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 Red cell System	2 Obligatory Paternal gene	3 Alleged Father x	4 Cumulative x	5 Random Man y	6 Ratio x/y	7 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.

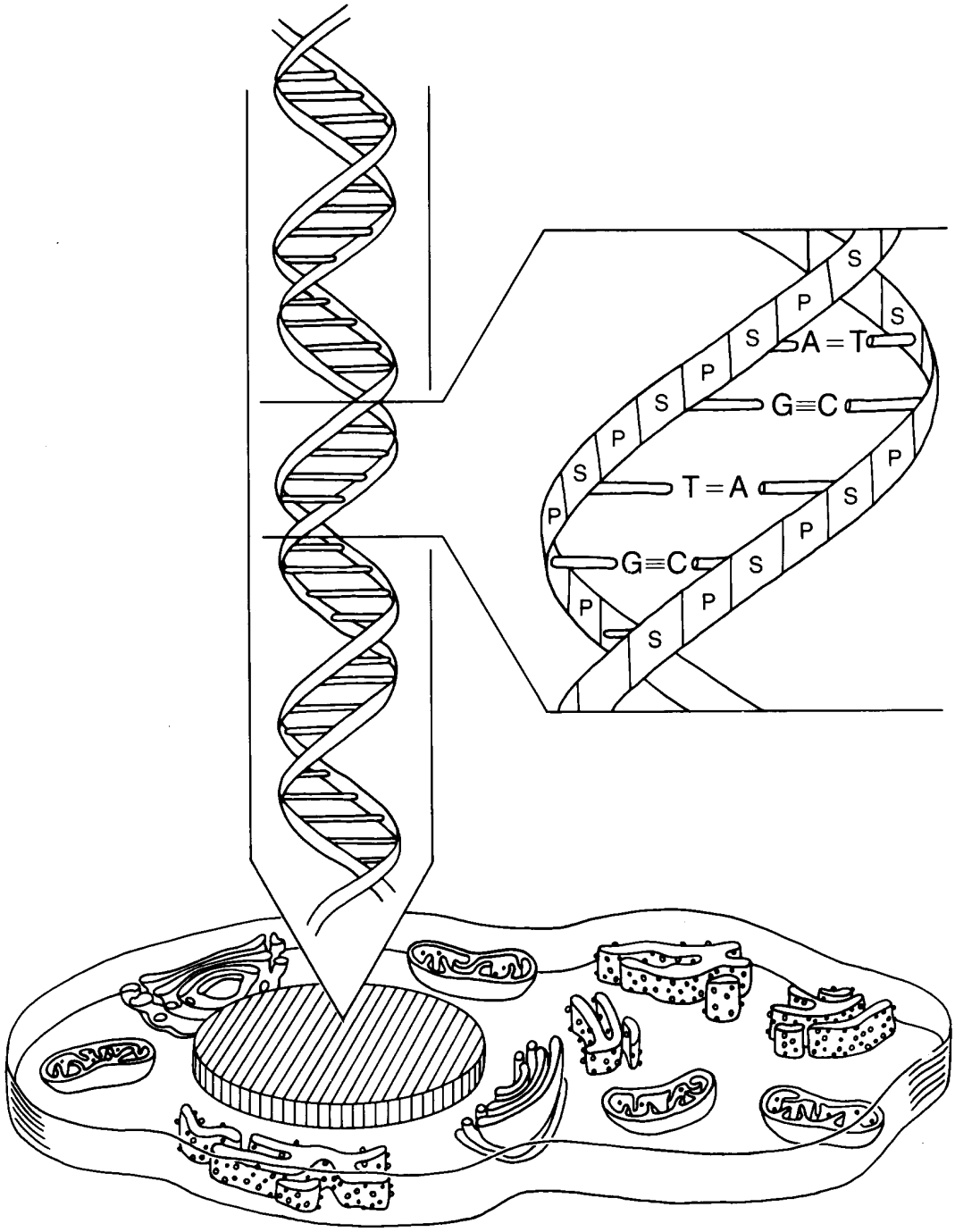
The sequence of base-pairs act as a four unit code, just like a dot and dash form the two unit Morse code. Because there are four bases, these can code for $4^3 = 64$ items, which covers the twenty or so amino-acids that go to making up proteins plus punctuation for starting and stopping production. 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.

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.)

