as with ICI, the SFSL (certainly in the immediate future), would be wholly dependent on the USA with no control over population statistics, quality of methodology, results, interpretation, or courts.

(iii) **Collaborative Research Inc.** This was undoubtedly the cheapest option, utilising colour linked detection rather than radioactive labelling. However, there was no support offered whatsoever for their technology. This would mean that the development of all local data, population statistics, etc. would have to be carried out here. In addition this technology had never been applied to stain material (only liquid blood). The SFSL harboured doubts that this technology was even appropriate to stain applications. Suffice to say that profiling of stains using Collaborative was untried, even in the American courts.

(iv) **Integrated Sciences (Lifecodes)** This company offered a training program with full Quality Assurance/Quality Control assessment and ongoing accreditation. Lifecodes in America had been involved in DNA profiling of stain material for a number of years, had generated the necessary population statistics and had a proven track record in the American courts. Their technique, like Cetus and ICI, involves radioactive probes which makes the technology expensive. They offered a full package which was aimed at making the laboratory in question fully autonomous, but with Lifecodes support and

backup. This package cost $56,000 and required the purchase of additional capital equipment.

As SFSL policy had always been to have responsibility for control of examination, analysis and interpretation, the Lifecodes option was identified as offering this scenario.

Around Australia at this time, significant interest had been aroused. Tasmania had sent some samples to Cellmark in the United Kingdom and both the Northern Territory and the Australian Capital Territory were gearing to sending their samples to Lifecodes in the United States. Only Victoria and South Australia were working toward developing local DNA profiling capability. Although in early to mid-1988 both agreed that due to cost considerations, ease of use, and control of testing, the Collaborative Research Inc., option was the most attractive, it soon became evident that sensitivity was a problem for its application to forensic crime samples. It was at this point (mid-1988) that SFSL opted to pursue radioactive labelled probes and aligned with the Lifecodes Corporation DNA regime.

In July 1988, Dr Gutowski took some urgent, important casework samples to Lifecodes Corporation in New York and worked through the process under the supervision of experienced DNA profiling practitioners. This procedure was followed for four reasons:

- to avoid the necessity and cost of calling Lifecodes practitioners to hearings or trials in Australia at some later stage;

- to ensure any subsequent evidence would be given by an experienced forensic scientist familiar with the local legal environment;

- to allow a first hand evaluation of Lifecodes; their methods, operational standards, personnel, training, data base, etc.; and

- to familiarise Dr Gutowski with the system used by Lifecodes and thereby serve as useful initial training toward that required by the company in eventually making the system available to SFSL.

An evaluation of this exercise resulted in consolidation of the Lifecodes system option and in September 1988 the Minister, Mr Crabb, promised $100,000 special funding for the development of DNA profiling in Victoria. Included in the prospectus outlining the proposed program were schedules for equipment and recurrent costs and a cost-benefit analysis. Cost-benefit considerations necessarily involved estimates of savings in investigation costs and court costs. Arguments as to the considerable expense of conducting DNA profiling have some merit in comparison to the relative costs of other forensic biological typing procedures but pale into insignificance when considered in the overall cost structure of the investigation and subsequent trial.

From this injection of funds the program was geared up to a fully fledged research and development effort using the Lifecodes DNA-Print System. The commitment to this system then determined a time frame also for the transition from research and development to an operating Molecular Biology Section and the associated recruitment of suitably qualified and experienced staff.

In November 1988, Dr Gutowski travelled to New York for the second time with urgent samples from major crime cases. At this time DNA extraction was perfected at SFSL and these samples were therefore processed at Lifecodes from this point. Between November and February 1989, SFSL capabilities were extended further until by the end of February the entire process was perfected locally.

Concurrently with the steady development of SFSL technical capability and the associated training of new `DNA-staff', we were informed that Lifecodes had been successful in their patent application for the DNA Print System in Australia. This meant that use of the Lifecodes system by SFSL was now subject to licence and patent conditions. Science and new developments therein had previously been virtually freely available, but DNA technology brought with it a whole new matrix of licences, permits, and patents. The SFSL found itself firmly embroiled in this matrix.

The SFSL did not (and still does not) agree with burdening new technologies with patents and licences, etc. This is particularly valid in applications such as forensic science, which provides a community service through the justice system, but it was clearly recognised that if a DNA service was to be provided in response to demand, then this approach had to be embraced. Lengthy legal discussions between SFSL, Departmental and Ministry personnel (including legal advisers) and Genetic Technologies Corporation (GTC, the Australian licensee of Lifecodes) parallelled SFLS's scientific progress and the legal agreement was finalised in February 1989.

With finance, legalities and scientific capability all in place by March 1989, SFSL embarked upon the Lifecodes Corporation accreditation program which required the 100 per cent successful profiling of 175 samples, 100 of which were totally blind. Quality of the work performed was of paramount importance and this was judged from the submitted DNA profile autoradiographs and the associated detailed documentation covering all aspects of the process, interpretation of results and conclusions. Evaluation and final assessment was made by Lifecodes Corporation in the USA.

As the accreditation program was designed in modules, the results of each had to be evaluated and accepted before the next batch of samples were forwarded. Needless to say this was a lengthy process as special clearances were required for new material to enter Australia. Accreditation was finalised in late June 1989 and the SFSL offered a full casework DNA profiling service from 1 July 1989.

Thus far 16 cases have been processed by the Molecular Biology Section, 9 sexual offences and 7 homicides and a further 34 cases are currently in progress. SFSL's estimates of annual service demand are in the order of 300 cases per annum at an estimated cost of around $100,000 (recurrent budget). Proposed new legislation in Victoria regarding police powers for gaining relevant biological samples could substantially increase the demand (estimated at 250 per cent) for this service beyond this projected figure.

# Fraudulent Frozen Meat - DNA in Action

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The export meat trade is regarded as a tough and competitive business, subject to the vagaries of drought, flood, exchange rate fluctuations, European and American politics, and sometimes unscrupulous activities by competitors.

The Department of Primary Industries and Energy (DPIE), Meat Inspection Branch, is responsible for the certification and monitoring of Australia's meat export industry. The Meat Inspection Branch is charged with the responsibility of ensuring that meat products are safe, wholesome, accurately described, and in compliance with the requirements of meat importing countries.

