

## **METHODS OF FORENSIC-DNA PROFILING**

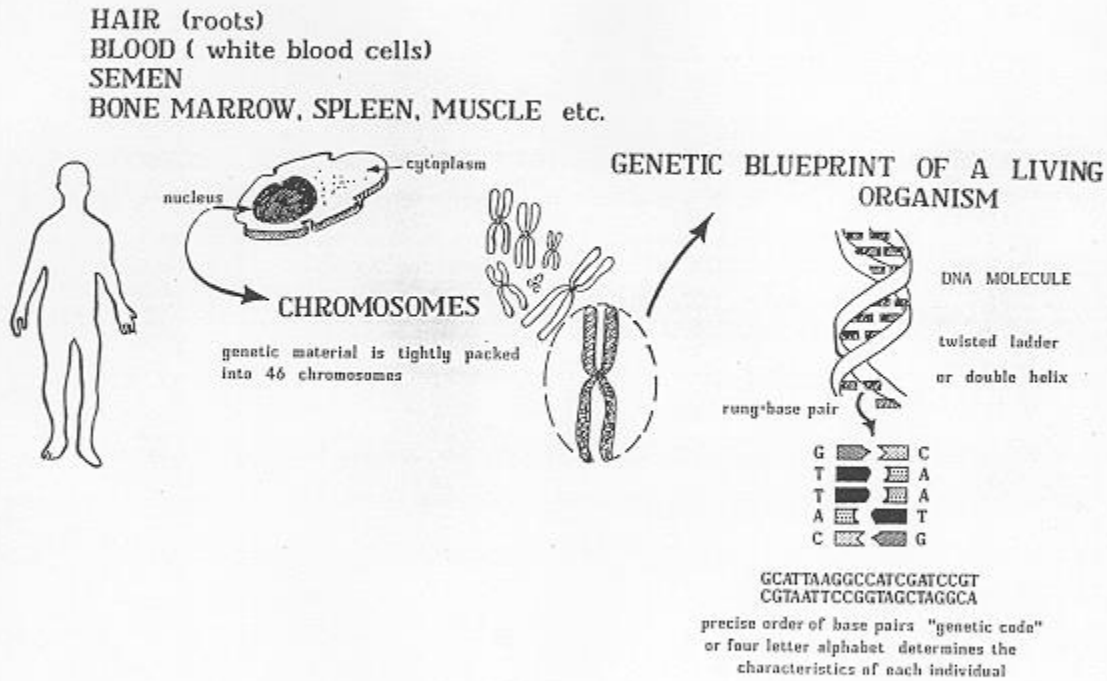
The current level of sophistication and expertise in the science and technology of molecular genetics has provided the basis for the "genome project," an international program to determine the sequence of all the base-pairs in the 23 pairs of human chromosomes. The approximately 3 billion base-pairs in the human genome incorporate both the specific sequences which constitute functional genes, and the 95% (or more) of human DNA which is non-coding; that is, which has no known genetic function. It is important to understand that the genome project is separate, and different, from forensic-DNA profiling, although some of the same technologies are used in both activities.

Another essential point is that the DNA profile, or "DNA fingerprint," of an individual as used in forensic-DNA profiling does not represent the genetic make-up of that person. It represents only a number of fragments of the person's DNA; these have been extracted, processed and utilized to form an individualized molecular-DNA "snapshot" that can be used for identification purposes. The forensic-DNA profile does not give any information on the individual's genetic make-up.

Forensic-DNA profiling can make use of any specimen that contains DNA. As shown in Figure 2, the list can include hair (with the root attached), blood stains, semen, bone marrow, or any other tissue or bodily fluid that has nucleated cells. In the use of blood stains, it is the DNA from the white blood cells that is used: mature human red blood cells do not have nuclei and so contain no DNA. Semen normally contains large amounts of DNA in the sperm cells, which makes it very useful for DNA typing, especially in cases of sexual assault. (If the rapist had been vasectomized, however, there would be no sperm cells and the specimen would not be useful with current RFLP technology.)