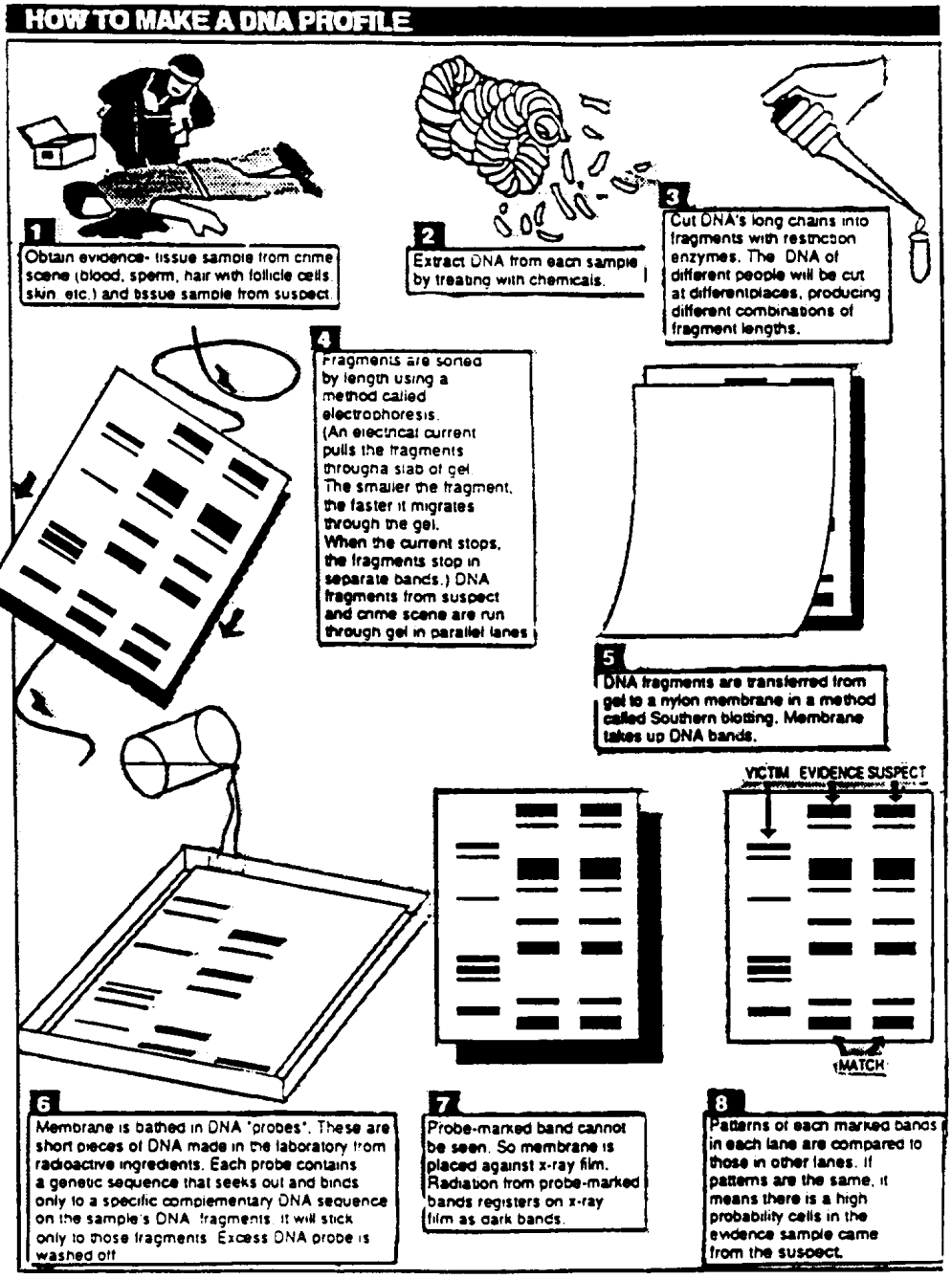
In step 2 the DNA is cut into fragments defined by having the cuts occurring at the same sequence of code every time.[7] The repeated sequences are kept within these fragments.

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 7×10^9 base pairs. This corresponds to 7×10^{-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

```
      _ cut
-C-C-T-N-A-G-G-          the N can be any base
-G-G-A-N-T-C-C-
          _ cut
```

   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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