Because of the competitive nature of the industry, and human nature, some meat exporters have been tempted, in the past, to work on the assumption that `what the customer doesn't know won't hurt'. This attitude is best illustrated by the 1981 meat substitution scandal in which a variety of circumstances and factors enabled the substitution of frozen beef meat packs with beef/kangaroo and possibly other species. The meat exporting companies concerned were able to engineer this scam through criminal activity, such as forging export certificates and stamps, and because the compliance testing and assay procedures at the time were not stringent enough to detect the substitution at source.

This situation has changed, and all export meat abattoirs now have their bonded product randomly sampled on site, using an 8 species Enzyme-Linked Immunosorbent Assay meat speciation system, developed, manufactured and marketed as Checkmeat by the Regional Veterinary Laboratory, Benalla. The introduction of the Checkmeat system by the Meat Inspection Branch has essentially eliminated meat substitution and paved the way for improved export surveillance of the quality of certified export meat products.

Some aspects of quality assurance testing are, however, difficult to assay objectively. The chronological age and sex of meat are two examples. Other parameters such as leanness and tenderness can be measured objectively if required, but are of course correlated with age.

The sex of slaughtered animals can be important in meat marketing. Bull meat is a premium product because of its water retention qualities and it is an ideal base for the manufacture of sausages and related small goods. For animal management reasons there are relatively few bulls available for slaughter, so there is a temptation by meat works to substitute bull meat with meat from culled dairy cows, which do not command the high sale price of bulls. Once the bull and cow meat has been boned out, the resultant meats can be mixed and labelled as bull meat with minimal chance of detection.

DPIE meat inspectors at export boning rooms know the weight of bull carcasses slaughtered and can calculate the yield and thus the expected kilograms of bull meat in frozen boneless bull meat packs. Instances have occurred in the meat trade where the weight of frozen boneless bull meat produced exceeded the calculated maximum yield from the available bull carcasses.

The problem for the Meat Inspection Branch is, therefore, how to assay frozen packs of bull meat to confirm the genetic sex of the product. If a meat pack is labelled `bull' meat there should be no genetically female meat present. Traditional methods of sexing meat such as gross and histological examination and hormonal assay are inadequate and technically difficult, if not impossible, on frozen tissue.

A.B. Technology Pty Ltd, in consultation with the DPIE Compliance Branch, and Bureau of Rural Resources staff, have developed a new assay which can be used to differentiate the genetic sex of meat from packs of frozen bovine meat. This method has the potential to be developed for use with other species.

A.B. Technology Pty Ltd have isolated and sequenced DNA from the Y chromosome of domestic ruminants, and have identified the conserved Y specific repeat sequences of bovine species in particular. These elements have been used to develop a rapid polymerase chain reaction based embryo sexing assay, used on single cells of split bovine embryos, enabling sexing of embryos before implantation. The assay has been modified for use in post mortem tissue.

Samples (50-100 mg) of frozen meat can be collected from clearly identified single muscle masses extracted from frozen meat packs. A clean surface within the muscle mass has to be prepared with a chisel, and then a small bit is used to collect the sample into a small plastic cup.

Measured amounts of these samples are denatured using sodium hydroxide to produce disassociated strands of bovine DNA. The disassociated DNA is assayed in parallel using rapid dot-blot hybridization with a y specific probe and a probe for a constant number of autosomal repeats that are specific for cattle. This allows confirmation of species of origin, that is bovine, and quantitative of total DNA. Both qualitative (autoradiography) and quantitative (scintillation counting of individual dots) analysis can be performed, allowing unequivocal evidence of the species and sex of each sample.

The technology could be used in routine compliance testing, of the type now undertaken with species testing, or in situations where substitution is suspected by Australian Quarantine and Inspection Service Compliance Branch Officers. The assay may also have application in the monitoring of product by importing countries, and theoretically could be used in conjunction with hormonal assays, to separate bull, steer, cow and heifer meat, and meat from animals treated with hormonal growth promotants.

# Potential Sources of Artifacts and Errors in generating a `DNA Profile'

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Discrepancies between independent analyses of a particular sample may be due to variations in any of the following procedural details. Some do not have a significant effect on the validity of properly controlled side-by-side comparisons; many more could be monitored routinely by analysis of internal `non-variable' control sequences.

These points are raised to underline: the need for standardised, controlled procedures; the need for accreditation and auditing; the desirability of providing full technical details to counsel. They should not be construed as arguments against the adoption of evidence provided by `DNA Profiling' in criminal and civil cases. On the contrary, it is hoped that their recognition will expedite the routine adoption of sound DNA-based evidence.

1. **Tissue Sample**

- origin
- quantity
- quality
- storage conditions
- human? (many probes react with DNA of many different species)
- continuity

2. **DNA Isolation**

- method
- reagent quality (e.g. phenol-induced damage?)
- DNA quality (degraded? denatured?)
- DNA storage conditions

3. **DNA Digestion**

- incomplete?
- excessive? (`star' activity)

4. **Electrophoreses of DNA**

_ gel type (agarose, polyacrylamide, other)
_ gel format (vertical, horizontal - `submarine' can lose small fragments)
_ buffer type, ionic strength, pH
_ gel concentration
_ overloading? similar loading for all samples? critical measurement is amount of DNA per cross-sectional area of gel track, affected by
- gel thickness
- width of sample loading wells
- depth of sample loading wells (if horizontal gel)
- amount and concentration of DNA loaded

_ field strength
_ uniform migration rate for all samples?

A photograph of the gel after electrophoresis (stained to reveal DNA) should be included in evidence.

5. **Transfer of DNA to Membrane**

_ membrane type, manufacturer and batch
_ gel pre-treatment
_ transfer solution
_ transfer method
_ transfer time

6. **Hybridisation**

_ buffer type, ionic strength, pH
_ accelerator?
_ denaturants?
_ `carrier' DNA?
_ volume
_ temperature
_ duration
_ physical method used (details)
_ probe
- origin and information re probe sequence
- is it a recombinant probe?
- DNA or RNA?
- single or double stranded?
- radioactive or non-radioactive label? (specifics)
- labelling method
- specific activity and concentration

_ is this a re-probe of a previously used (stripped) membrane?

7. **Washing**

_ buffer type, ionic strength, pH
_ volume
_ temperature
_ duration
_ number of washes under each set of conditions

8. **Autoradiography**

(If non-radioactive probes are used this is redundant, but attention must then be given to the additional steps involved in developing the profile pattern).

