DNA Fingerprinting

Presented By
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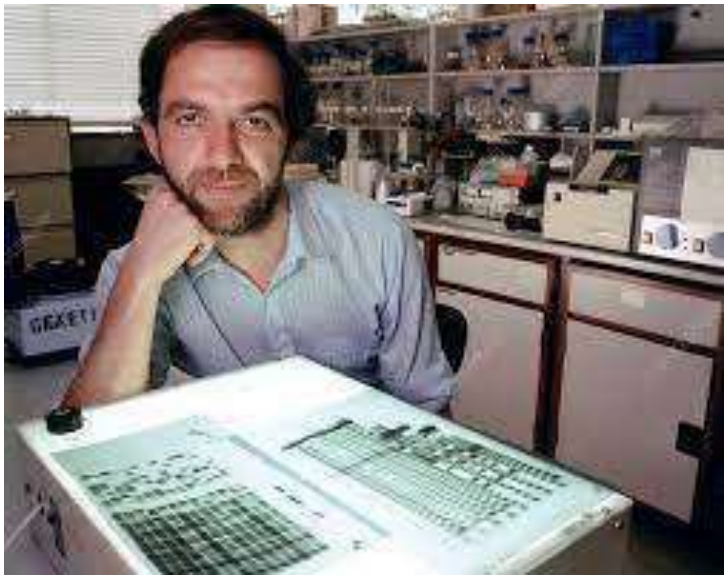
Introduction

DNA fingerprinting is a technique that shows the genetic makeup of living things. It is a method of finding the difference between the satellite DNA regions in the genome."

Or

DNA profiling is a process used to determine the nucleotide sequence at a certain part of the DNA that is unique in all human beings.

