# DNA fingerprinting

In 1984, Alec Jeffreys developed the technique of DNA fingerprinting in his laboratory at the University of Leicester. These techniques have revolutionised the way that the police solve crimes.

Alec and his team were studying inherited variation and had shifted their focus from the products of genes[?], specifically blood groups, to DNA[?] itself. As humans we share around 99.9 per cent of the same genetic material. But what Alec was interested in was the remaining percentage, the part that contains our unique genetic code. Alec wanted to find this code and understand it.

Alec started by examining the DNA sequence found in the myoglobin gene of seals.

The myoglobin enables them to hold oxygen in their bodies for long periods of time. The myoglobin gene has developed and adapted over millions of years, mice have a similar gene, and so do we. Within the DNA sequence of the seal myoglobin gene Alec identified a repeating sequence or 'stutter' in the sequence. He realised that these stutters were unique to an individual and therefore could be used to distinguish one seal from another. Perhaps similar sequences in humans could be used to distinguish one person from another.

The scientific name for these stutters is a minisatellite. These are short, repetitive sequences of DNA, (usually 10 to 60 base pairs[?] long), that occur at more than 1,000 locations throughout the human genome[?]. Minisatellites are highly variable so tend to differ from person to person, which means that by comparing minisatellites it is possible to identify a particular individual.

he tried to cut the DNA up into smaller and smaller pieces using specific enzymes[?]. But time after time he came up against a wall – the enzymes weren't cutting the DNA as much as he hoped. This meant he wasn't able to get a clear picture of the stutter.

In September 1984 he decided to try something different based on his knowledge of the myoglobin gene. He took a DNA sample from one of his lab technicians and placed it alongside DNA from their mother and father as well as the DNA from a tobacco plant, a cow and a seal.

What Alec found was that the probe had bound to all the minisatellite sequences that had a similar sequence to his probe. This resulted in a pattern of dark bands that was completely unique to the individual. The technician shared parts of their pattern with their mother and father showing that they were related. In contrast, the tobacco, cow and seal all had completely different patterns, showing that they were not related at all. Finally, the penny dropped and Alec realised he had produced the first DNA fingerprints.

In 1983 , first criminal case was solved by dna finger printing method.

## The Steps of DNA Fingerprinting

DNA fingerprinting involves a number of intensive and important steps in order to fully complete and develop and DNA fingerprint of a father, a suspect or a person involved in an immigration problem.

1. The process of DNA fingerprinting starts with isolating DNA from any part of the body such as blood, semen, vaginal fluids, hair roots, teeth, bones, etc.

2. Polymerase chain reaction (PCR) is the next step in the process. In many situations, there is only a small amount of DNA available for DNA fingerprinting. Because of this, in a test tube, DNA replication is must occur to make more DNA. The DNA and the cells will undergo DNA replication in order to make more DNA to be tested.