_ X-ray film
  - manufacturer and batch
  - type (particularly as it affects speed of image development)
_ exposure time
_ exposure temperature
_ intensifying screen(s)?
_ texta touch up?

It is recommended that evidence for each comparison set includes two autoradiograph exposures differing markedly in sensitivity; the autoradiographs should be available for examination and confirmation of the accuracy of photographic records.

9. **Photography**

(less significant if original autoradiographs can be examined)

_ photographic and development conditions
_ filters? (particularly in photography of colour (non-radioactive) signals)
_ evidence of differential enhancement?

10. **Interpretation**

_ relative weighting given to matches of large, medium and small bands - far less weight should be given to bands of low or high mobility
_ criteria used to establish/eliminate matches
_ population base used in comparisons
_ lies, damned lies, statistics

# The Tracktel Electrophoretic Pattern Image Processing System

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Image processing techniques provide the opportunity for rapid, accurate, automated analysis of DNA electrophoresis gels. Forensic Science Technology International Pty Ltd (FSTI) has developed the TRACKTEL system which exploits the latest available technologies and provides biological scientists with a means of storing, retrieving and comparing results of DNA analysis, including paternity tests.

In the forensic environment, FSTI's TRACKTEL system provides enhanced control, reporting and security functions to ensure that the rigid quality assurance and security procedures associated with material of a potentially evidentiary nature are fully maintained. TRACKTEL also enables the development of cumulative population databases, against which individual results can be measured.

The fundamental architectural basis of all FSTI's technology provides the capability for TRACKTEL systems to be networked. Consequently, the potential exists for a national database network of DNA results to be established.

# Macro Mini Micro Satellite VNTR Polymorphism: Theory and Application

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**DNA as a Means towards Human Individualisation**

Single stranded deoxyribonucleic acid (DNA) is a long linear polynucleotide constructed from four different nucleotide monomers. The ordering of these monomers defines DNA sequence. Double stranded DNA consists of two such polymers held together by hydrogen bonding via specific nucleotide pairs, such that the sequence of one strand defines the sequence of its homologous strand. The resultant double stranded DNA is twisted in a helix and structurally stabilised by packaging around nuclear proteins, the histones. The combined complex is then called chromatin. Chromatin may be packaged and condensed to varying levels of compactness and microscopically it is seen in its most compact state as chromosomes. The DNA in the human genome is distributed unequally among 23 pairs of chromosomes, these totalling some 6 x 109 nucleotides per diploid genome.

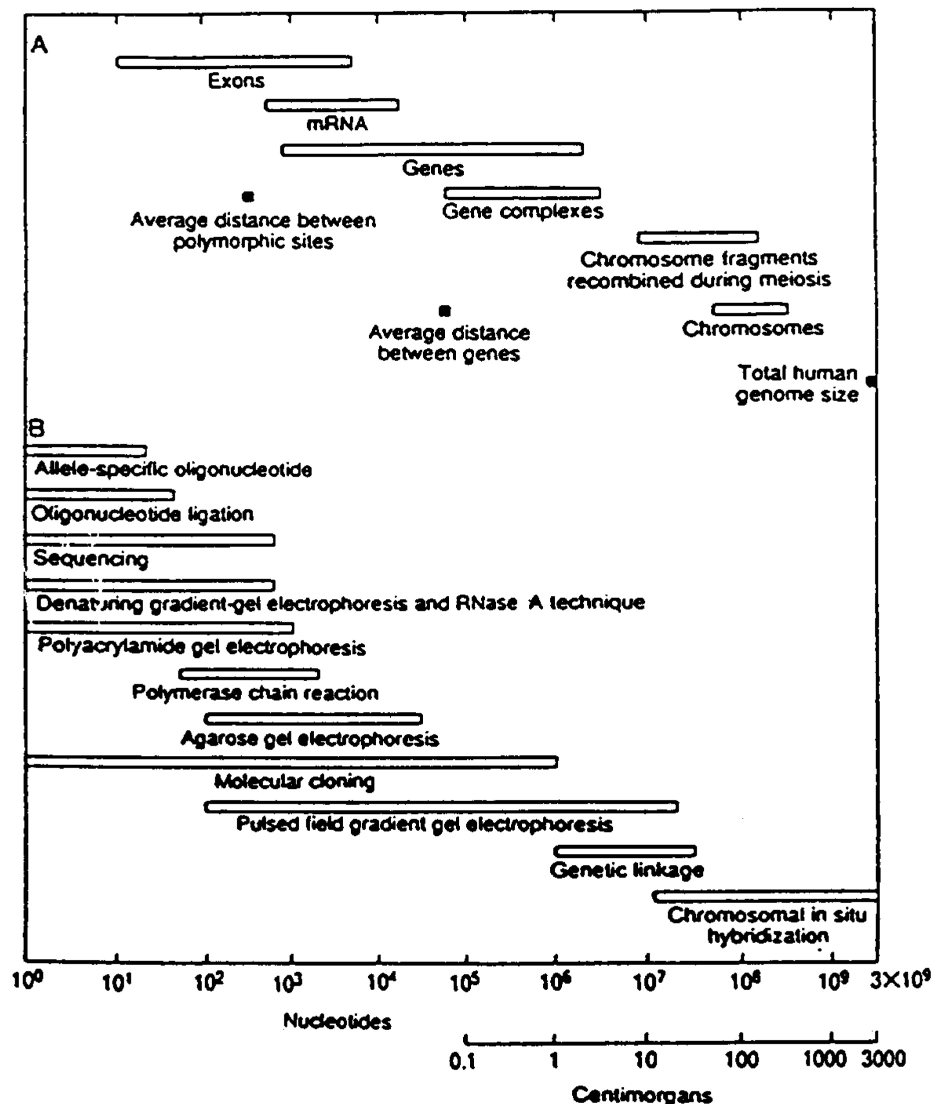
Two important practical points arise from this. Firstly, removal of the protein scaffold upon which the DNA is supported is generally necessary before any DNA analysis is possible. This process inevitably leaves the DNA more susceptible to mechanical or enzymatic damage, thus disrupting its polymeric integrity. Therefore the quantity and integrity of DNA are major factors in determining a suitable method for its analysis (Figure 1). The DNA recoverable from forensic specimens may be extremely limited, both in quantity and integrity. Such samples require specialised methods of DNA analysis for their association (or dissociation) with any putative source.