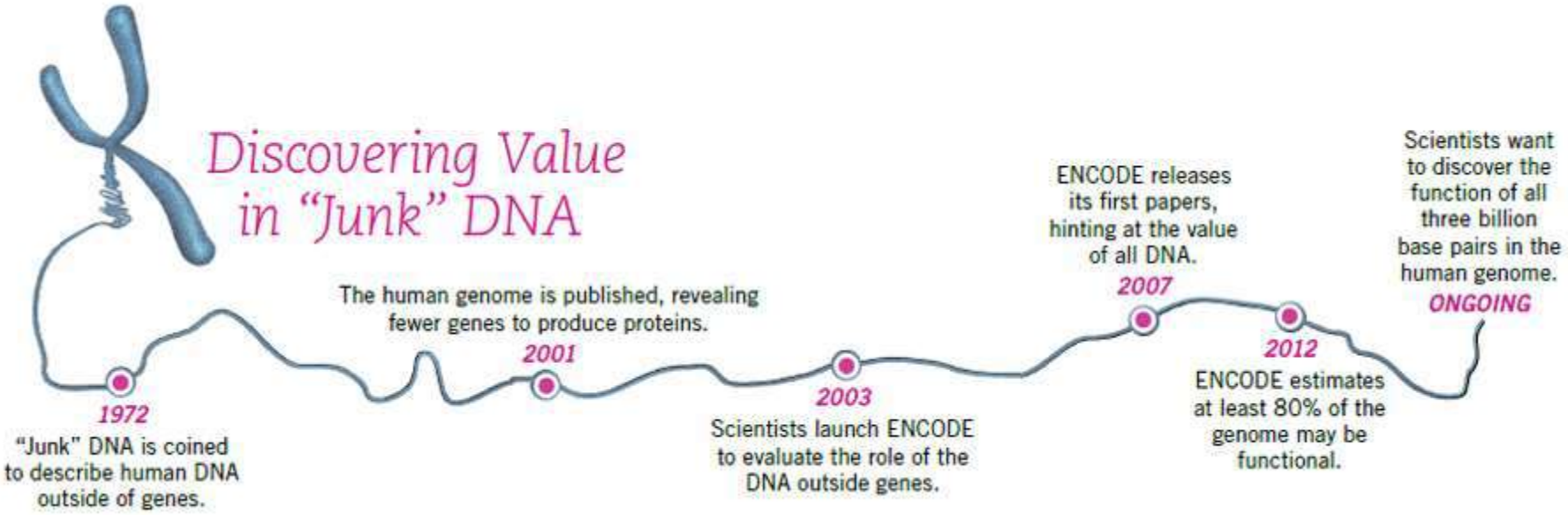


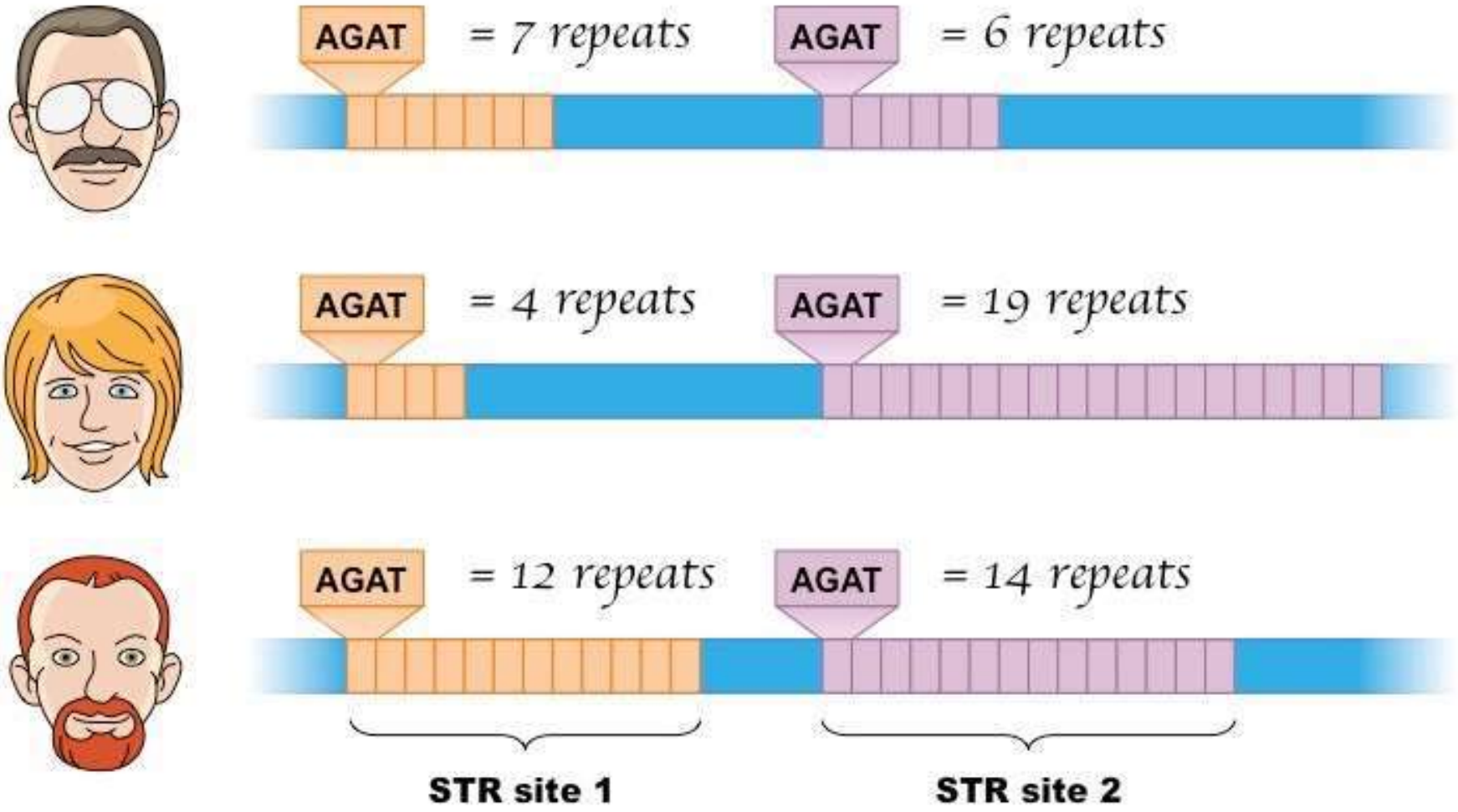
•The process of DNA fingerprinting was invented by **Sir Alec Jeffrey** at the University of Leicester in 1985.

Principle of DNA Fingerprinting

The DNA of every human being on the planet is 99.9% same. However, about 0.1% or 3 x 10^6base pairs (out of 3 x 10^9 bp) of DNA is unique in every individual.

Human genome possesses numerous small non-coding but inheritable sequences of bases which are repeated many times. They do not code for proteins but make-up 95% of our genetic DNA and therefore called the —junk DNA.




AGAT
= 7 repeats
AGAT
= 6 repeats
AGAT
= 4 repeats
AGAT
= 19 repeats
AGAT
= 12 repeats
AGAT
= 14 repeats
STR site 1
STR site 2