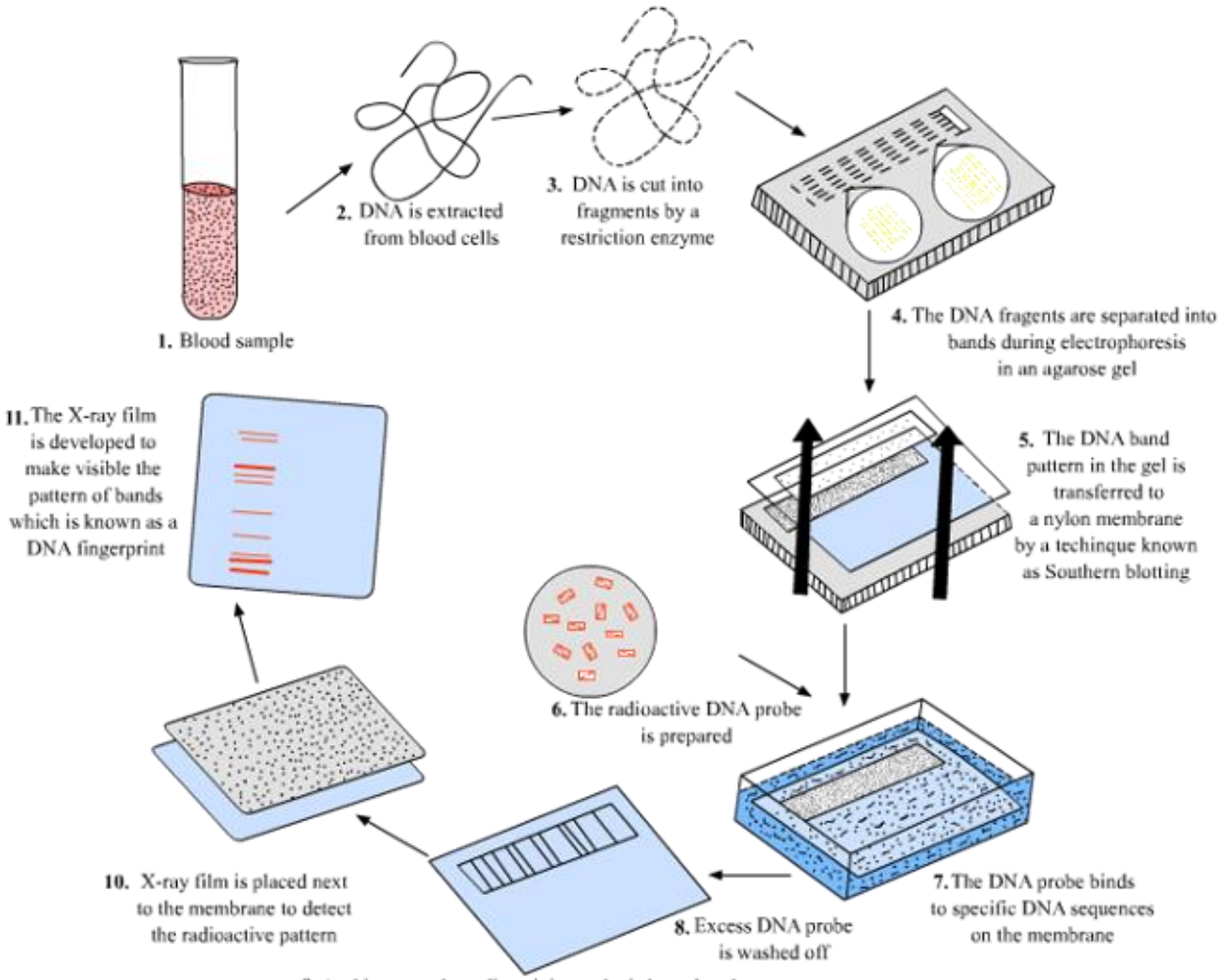
3. After the DNA is isolated and more copies of the DNA have been made, the DNA will be tested. The scientist will treat DNA with restriction enzymes (an enzymes that cuts DNA near specific recognition nucleotide sequences known as restriction sites).
   -This will produce different sized fragments which are known as restriction fragment length polymorphisms (RFLPs).
   -These fragments can then be observed doing an experiment called gel electrophoresis which separates DNA based on fragment sizes.

4. Gel electrophoresis is the next step in this process of DNA fingerprinting. During gel electrophoresis, an electrical current is applied to a gel mixture, which includes the samples of the DNA.
   -The electric current causes the DNA strands to move through the gel. This separates the molecules of different sizes.
   -The fragments of separated DNA are sieved out of the gel using a nylon membrane (treated with chemicals that allow for it to break the hydrogen bonds of DNA so there are sing strands).

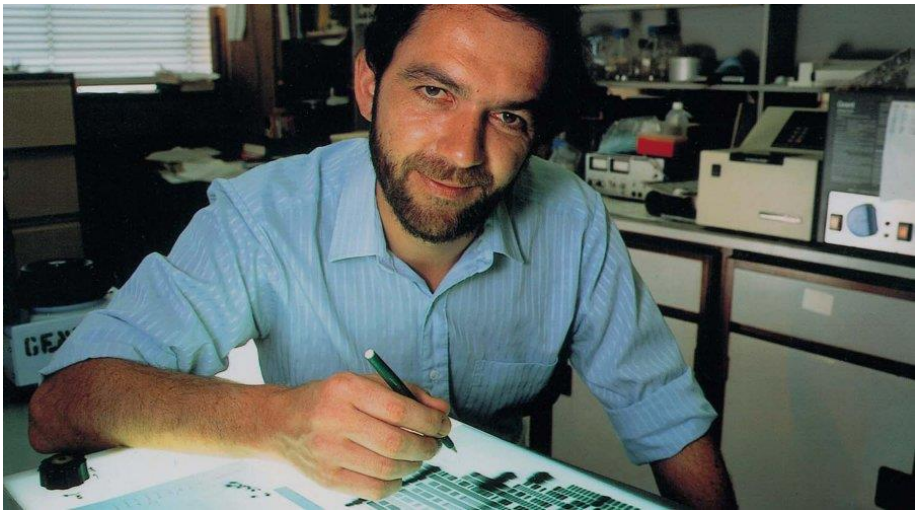
5. The DNA (single stranded) is cross-linked against the nylon using heat or a UV light.