Secondly, the fidelity of DNA base pairing: G-C, A-T is essential, not only to life itself, but also to all in vitro DNA manipulations: for example, DNA probe preparation, `Southern' hybridisations, DNA sequencing or the polymerase chain reaction (PCR) amplifications. An appreciation of the experimental conditions which influence and maintain the fidelity of this pairing is paramount.

These two points serve as a platform from which DNA technology is launched. The first is its major limitation (loss of DNA sequence destroys its only distinguishing feature) and the second is its major strength (the ordering of nucleotides can be mapped and measured by the precision of its hydrogen bonding).

*Figure 1*

**Diagnostic techniques and the human genome. (A) The ranges of sizes of informational units of the human genome in nucleotide or centiMorgans. (B) The size ranges over which various diagnostic techniques are useful.**




Source: Landegren et al. 1988.

### Repetitive Sequences in the Human Genome

The genomes of higher eucaryotes, including humans, may be divided into different classes based very broadly on known functional properties. The so-called `coding regions' contain DNA sequences (genes) which determine primarily the amino acid sequences of the proteins for which they code, and also the degree of expression of the gene in any tissue at any time.

`Non-coding' DNA generally contains DNA sequences for which no function has yet been established or possibly for which no function exists. Such sequences may either be single copy (`spacer DNA' between the coding regions of the genome) or exist in multiple copies, thus being called **repetitive** DNA (reviewed Fowler et al. 1988).

Some 20 per cent to 30 per cent of the human genome is comprised of repetitive sequences, these having no clearly ascribed functional attributes.

A system of nomenclature and classification of repetitive DNA thus evolved, this system being largely based on the structural organisation and reiteration frequency of each species. There are two broad classes, namely: the tandemly repetitive sequences of which there are now recognised a number of different size classes, and the interspersed repetitive sequences, again for which there are different classes (Figure 2).

The distribution of all the major repetitive DNA sequences in the genome is remarkably orderly, rather than random. The centres and tops (telomeres) of chromosomes are constructed predominantly of tandem repeats. Human centromeric and pericentromeric DNA consists predominantly of `classical' macro-satellite sequences: Satellite I, II, III, IV and alphoid. These have evolved by a combination of saltatory replication, non-reciprocal exchange events and sequence divergence so that many such sequences assume a chromosome-specific character. They are also species-specific or near-specific. Telomeric tandem repeats, by contrast, are generally conserved in sequence and unlike centromeric sequences are common to all mammalian chromosomes. The long arm of the Y-chromosome also contains a major block of a `classical' satellite which may be used to sex the genome.

Dispersed between the centre and tips of human chromosomes are interspersed tandem repeats, as well as SINES and LINES (Figure 2). None of these repetitive sequences are, however, randomly dispersed. SINES and LINES tend to be mutually separate, their location correlated to distinct chromosome bands (Korenberg & Rykowski 1987).

The interspersed tandem repeats, particularly those associated with hypervariation are predominantly `clustered' towards chromosome ends and are often within the terminal G bands (Royle et al. 1988). This location, either by coincidence or consequence, is the region of highest frequency of recombination in the genome. This is demonstrated by chromomere maps of diplotene spermatocytes and shows both non-random location and higher frequency of chiasmata towards the ends of chromosomes (Fang & Jagiello 1988).

## Polymorphism in Tandemly Repeated DNA

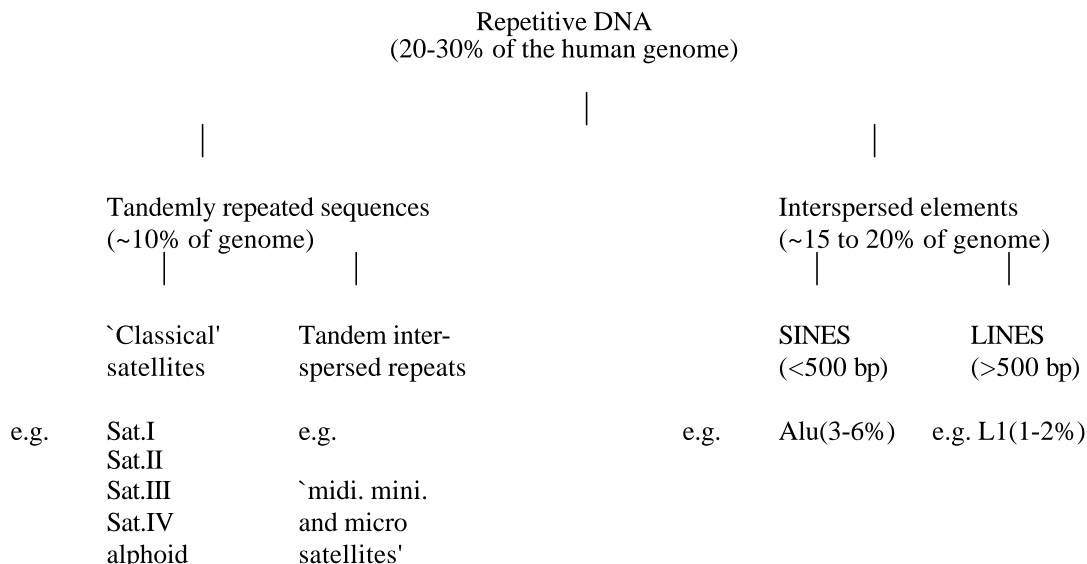
All such polymorphisms associated with tandemly repeated DNA are revealed by restriction enzymes creating DNA fragment lengths which contain variable numbers of the repeated monomeric species. There are, in general, two contracting causes by which this occurs (Fowler et al. 1988). In one case the enzyme restricts the DNA **externally** to the block of tandem repeats and in the other the DNA is cut **within** the lengths of tandemly repeating units.

The consequence of this is that in the first mechanism any enzyme which cuts the DNA in regions flanking (but not within) the tandem repeat will reveal the variation which may exist in the number of repeats, and hence the fragment size. The second mechanism is enzyme specific, the polymorphism being generally due to an enzyme which cuts (or fails to cut) infrequently into the higher order repeat units of each tandem array.

By way of nomenclature, polymorphisms arising by the first mechanism have been referred to as `minisatellites' or hypervariable regions (HVRs) (Jeffreys 1987) or variable number of tandem repeats (VNTRS) (Nakamura et al. 1987). Polymorphisms arising by the second mechanism have also been referred to as VNTRs. The preferred term VNTR will generally be used in this paper.