DidYouKnow

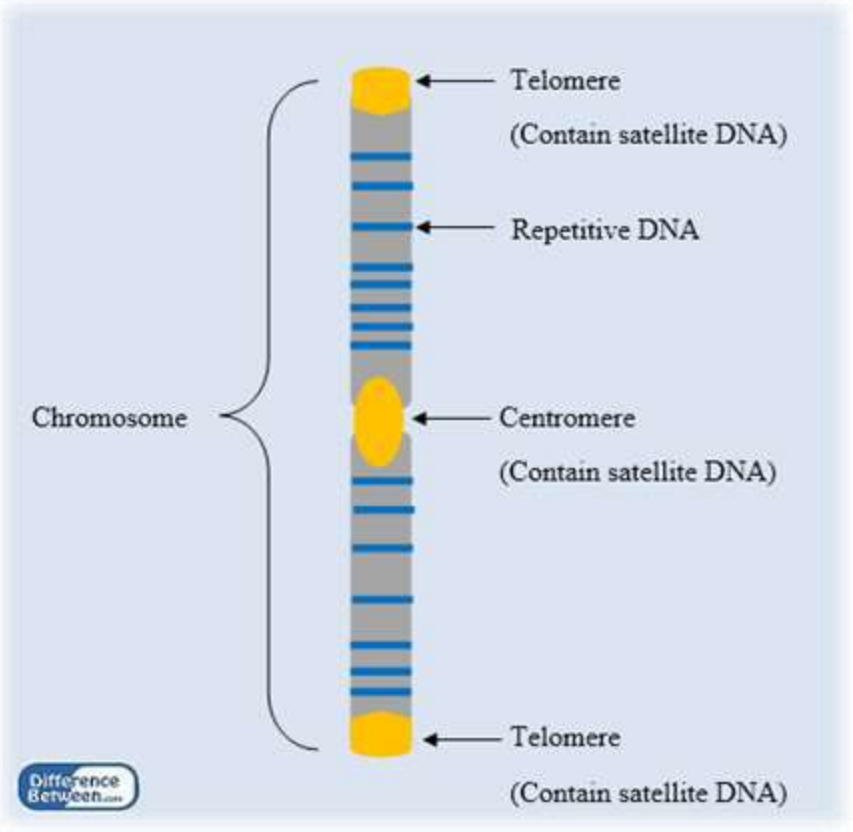
National DNA Day APRIL 25

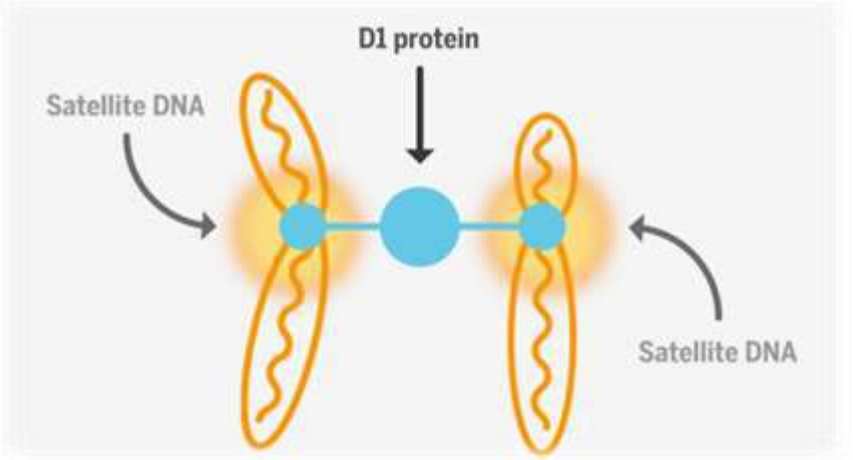
98% of your genome has no known function. It is the remaining 2% that codes for genes and ultimately, proteins. While often referred to as junk DNA, it is anything but. This is because some non-coding DNA can regulate gene expression.

They can be separated as satellite from the bulk DNA during density gradient centrifugation and hence called satellite DNA.

In satellite DNA, repetition of bases is in tandem. Depending upon length, base composition and numbers of tandemly repetitive units, satellite DNAs have subcategories like microsatellites and mini-satellites.

Satellite DNAs show polymorphism. The term polymorphism is used when a variant at a locus is present with a frequency of more than 0.01 population.





A microsatellite is a repeating sequence of 2-6 base pairs in the genome. Since it is a type of tandem repeats with short sequences of nucleotides, microsatellites are also known as **short tandem repeats (STRs)**. The repeats of single nucleotides are called single nucleotide polymorphism (SNP). Furthermore, microsatellites occur throughout the genome. In the human genome, the dinucleotide repeats occur in every 30, 000 base pairs.