Figure 2

Sources of DNA for Forensic DNA Typing



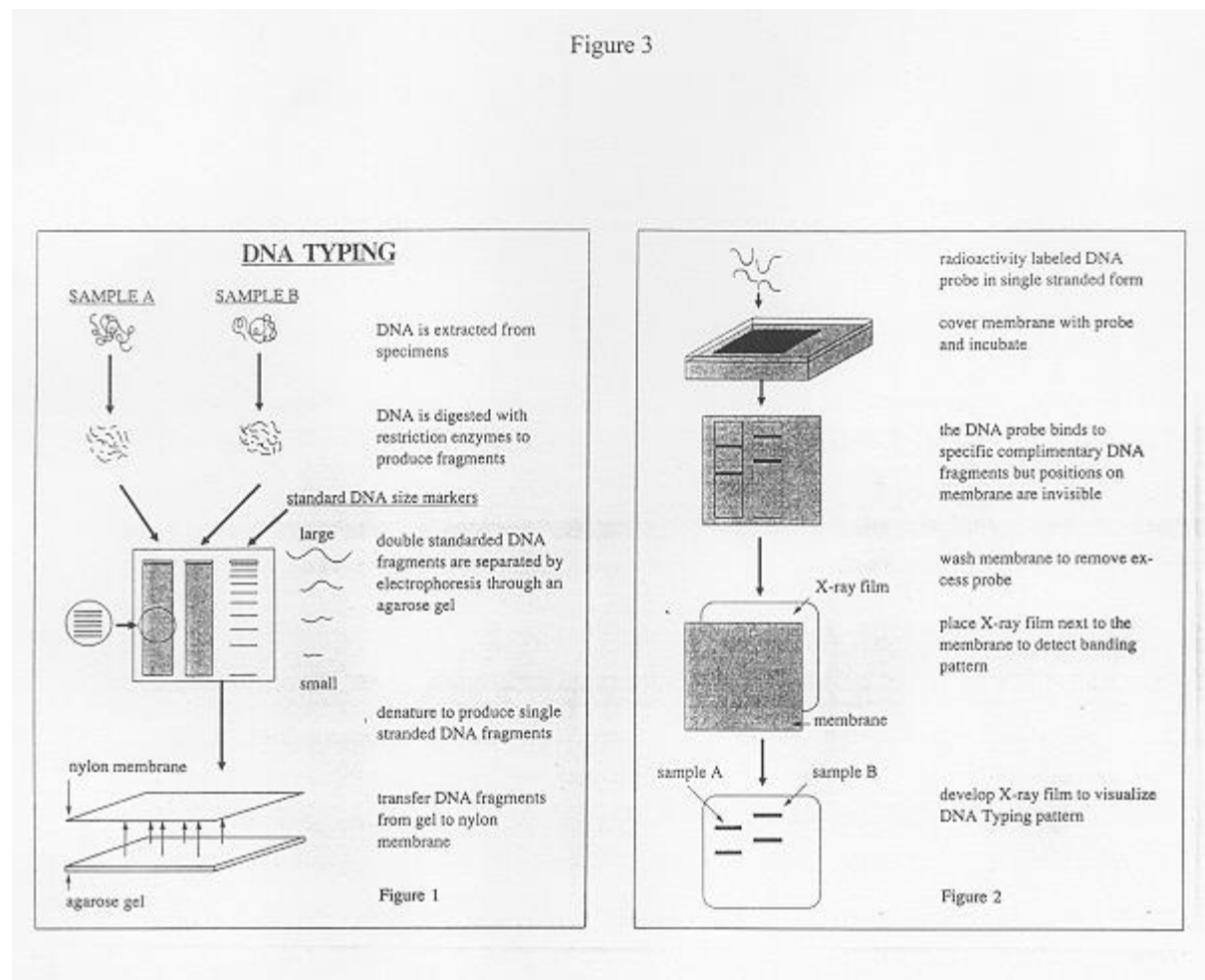
Source: RCMP Central Forensic Laboratory.

The standard forensic-DNA typing technology initially used in Canada was the RFLP technology; this is now being replaced by the newer PCR/STR (polymerase chain reaction/short tandem repeat) technology. As of May 1997, the RCMP's Central Forensic Laboratory in Ottawa, as well as the laboratories in Regina and Vancouver, had converted to PCR/STR from

RFLP; the RCMP laboratories in Halifax and Edmonton were still using the RFLP technology; and the Winnipeg laboratory was using both technologies. Full conversion of the RCMP forensic laboratory system to PCR/STR analysis is expected to be completed in early 1998.<sup>(17)</sup> The Centre for Forensic Sciences in Toronto also uses the PCR/STR technology.