*Figure 2*

**Classification of repetitive DNA in human genomes. Examples of tandem repeat sequences**




(`Classical' satellites mostly found at, or near, chromosome centromeres (`heterochromatin') - likely to be non-mobile. Smaller satellites dispersed throughout genome - maybe clustered in some regions, and defining telomeric ends.

(Single units scattered throughout the genome - likely to be mobile genetic elements - maybe clustered in some regions.)

SINE short interspersed element
LINE long interspersed element

Sequences:
Sat. III (TTCCA)nTTCGGGTTG (where n = 7 - 13)
Sat. II GATTCCATTCGATGAT 16 mer': GATTCCATTC 10 mer' (human specific)
Sat.alphoid ~ 171 bp repeat: ancestor ? GAAAC(A/T)TTCT

Telomere TTAGGG repeat.

VNTR sequences (examples discussed):

YNH24 CAGGAGCAGTGGGAAGTACAGTGGGGTTGTT
YNZ22 TGGAGTCTCTGGGTGTCGTGCGTCAGAGT
(Source: Nakamura et al. 1987)

Apolipo.B
TTTTATAATTAATATTTATAAT
(Source: Boerwinkle et al. 1989)

### *VNTR polymorphism in tandem interspersed repetitive sequences*

The polymorphism in all such examples is an insertion/deletion mutational event, thereby lengthening or shortening the overall fragment length. The length of repeat units inserted or deleted is typically between 64 and 2 bp. Tandem arrays of such units may exist at either a unique or a number of dispersed genomic sites. Examples are listed in Fowler et al. 1988.

The cause of a loss or gain in the number of repeat units at any genomic site may be either integral numbers of unit slippage at replication and/or unequal recombination between the tandemly repeated sequences (Jeffreys 1987). This recombination may indeed be directed by a particular GC rich `core sequence' which (with some sequence diversity) has been found to be embedded in many of the GC Rich `minisatellites'. Jeffreys' conclusion is that such core sequences, 11 to 16 bp in length in his examples, either promote the initial duplication of DNA immediately adjacent to it and/or assist in changing the number of repeat units so created by unequal recombination or faulty replication (Jeffreys 1987). However, oligonucleotide probe sequences constructed complementary to some of the individual repeat units within a VNTR array do not always detect polymorphisms at high stringency conditions (Ali & Sher 1988). This suggests that more than the mere presence of a `core' sequence is required to generate the polymorphism.

Cytogenetic evidence appears to support the view that VNTR loci are sites for high frequency meiotic cross-overs (Chandley & Mitchell 1988). The multi-locus probe sequences 33.15 (Jeffreys 1987) were found to hybridise in situ principally at or around chiasmata, autosomal sites at which crossing over had occurred. There is also the observation that some `core' GC-rich VNTR sequences bear moderate sequence homology to the so-called `Chi' (cross-over hotspot instigator) sequence (Jefferys et al. 1985) believed to be active in procaryotes. The `Chi' sequence is postulated to be a target for a protein assisted recombination event between homologous DNA sequences, perhaps initiating alteration in the number of repeats and providing the inter-individual variation.

More recent results have demonstrated sequence similarity in the core sequence of some GC-rich hypervariable VNTRs and a protein binding site in yeast (White et al. 1988). This site is believed to bind cross-stranded structures during recombination in yeast and therefore may serve a similar role in higher eucaryotes (Chandley & Mitchell 1988).

Noting the similarity of the `Chi' sequence with other potentially recombinogenic sequences such as the X gene region of the hepatitis B virus (a sequence believed to be responsible for viral integration into human genomes) Nakamura et al. (1987) screened a human genomic library with oligonucleotides representing such potentially recombinogenic sequences. The result was a spectacular haul of over 80 new VNTR loci and further related examples (Nakamura et al. 1988).

Though there is evidence that such VNTR loci may be involved in recombination processes, the conclusion that VNTR variation is a direct result of unequal exchange between homologous chromosomes at meiosis is not supported by molecular evidence (Wolff et al. 1988; Wolff et al. 1989). In particular, where there are examples of spontaneous generation of new allele lengths at VNTR loci, there appears to be no exchange of flanking sequences as might be expected by normal recombination. Rather the new allele was generated without exchange of flanking DNA, and thus allele length variation probably arises by mechanisms such as unequal sister chromatid exchange, polymerase error during replication or single stranded exchanges by gene conversion.

There are now estimated to be at least 1500 VNTR loci in the human genome (Jeffreys 1987). These include both GC and AT rich `cores' in the sequence repeats, suggesting that there may be different classes of sequences which may serve as primers; by whatever mechanism; to VNTR tandem repeat formation. The AT rich VNTR

sequences (*see* review Fowler et al. 1988) show no sequence homology with the `Chi' sequence, and thus other explanations for their formation and propagation must be proposed.

More detailed examination of sequences flanking hypervariable VNTR sequences lead to one such proposal (Mermer et al. 1988). This investigation found two random repeats to be flanked by an interspersed repeat named Mst II, this showing homology to THE `1 and O' interspersed sequences, all being examples of SINEs. Though there was no firm evidence showing such sequences flanking VNTRs are mechanistically important to VNTR generation and variation, the association between VNTR repeats, and interspersed repeats immediately flanking them, has been confirmed for a number of other VNTR loci. This included clustered segments of Alu and LI sequences, retroviral long terminal repeat sequences (Armour et al. 1989). Their relevance to the generation of the tandem repeat array is unknown, any cis-acting function perhaps being unlikely given their relatively poor sequence conservation (Armour et al. 1989). However these flanking sequences could be an indication as to mechanisms of VNTR dispersal in the genome, as some virus have internal tandem repeat arrays (Lupton & Levine 1985).

Population frequency data and evidence of ethnic variation exist for the variable length polymorphisms associated with the 3' region of the HRAS-1 onco-gene and the `D14S1' region (Baird et al. 1986). Other loci, in particular those described by Nakamura et al. 1987 have been mapped to individual chromosomes and extensively studied for both their inheritance and allele frequencies (over 100 citations may be found in Nucleic Acids Research during 1987/88 which details this work). As the more useful of these sequences become internationally available as commercial products, so will this population data expand.