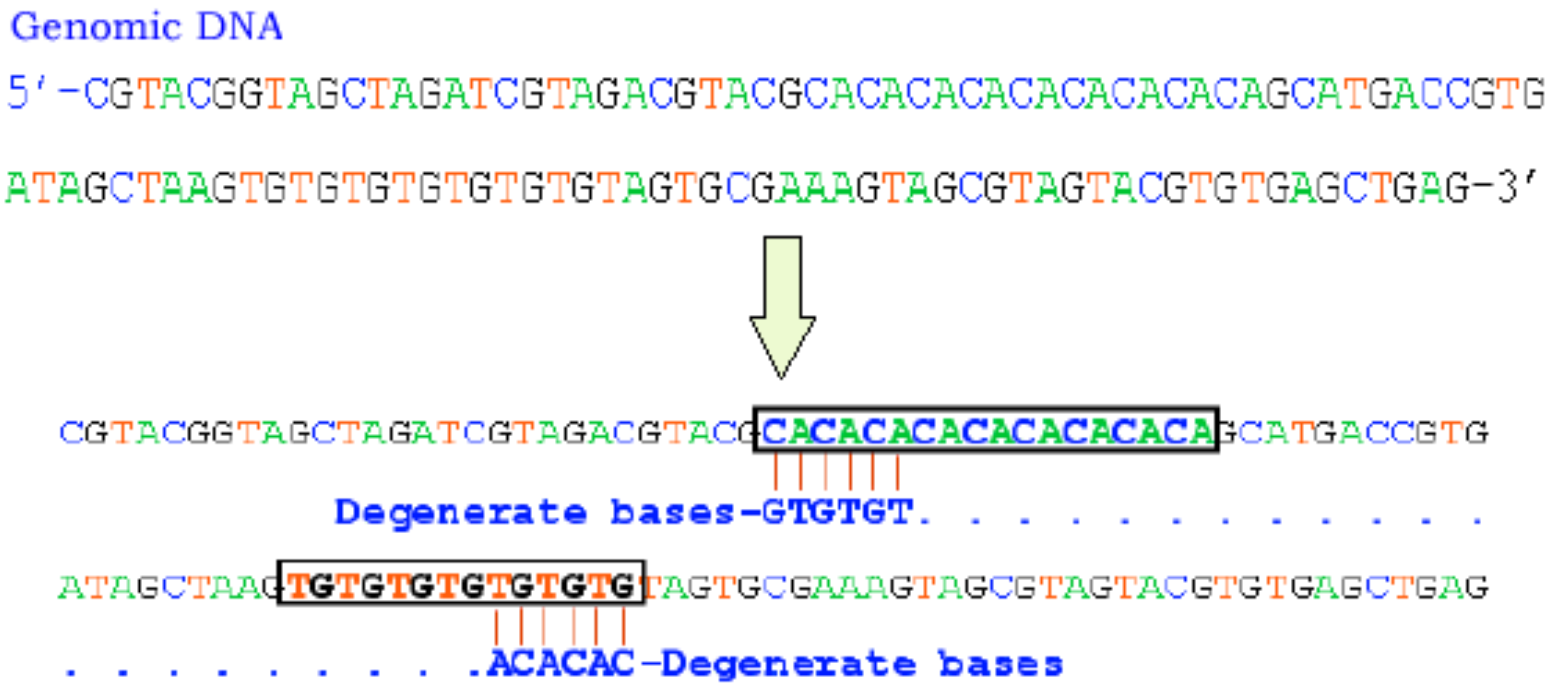
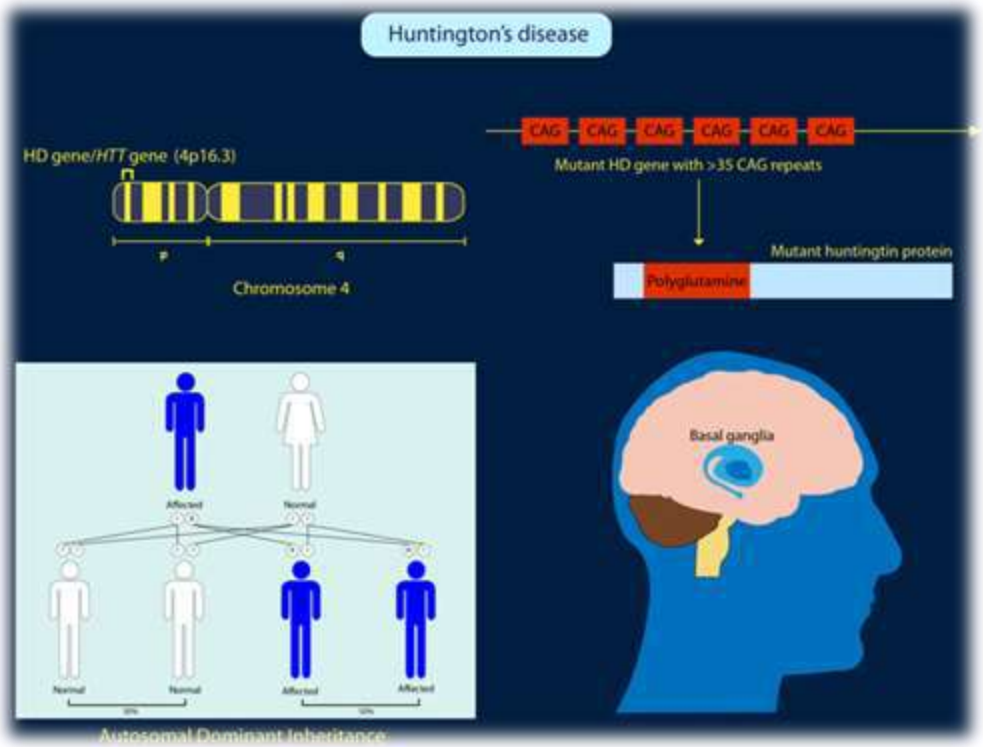
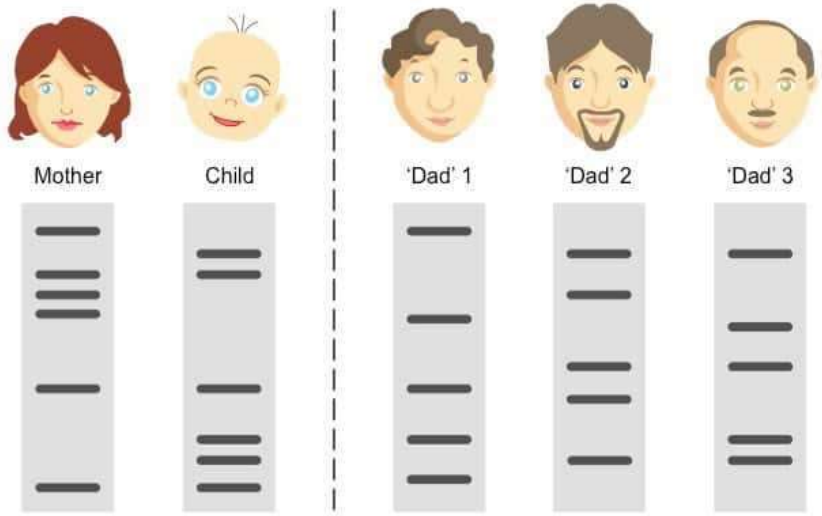