6. The probe shows up on photographic film because the strands of DNA decay and give off light. In the end it leaves dark spots on the film which are also known as the DNA bands of a person. What make up the fingerprint are the unique patterns of bands. The pattern of bands are different because we are all different and unique (other than identical twins).

7. Once the filter is exposed to the x-ray film, the radioactive DNA sequences are shown and can be seen with the naked eye. This creates a banding pattern or what we know as DNA fingerprints. This technique is called southern blotting.




**Steps in DNA fingerprinting techinque**



## Technical aspects of minisatellite DNA fingerprinting methodology

The first protocols for visualizing multilocus DNA fingerprints [7] used relatively long 'minisatellite' (VNTR) DNA probes. Probes were hybridized to restriction enzyme-digested DNA that had been size-separated and bound to a nylon membrane. These early probes consisted of concatenates of short (approximately 16 bp) 'core' repeats that were found to be both highly conserved and also distributed throughout the genome (Figure 1). These conserved core regions were found within the highly repetitive minisatellite repeat sequences. Some of the most commonly used probes were derived from an intron of myoglobin, and were referred to as '33.15' (consisting of 29 repeats of a minisatellite core with 128 bp of flanking sequence) and '33.6' (consisting of 18 repeats of a 37 bp sequence unit. The 37 bp sequence unit comprised three repeats of a 11 to 12 bp core plus two base pairs) (sourced from Jeffreys' United States Patent: US5413908.