Some VNTR core sequences appear to show remarkable conservation throughout nature. The most interesting example thus far being a `minisatellite' found in the protein III gene of the `wild' type M 13 phage (a bacterial virus) used to locate VNTRs in human, bovine, equine, murine, canine, plant and micro organism genomes (Vassart et al. 1987; Georges et al. 1988). Bkm (GATA, GACA) and other short simple repeats (CAC, TCC, CT) are also widespread in nature and display multilocus hypervariable VNTR profiles in evolutionary diverse genomes (Epplen 1988).

*VNTR polymorphism in tandem centric repetitive sequences*

Each of the `classical' or `macro' satellites generally consist of a diverged `family' of sequences, with particular restriction fragment lengths arising from specific chromosomes and the sequences of individual family members (in any one class) being chromosome specific (or nearly specific) in origin.

Alphoid DNA is the most comprehensively researched example. The basic unit is 171 bp, but the chromosome `domains' in which such sequences exist show a complex hierarchy of structure. This may commonly be two alphoid repeats multiply repeated to form a single domain, or five, or as many as 16 such units forming the blocks by which such domains expand (Willard & Waye 1987). This higher order repeat organisation is probably created by processes such as non-reciprocal recombination and length fixation, gene conversion and possibly sequence excision and reintegration (Thompson et al. 1989). Such events, in combination with random sequence divergence specific to individual chromosomes has meant many individual alphoid sequences have evolved to be chromosome-specific or near specific in sequence. This may be demonstrated by both in situ or `Southern' analyses (Willard & Waye 1987; Alexandrov 1988).

Relatively complex VNTR polymorphisms exist in alphoid sequences (*see* review Fowler et al. 1988). In alphoid-derived VNTR polymorphisms, the variable fragment lengths are often present within more complex patterns, a portion of the fragment

lengths being constant between different individuals. In addition there is some quantitative (copy number) variation.

The distinction between alphoid VNTR polymorphisms and those of tandem interspersed VNTR examples is that in the former, the polymorphism appears to be the result of either point mutational or more complex insertion/deletion/cross-over and fixation events within the long tandem arrays (Waye et al. 1987). They are most likely to be detected using restriction enzymes which cut infrequently (or due to a site deletion fail to cut) into the higher order repeat units of each tandem array (Willard et al. 1986).

Like alphoid sequences, Satellite III and Satellite II sequences exist in chromosome specific `domains' (Fowler et al. 1989). Satellite III displays a complex TaqI-specific restriction fragment length polymorphism. The primary probe sequence that was developed to demonstrate this polymorphism is coded 228S. This polymorphism is best represented by TaqI restriction fragments in the size range about 25 to 4 kb. Such fragments are herein called the TaqI-deficient-polymorphic-sequence (TDPS).

The TDPS are highly discriminating of individual genomes and family studies show them to be inherited in a Mendelian manner.

The primary cause of the Satellite III polymorphism appears to be a specific C to G point mutation in the pentameric repeat unit, 5' TTCCA 3', typical of Satellite III. However, less frequent mutational events such as non-reciprocal recombination cannot be excluded, although this is unlikely to be responsible for the bulk of the polymorphism.

The primary probe 228S specifically locates region 9qh at limiting probe concentration and extreme stringency. However the genomic location of the TDPS, based upon the examination of a limited range of somatic cell hybrids, appears to be predominantly the centromeric heterochromatin of chromosomes 20, 21 and 22. These regions may therefore represent the largest blocks of homogeneous Satellite III in the genome and are thus likely to be the locations for more recent Satellite III amplification events.

These TaqI-deficient polymorphic regions differ from previously described Satellite III enriched `domains' in the genome where the TaqI site frequency is far greater, namely the long arm of the Y chromosome and so-called K-`domain' regions located on the short arm of chromosome 15. Minor contribution to the TDPS may also arise from chromosomes 9 (9qh), 15 and possibly chromosomes 13, 14, 5 and 1.

The autosomal blocks of Satellite III sequence from which the TDPS are derived are highly homogeneous in sequence being completely or largely resistant to digestion with a range of common restriction endonucleases. The TDPS are thus apparently only rarely and intermittently interrupted by either non-satellite III sequences or regions of Satellite III which have a low frequency of `rare' restriction sites. This suggests the TDPS are recent in their amplification, having incurred only limited levels of transposition invasion and having accumulated, by random drift, little degeneracy in their sequence.

Digestion of human DNA with HaeIII, for example, and field inversion electrophoresis shows these blocks of autosomal Satellite III DNA to range in length between 0.2 mb and at least 2.0 mb. This confirmed the unusual sequence homogeneity of such sequences, but also revealed megabase-scale inter-individual VNTR length variations, as did digestion with a number of other restriction enzymes. Thus the TaqI-specific RFLP is embedded within a larger scale VNTR polymorphism associated with mega-base sized blocks of Satellite III.

The TaqI-specific Satellite III polymorphism represents an alternative method to those based upon `minisatellite' hypervariation for either the discrimination of individual genomes in criminal forensic studies or in establishing parentage.

The probe 228S is now patented and marketed by Bresatec Limited as Polysat 3TM. Its principal practical advantage is the use of the same probe for either sexing

human genomes (3.4 kb Y-specific fragment in HaeIII digests) or `individualisation' (TaqI digests). The probe will only hybridise to higher primate DNA, and is therefore virtually human specific. Results may be achieved using non-isotopic probe methods, trials being conducted with photobiotinylated probes.

The principal practical disadvantages are the complexity of the polymorphic pattern, its interpretation and comparison. The size range of the SatelliteIII/TaqI fragments require the source DNA to have a high initial integrity, whereas both the quantity and quality of DNA recoverable from typical forensic specimens may often be limiting. There is also no potential for in vitro amplification of the satellite III polymorphism as is now possible with some single locus `micro satellite' sequences.

## PCR Technology and `Micro-Satellite' Polymorphisms

With the exception of parentage studies, the quantity and integrity of DNA recoverable from typical forensic specimens is often limiting. Thus the only means of analysis of such samples is to examine for inter-individual point or length variations within small genomic fragments. Efficient PCR amplification is possible for fragments 1 kb in size (and even larger) for example VNTR 3' to the apolipoprotein B gene (Boerwinkle et al. 1989), and YNZ22 (Horn et al. 1989). The quantity of PCR product so generated may be detected by an oligonucleotide probe using non-radioactive DNA detection systems (as is the policy in the State Forensic Services in South Australia). These oligonucleotide probes may be designed so as to confirm the authenticity of the amplification products.