Fig: Locus specific Microsatelite DNA sequence

Moreover, microsatellites are a highly mutative region in the genome. Unique microsatellite sequences occur within families. Therefore, we use the analysis of microsatellites for paternity testing. Furthermore, the extension of trinucleotide microsatellite repeats causes severe human disorders like Fragile X syndrome and Huntington's disease.





Minisatellite is a repeating sequence of 10-100 base pairs in the genome. Here, the repeating unit is somewhat large and it is called a **DNA motif**. Another name for minisatellite is **variable number tandem repeats** (VNTRs). The number of VNTRs is highly variable among individuals. The repetitive unit of a minisatellite is GC rich.

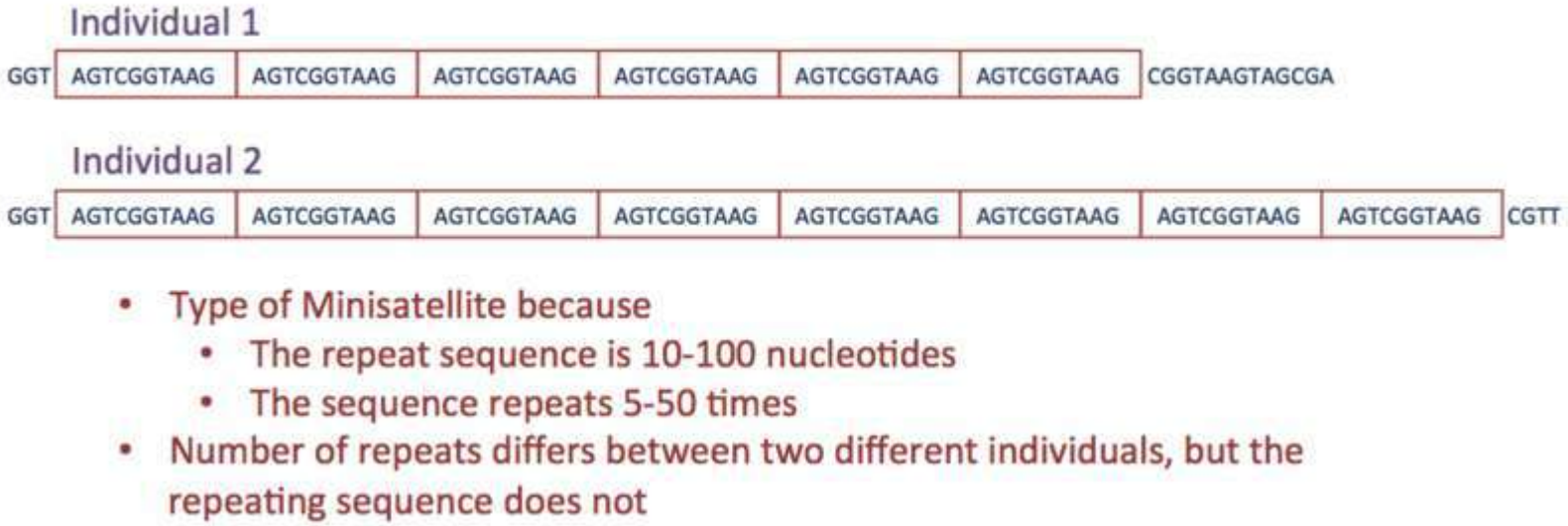
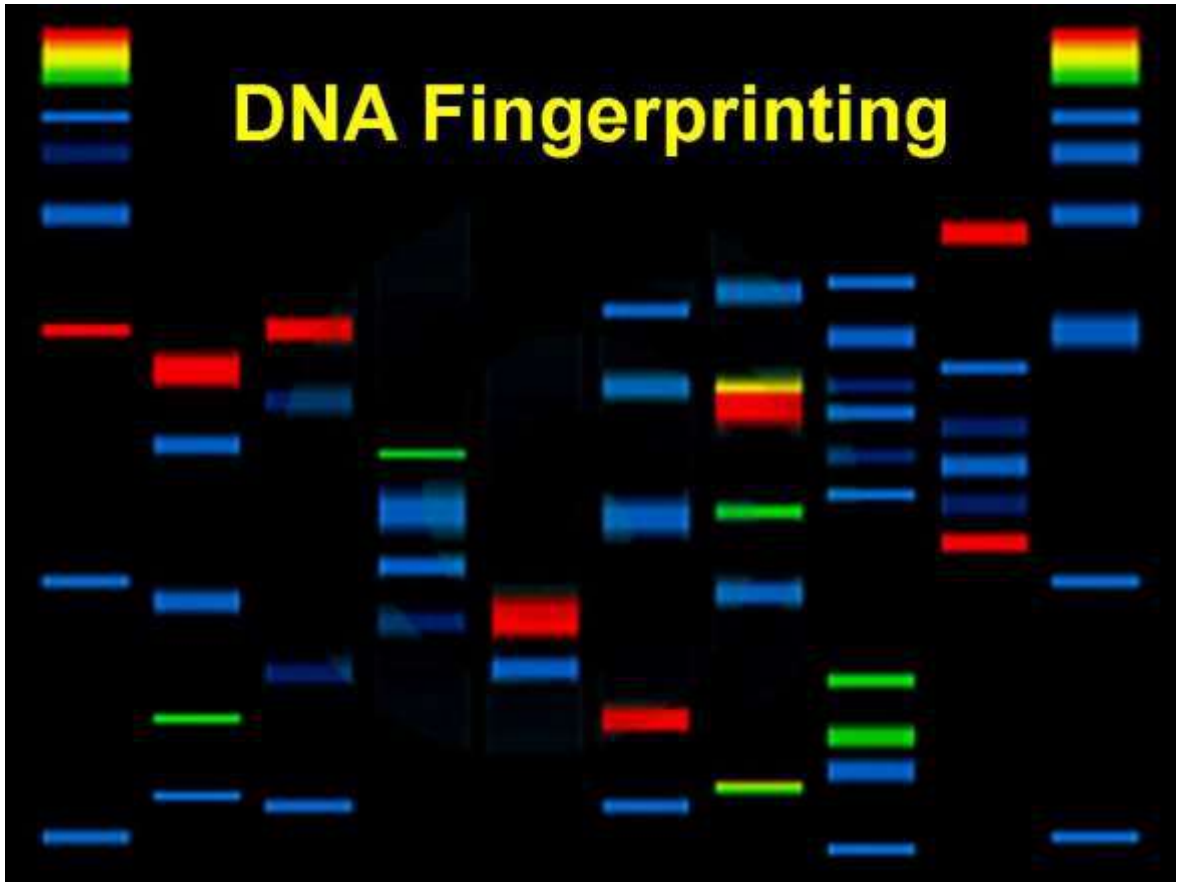