### **A. RFLP (Restriction Fragment Length Polymorphism) Analysis**

The following description of RFLP analysis is illustrated in Figure 3.



First, the DNA is extracted from the specimens, using established procedures. In the next step, the extracted DNA is broken into fragments, using restriction enzymes. Although there are several hundred such enzymes, or REs, available today, the laboratories of most North American law enforcement agencies and governments (Canada included) selected one specific RE (called

"*Hae*III") in order to achieve uniform results and to facilitate "networking" of DNA-typing information.

After the extracted DNA has been digested by the enzyme, the various fragments are sorted according to size, using a technique called **agarose gel electrophoresis**, initially used in genetics research and adapted to forensic use. Agarose gel is a jelly-like material containing pores through which the DNA molecules can pass. The digested DNA samples are loaded into slots at one end of a flat slab of the gel. An electric current is applied across the gel causing the DNA fragments to migrate through the material. The smaller fragments migrate farther than the larger ones, to give the end result of an orderly array of fragments separated by size.

In the next step, the DNA fragments are **denatured** by soaking the gel in an alkali solution. In denaturing, the hydrogen bonds holding the two sides of the double helix of the DNA together are broken, with the result that there are now **single-stranded** DNA fragments arrayed on the gel in place of the original double-stranded fragments.

Because the agarose gel is not sufficiently stable to be used in the rest of the RFLP-typing procedure, the DNA fragments are transferred to the surface of a thin nylon membrane. This technique, called "Southern blotting" or "Southern transfer," is named for Edwin Southern, the scientist who developed it. When the DNA is fixed to the nylon membrane, the fragments are ready to be analyzed.

The subsequent analytical technique is called **nucleic acid hybridization**. The term is explained as follows. Hybridization is a process that involves pairing the single-stranded DNA (nucleic acid) fragments on the nylon membrane with specific complementary DNA strands; the reader will recall from the earlier discussion that the double-stranded DNA molecule comprises two complementary, rather than identical, strands.

The hybridization is carried out with strands of DNA which have been labelled with a radioactive isotope, usually an isotope of phosphorus. These strands are known as **DNA probes**, so-called because their base sequences are known and they are used specifically to bind only to those DNA strands containing complementary sequences. Because the probes carry a radioactive

label, the newly hybridized strands can be visualized as images on an x-ray film. The visual result is often compared to a supermarket "bar code."

The specimen in question can then be compared with known specimens through their x-ray images. If there is a difference in the patterns between the DNA from the suspect individual and the DNA from the specimen taken from the crime scene, the suspect will be exonerated. If the patterns match, the prosecution can use this fact as evidence linking the suspect to the crime scene.

### **B. PCR/STR (Polymerase Chain Reaction/Short Tandem Repeat) Analysis**

As noted above, the RFLP technology is being replaced in Canada by the newer PCR/STR technology.

In some basic respects, PCR/STR technology is similar to the RFLP technology described above. The selection and extraction of the DNA is the same, and in both technologies the selected fragments of DNA are placed in a special gel and sorted by size through the use of an electric current. However, with PCR/STR, a much smaller amount of DNA in a sample is adequate to carry out the profiling; even badly degraded DNA, such as might be obtained from decayed or severely burned bodies, can be used. In fact, enough DNA can be extracted from a single hair follicle, or from a saliva trace on a cigarette butt or envelope, to carry out the profiling using this technology.<sup>(18)</sup> Additional advantages of PCR/STR are that the technology is less susceptible than earlier PCR-based methods to being compromised by contaminants, and there is a "significant advantage in deciphering the origin of specific DNA profiles from complex sample mixtures...i.e., mixed blood stains, commingled remains and sexual assault samples."<sup>(19)</sup>

The principal difference between the two technologies is the use of polymerase chain reaction (PCR) to amplify the amount of DNA in the sample. A second important difference is that the PCR/STR technology lends itself well to the use of fluorescent labels for detecting the DNA bands visually, and several such systems have been developed. Also, fluorescence is amenable to automated detection, which greatly facilitates subsequent analysis of the forensic-DNA profiles and the storage and retrieval of data.<sup>(20)</sup>

Dr. Fournery describes the use of automated fluorescent detection, as follows:

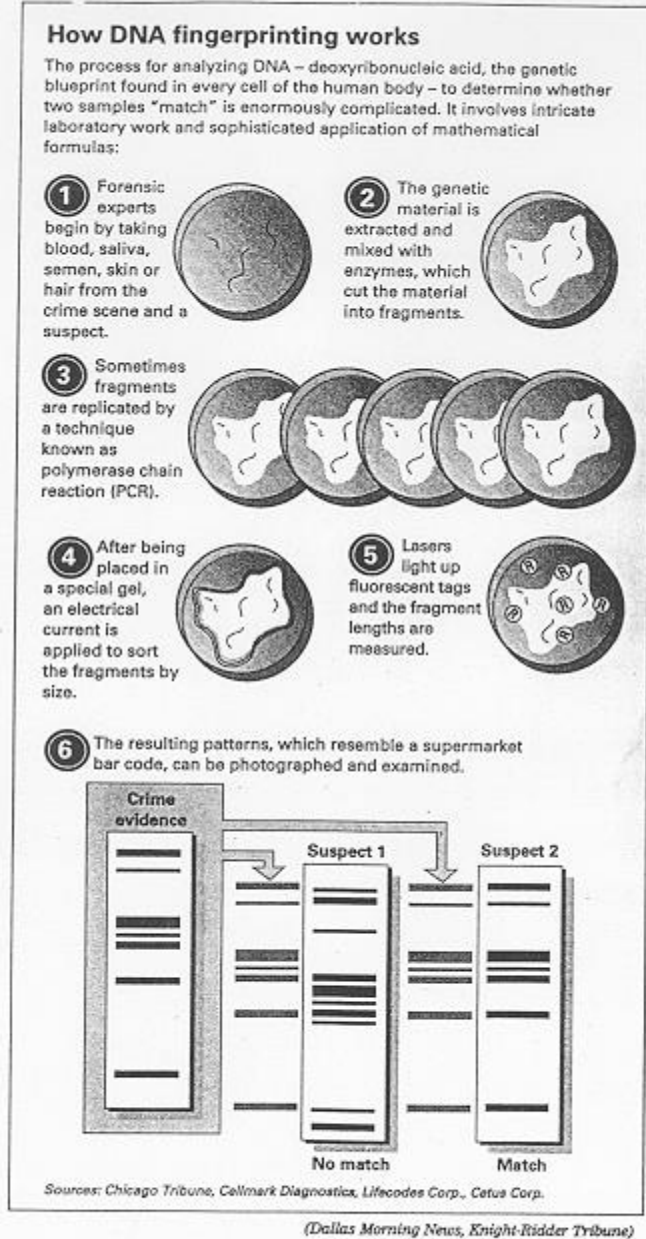
A major tool employed by both clinical diagnostic laboratories and numerous larger forensic laboratories has been automated fluorescent detection of DNA fragments using DNA sequencers...Essentially, several DNA fragments can be labelled simultaneously with a different fluorescent tag in a single reaction tube (multiplex analysis) during the (PCR) amplification process. Automated detection incorporates the technique of "real time analysis" of DNA fragments as they migrate through a polyacrylamide gel past a laser window which excites the fluorescent tag (fluorochrome) of the fragment and detects the specific enhanced light using an array of CCDs (charge coupled devices). DNA fragments are precisely sized ... calibrated and entered into a digital computer base ...

... a major characteristic of this detection method is the precision and accuracy afforded through the use of an internal sizing standard run in the same lane (of the gel) as each STR sample. The internal lane standard is recognized by the computer and used to generate a fragment size calibration curve, thereby providing an accurate quantitation of the amount of a fluorescent signal (from the tagged fragment) and a precision standard for evaluating any potential aberrant electrophoretic migration patterns. With the aid of the computer and precise digital sizing data, the forensic scientist evaluates each fragment with regards to match or nonmatch.(21)

PCR/STR profiling systems are extremely sensitive and capable of analyzing a DNA sample as small as 1 ng (1 nanogram = one- billionth of a gram); however, DNA samples of 2-8 ng are considered optimal for the processing of numerous forensic samples in the multiplex format described above.(22)

A simplified illustration of the PCR/STR technology is shown in Figure 4.

Figure 4



Source: The Globe and Mail, 19 July 1997, p. A6

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