Therefore, although conventional `Southern' analysis of VNTR RFLPs will be of use in some criminal cases, in the longer term these methods are likely to be replaced by the speed and sensitivity of PCR technology. For example, our initial results have shown that the very few sperm recovered some 30 hours post-intercourse will still provide sperm-specific VNTR amplification products. Such results, although of only moderate discriminating value, are less difficult to interpret than conventional RFLP alleles. For example, test allele lengths may be compared with a finite number of standard alleles run as internal controls. A further advantage is that only a fraction of the PCR product is analysed, reserving the remainder for any later comparative or confirmatory tests.

## Summary and Conclusions

It is envisaged that conventional `Southern' analysis of VNTR polymorphisms will have use in parentage testing once statistically significant numbers of individuals have been analysed and appropriate QA evaluations made, for example family studies and localised population surveys. Such DNA markers will replace less informative conventional genetic markers presently used in parentage enquiries.

As far as criminal samples are concerned, a different strategy is advocated. This is based upon the view that the methods adopted should be primarily selected for their ability to achieve results from small and degraded samples of DNA, rather than primary consideration being given to levels of inter-individual discrimination. Indeed, experience now shows that comparison of hypervariable allele sizes and their meaningful statistical evaluation are two major impediments in the legal admissability of such data.

Although PCR methods will not initially provide the same levels of discrimination as conventional multi-locus and single locus VNTR probes, this will not remain the case. An expanded set of `micro' VNTR loci (for example Weber & May 1989) and HLA loci will soon be rapidly adapted to forensic use. These in the longer term will

undoubtedly prove to be the most efficient means of generating reliable associative and reconstructive evidence particularly in dealing with the ever-increasing case numbers of sexual assault examinations.

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# DNA Fingerprinting by PCR Amplification of HLA Genes

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The term `DNA fingerprinting' could be applied to any approach to detecting individual variation through analysis of DNA. It has, however, come to be associated with a particular method based around the technique of `Southern' blotting. The application of this procedure has been widely acclaimed as a major breakthrough in forensic science, although its use has not been without criticism in some instances (Lander 1989). The power of the method to discriminate between individuals derives from the use of DNA probes that identify highly variable regions of the genome, usually short tandemly repeated sequences or minisatellites (Jeffreys et al. 1985; Nakamura et al. 1987; Wong et al. 1987). These regions are sufficiently variable that the probability of false identity is negligible, thus allowing positive identification to be made with confidence (Gill et al. 1985; Jeffreys et al. 1985).

Because this approach is potentially so powerful, one might be tempted to think that we have gone as far as we need in our efforts to develop methods of identifying individual genetic variation for forensic purposes. Existing methods do, however, have a number of limitations and new techniques are now available that can overcome these. These techniques are already widely used in many areas of human genetics and will undoubtedly become the principle method of analysis in forensic studies in the near future. The techniques are based around a procedure known as the polymerase chain reaction (PCR) (Saiki et al. 1985; Saiki et al. 1988). This procedure allows the multi-million-fold replication, in vitro, of specific DNA regions. Starting with as little as the unpurified DNA contents of a single cell, PCR produces, within hours, microgram amounts of a specific segment of DNA, enough to allow genetic variation to be analysed in a number of ways.

Effective use of this approach in forensic context requires firstly, the appropriate choice of highly variable regions of DNA for amplification by PCR and secondly, the development of efficient and sensitive methods for detecting variation in the PCR amplified product. This paper will describe the PCR approach to detecting genetic variation and discuss how the above requirements can be met to allow the approach to be effectively used in forensic investigations. Before doing that, some aspects of the currently used `Southern' blotting method will be considered to highlight its limitations and allow it to be compared with the PCR approach.

## Limitations of the Present Method

There is a requirement in `Southern' blotting analysis for 0.05 - 1 Âg of purified DNA, depending on the nature of the DNA probes being used to detect variation (Jeffreys et al. 1988). This amount of DNA can be obtained from approximately 10,000 - 200,000 nucleated cells, or from one or two hair roots, 2 - 40 Âl blood, or 25 - 500 Âl saliva. More than this may be required as some DNA is usually lost during purification. There will also be a requirement for more DNA when the analysis needs to be duplicated (for example, by prosecution and defence) or repeated (because of experimental failure or ambiguous results) or when the DNA is cut with more than one enzyme in the course of the analysis.

This lower limit to the amount of DNA required for `Southern' blotting does not pose a major problem in areas of civil law such as paternity testing or the resolution of immigration disputes. If a sample can be obtained in these situations it can usually be obtained in sufficient quantity. The limit does however have considerable implications in criminal investigations, where only small amounts of residue may be available for analysis.

It is not unusual for the results of `Southern' blot analysis to be other than completely clear and sometimes experiments need to be repeated. There are many possible causes of experimental failure. One, tissue degradation, is of particular importance in the forensic context because it is likely to be encountered and it may not be possible to overcome.

When DNA is degraded it is cut into fragments. The sizes of these fragments decrease as degradation progresses. Eventually a point is reached where there are no longer any fragments remaining that are long enough to span the distance between the sites in the DNA that are cut by restriction enzymes. The result is that the restriction fragments that normally occur in a region of DNA being studied are no longer detectable when probed with DNA from that region. `Southern' blot analysis is thus limited not only by the amount of required DNA, but also by a need for relatively undegraded DNA.

## A Better Alternative

The PCR approach is substantially less limited in both respects. PCR amplification can be achieved from a single cell (Jeffreys et al. 1988; Li et al. 1988), that is from 10,000 - 200,000 times less DNA than is required for `Southern' blots. PCR amplification can also be achieved from DNA that is extensively degraded. The extent of permissible degradation depends on the length of the DNA fragment being amplified, however fragments of only a few hundred bases are large enough to permit extensive genetic variation to be analysed following amplification. As long as there are some fragments of at least this length spanning the target region in a sample of DNA, successful amplification is possible. This compares with the `Southern' blot requirement for substantial numbers of fragments at least tens of thousands of bases long. The extent to which PCR can be successfully carried out on degraded material is illustrated by the amplification of DNA from the 13,000-year-old remains of the extinct giant sloth (Pääbo 1989) and the brain of a 7000-year-old mummy (Pääbo et al. 1988) as well as the remains of many other long-dead specimens (Pääbo 1989; Pääbo & Wilson 1988; Kocher et al. 1988).