Figure 2: Minisatellites

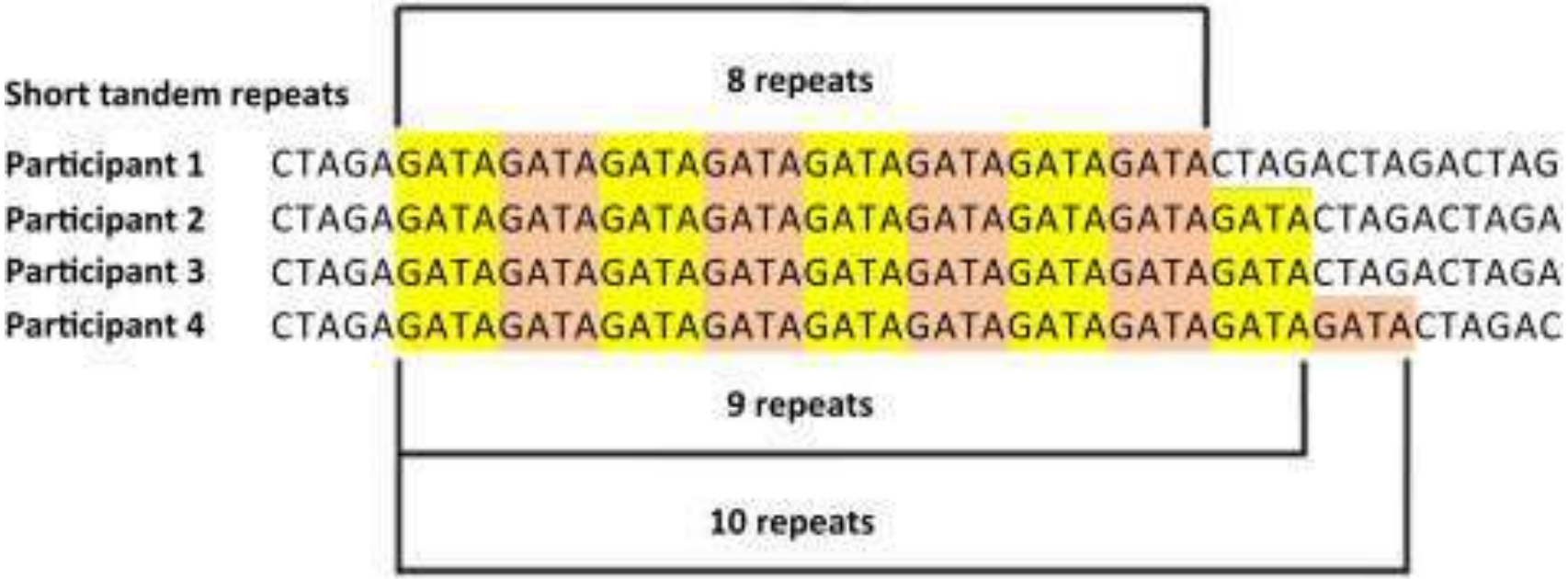
Due to the highly variable nature of minisatellites among individuals, scientists use them for DNA fingerprinting. They also use minisatellites as genetic markers during the linkage analysis. Some minisatellite sequences are involved in the formation of ras oncogene-associated cancer.