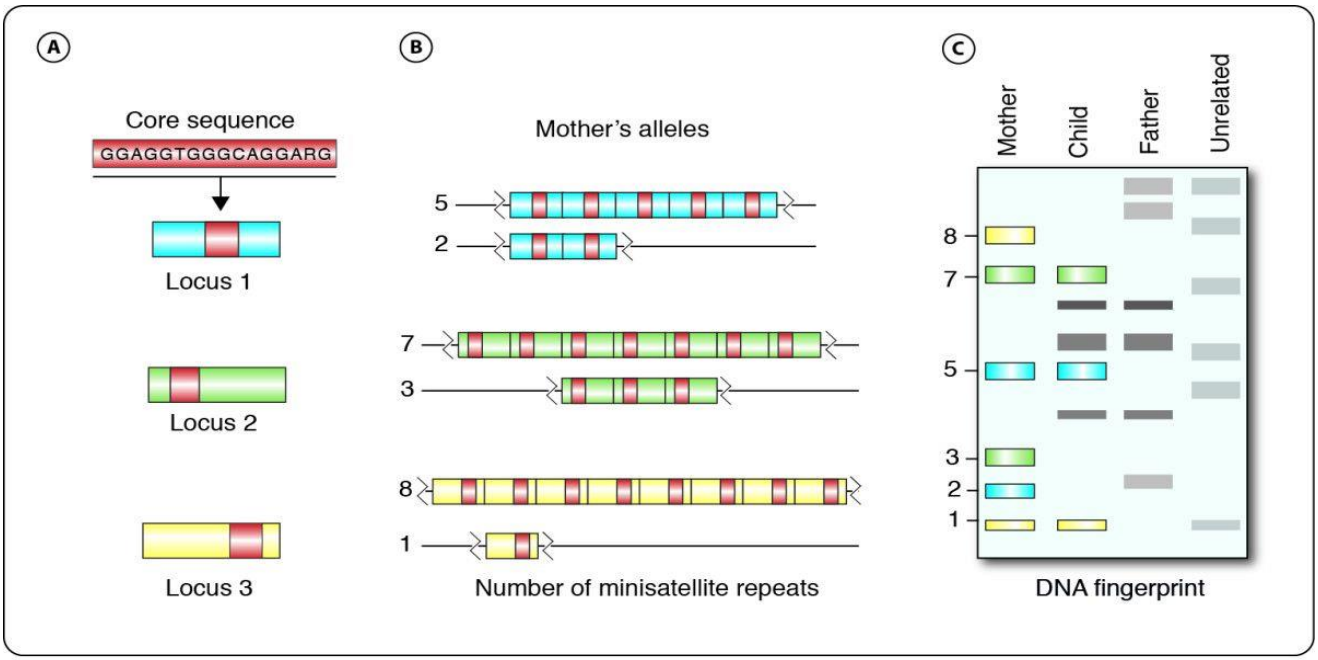



Fig 1. Chambers et al. 2013 I Investigative Genetics

Figure 1

**Minisatellite repeat units are characterized by an approximate 16 bp core sequence in humans and other animals. (A)** A core minisatellite repeat is present at three loci. **(B)** The number of minisatellite repeats at these loci are shown for one individual (the mother) who is heterozygous at each of the three loci. Locus 1 genotype: 5, 2; locus 2 genotype: 7, 3; and locus 3 genotype: 8, 1. **(C)** Representation of an autoradiograph showing restriction fragment profiles of four individuals at these three loci. At each locus in the child's profile, one allele is shared with the mother and the other is shared with the father, as would be expected when maternity and paternity have been correctly identified. Note that the unrelated individual shares only a small number of bands with the individuals from this family.

## DEFINITIONS

The following definitions used in the present invention and the above-mentioned earlier specifications may be of assistance.

Hypervariable :A region of human or animal DNA at a recognised locus or site is said be hypervariable if it occurs in many different forms e.g. as to length or sequence.

Restriction Fragment Length Polymorphism (RFLP): Is genetic variation in the pattern of human or animal DNA fragments separated after electrophoresis and detected by a probe.

Minisatellite: A region of human or animal DNA which is comprised of tandem repeats of a short DNA sequence. All repeat units may not necessarily show perfect identity. (Probes of the invention comprise minisatellites which are polymorphic).

Polymorphic: A gene or other segment of DNA which shows variability from individual to individual is said to be polymorphic.

Core (Sequence): Originally used in the sense of consensus core sequence, but extended to any repeated or variant sequence derived therefrom.

Consensus Core (Sequence): A sequence which can be identified as a substantial or perfect match between the repeat units of two or more minisatellites of differing origin or loci.

Repeat (Sequence): A sequence which is a perfect or imperfect tandem repeat of a given core sequence or segment containing the core sequence.

Defined Core (Sequence): A core sequence fully consistent with one of formulae (2) to (8) within its own length.

Variant (Core Sequence): An actual core sequence which differs from a defined core sequence to a minor extent (>50% homology).

Perfect Repeat (Sequence): A sequence which is an exact tandem replication of a given core sequence or of a segment containing the core sequence.

Imperfect Repeat (Sequence): A sequence in which at least one unit differs in base pair substitution and/or length from at least one other unit. (There will normally be at least three tandem repeats in a probe sequence within which there will normally be at least one defined core sequence and at least one variant).

## Significance of fingerprinting

the probes of the invention are useful in the following areas:

1. Paternity and maternity testing in man.

2. Family group varification in e.g. immigration disputes and inheritance disputes.

3. Zygosity testing in twins.

4. Tests for inbreeding in man.

5. General pedigree analysis in man.

6. Identification of loci of genetic disease in man, thereby enabling specific probes to be constructed to detect a genetic defect.

7. Forensic medicine

(a) fingerprinting semen samples from rape victims

(b) fingerprinting blood, hair and semen samples from e.g. soiled clothing

(c) identification of human remains.

8. Cell Chimaerism studies, e.g. following donor versus recipient cells after bone marrow transplantation.

9. Livestock breeding and pedigree analysis/authentication. (This could include, for example, the routine control and checking of pure strains of animals, and checking pedigrees in the case of litigations involving e.g. race horse and dog breeding). Also to provide genetic markers which might show association with inherited traits of economic importance.

10. Routine quality control of cultured animal cell lines, checking for contamination of pure cell lines and for routine identification work.

11. Analysis of tumour cells and tumours for molecular abnormalities.

12. It is anticipated that the polynucleotides or probes derived therefrom have a potential use in plant breeding.