PCR has a number of additional advantages over `Southern' blotting. The small amount of DNA required for PCR will make it easier for multiple independent tests to be conducted. It will also greatly facilitate the acquisition of DNA from suspects and other individuals, as this can be done non-invasively by sampling hair or mouthwash (Higuchi et al. 1988; Lench et al. 1988). This should make consent much easier,

obviate the need for medical supervision and reduce the risk of infection (by, for example, hepatitis B or HIV).

The PCR and associated techniques do not involve recombinant DNA nor do they need to involve radioactive material (Bugawan et al. 1988; Kaiser et al. 1989). There is thus no need for recombinant DNA or radioactive waste disposal and containment facilities. The techniques are much faster, taking a total of less than two days, as compared with at least the best part of two weeks for `Southern' blotting. They are less labour intensive and considerably cheaper.

## The Choice of Genes

There are two reasons why the minisatellites that are used as hybridisation probes in the `Southern' blot method are not well suited as targets for PCR (Jeffreys et al. 1988). First, they are generally too long. There is a limit of a few kilobases to the length of DNA that can be amplified by PCR and many minisatellites are longer than this. Second, because minisatellites consist of repeated sequences, out of register alignment may occur during PCR amplification making results difficult to interpret.

A better source of variation are the genes of the HLA or Major Histocompatibility gene complex. These genes have three important attributes:

1. The genes are extremely variable. The products of the HLA genes are the histocompatibility antigens responsible for the phenomenon of rejection in organ transplants. The reason that suitable donors are so hard to find for transplants is that we are almost all different from each other with respect to HLA genotypes. This variation which presents such a problem to transplantation biologists and surgeons does, of course, have enormous potential usefulness in forensic genetics. There are at least six highly variable genes in the HLA gene complex which have from 11 to 57 known variants (Bodmer et al. 1987). When all of these variants are considered, bearing in mind that each individual has two sets of the genes, there are at least 240,000,000,000 possible HLA genotype combinations. In fact variants of the different genes tend to occur in characteristic combinations, and thus not all the potential genotype combinations are found.

2. Much is known about the genes and their variants. In addition to their role in transplant rejections, HLA gene variants are associated with many autoimmune diseases such as insulin-dependent diabetes mellitus, multiple sclerosis and rheumatoid arthritis. Largely because they are clinically important in these two ways, the genes have been extensively investigated. Specifically, from the forensic perspective we know two important things: firstly, the sequences of the variable regions of a large proportion of the variants (Bell et al. 1987; Todd et al. 1987; Bugwan et al. 1988; Parham et al. 1988, Pohla et al. 1988); that is, we know the precise molecular nature of the variation. This has important implications in terms of the way in which the variation can be assayed; and secondly, we know the frequencies of many of the variants in different human populations (Albert & Baur 1984) including most of the major ethnic groups occurring in Australia. This is important in terms of determining the probabilities of random misidentifications in forensic comparisons.

3. The genes are widely studied. There is a large body of scientists and clinicians whose work in some way involves the HLA genes. International Histocompatibility Workshops are held every four years in addition to regional workshops and society meetings at which information is shared and, more

importantly, nomenclature and procedures for identifying and assaying variations are assessed and standardised. Data on HLA variation obtained in a forensic context can thus be understood and evaluated within an already existing, well-organised scientific infrastructure and against an extensive background of scientific knowledge. Nothing comparable exists for any other region of the human genome.

This has two important implications:

_ The scientific infrastructure makes it possible for data obtained using different assay procedures at different times and in different laboratories to be compared and communicated by means of a well-established and standardised, internationally recognised system of nomenclature (Bodmer 1987). It also provides a good source of expert advice and opinion.

_ The extensive underpinning of scientific knowledge about the HLA system should make interpretation of data on HLA variation obtained for forensic purposes less subject to ambiguities and more open to critical evaluation.

**Assaying the Variation**

Knowledge of the sequences of HLA variants allows three options for assaying the variation and makes possible comparisons of data obtained using the different assay procedures.

The three procedures are:

_ Dot-blot hybridisations with sequence specific oligonucleotides (Bugwan et al. 1988).

_ Digestion at sequence-specific restriction sites (Saiki et al. 1985).

_ Direct sequencing (Gyllensten & Erlich 1988).

If the sequence of a particular variant is known, then a short segment of DNA, or oligonucleotide, complementary to the region of the variant that distinguishes it from other variants, can be synthesised and used as a means of testing for the presence of the variant through hybridisation. If the variant is present the oligonucleotide hybridises, otherwise it does not. If the oligonucleotide is labelled either radioactively or non-radioactively and the amplified PCR product is bound to a membrane, the occurrence of hybridisation, and thus of the variant in question, can be identified. This procedure is known as dot blotting. By using a series of oligonucleotides specific to each of the known variants, sample genotypes can be determined.

Another approach is to identify restriction sites that are specific to particular variants and digest the PCR product with restriction enzymes that produce variant specific restriction fragments which can be separated by electrophoresis and visualised by fluorescent staining.

Both these methods are indirect and the results obtained require extrapolation about the underlying sequence. A third, more direct approach is to determine the sequences of the PCR amplified variants. In addition to being more direct this approach has the advantage that it allows previously undescribed sequence variation to be immediately identified.

The three approaches to detecting variations are based on different principles. Results obtained using one method can thus be used to check those obtained using another.

The results obtained from all three methods can be interpreted in the context of the existing system of HLA nomenclature and stored on computer in that form. Thus data obtained by any of the methods can be compared to those obtained by any other.

## Concluding Remarks

At present these methods can be applied to the HLA class II genes. In principle they could also be applied to class I genes although there is still much work to be done before that will be possible. The importance of developing methods of detection of class I variation is that they will greatly increase obtainable exclusion probabilities, and thus provide better quality evidence in criminal investigations.

There is a need for research and development in this area as well as in the implementation of a system for detecting class II variation. The potential advantages of the PCR approach are so great that this research should be encouraged. Furthermore, the establishment of laboratories and other facilities, and the consideration of the issues of standardisation and evaluation of data should be made with the expectation that the PCR technology will be firmly established as the main means of detecting genetic variation in forensic investigations within the next few years.

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