Variations occur due to mutations. These mutations in the non-coding sequences have piled up with time and form the basis of DNA polymorphism (variation at genetic level arises due to mutations).

The junk DNA regions are thus made-up of length polymorphisms, which show variations in the physical length of the DNA molecule.
At specific loci on the chromosome the number of tandem repeats varies between individuals. There will be a certain number of repeats for any specific loci on the chromosome.

Depending on the size of the repeat, the repeat regions are classified into two groups. **Short tandem repeats (STRs)** contain 2-5 base pair repeats and **variable number of tandem repeats (VNTRs)** have repeats of 9-80 base pairs.



Short tandem repeats (STRs)

Variable Number Tandem Repeats

- Tandem repeats occur in DNA when a pattern of one or more nucleotides is repeated and the repetitions are directly adjacent to each other.

- An example would be:
  AATTTTCCGGCCCCAAAATTCCAATTTTCCGGCCCCAAAATTCCAATTTTCCGGCCCCAAAATTCC
- These can be found on many chromosomes, and often show variations in length between individuals.

- The number of elements in a given region may vary, hence they are known as **variable number tandem repeats.**

- Each variant acts as an inherited allele, allowing them to be used for personal or parental identification.

- Scientists use polymorphic loci that are known to contain VNTRs/STRs in order to differentiate people based on their DNA.

Since a child receive 50% of the DNA from its father and the other 50% from his mother, so the number VNTRs at a particular area of the DNA of the child will be different may be due to insertion, deletion or mutation in the base pairs.

As a result, every individual has a distinct composition of VNTRs and this is the main principle of DNA fingerprinting.

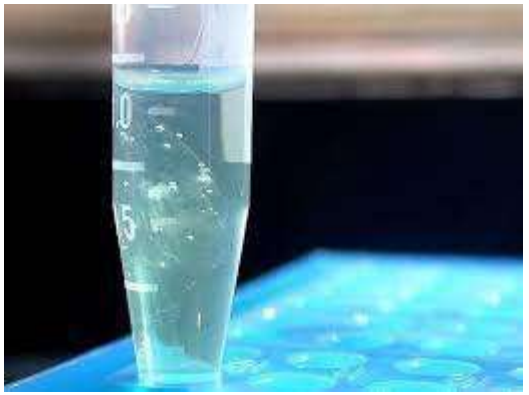
As single change in nucleotide may make a few more cleavage site of a given nucleotide or might abolish some existing cleavage site.

Thus, if DNA of any individual is digested with a restriction enzyme, fragments pattern (sizes) will be produced and will be different in cleavage site position.

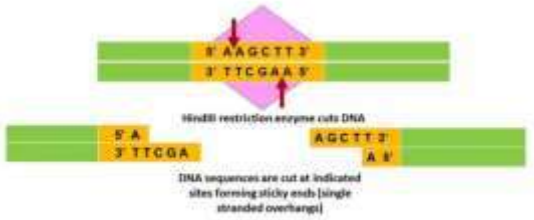
Methods of DNA Fingerprinting

A. Restriction fragment length polymorphism (RFLP)

The first step in this process is to **isolate the DNA** from the sample material to be tested. The sample size for RFLP test must be large enough to get the proper result.



Once the required size of the sample is available, the DNA is isolated from the sample and is **subjected to restriction digestion** using restriction enzymes.



The digested DNA sample is then **separated by agarose gel electrophoresis**, in which the DNA is separated based on the size.

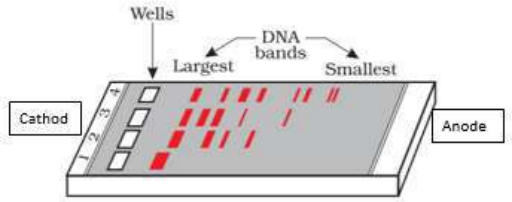


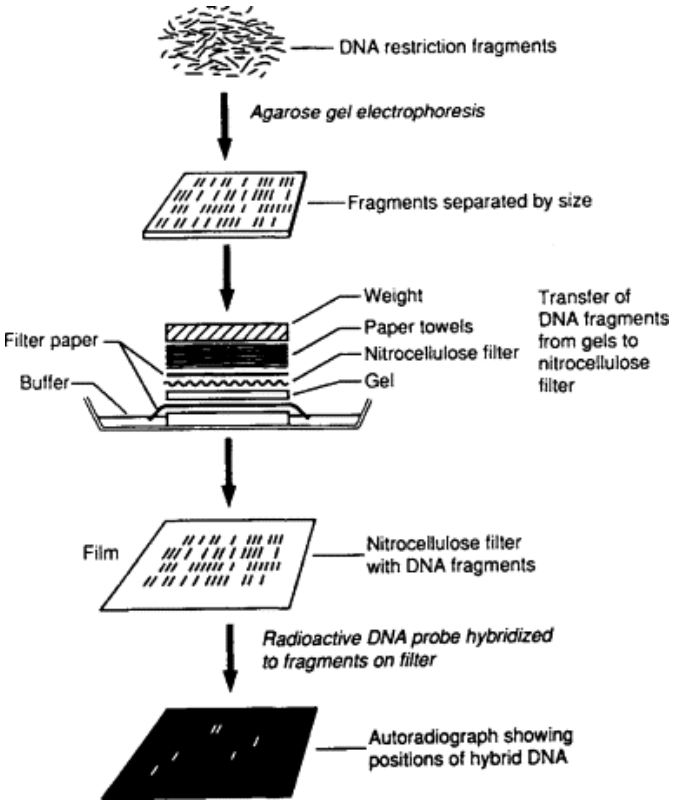
Figure 11.3 A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

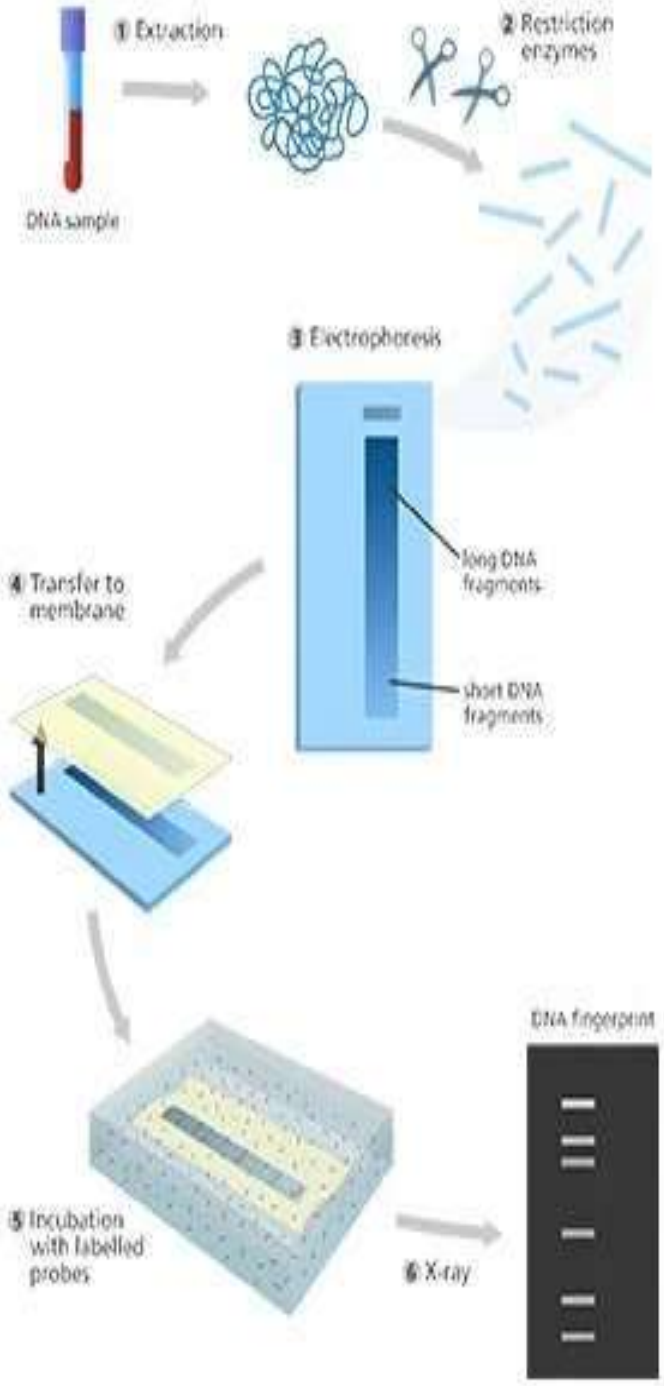
The next step is **transfer of separated DNA from gel slab onto the nitrocellulose membrane** to hybridize with a labeled probe that is specific for one VNTR region (radio activity labeled complimentary sequence for VNTR region nucleotide sequence).

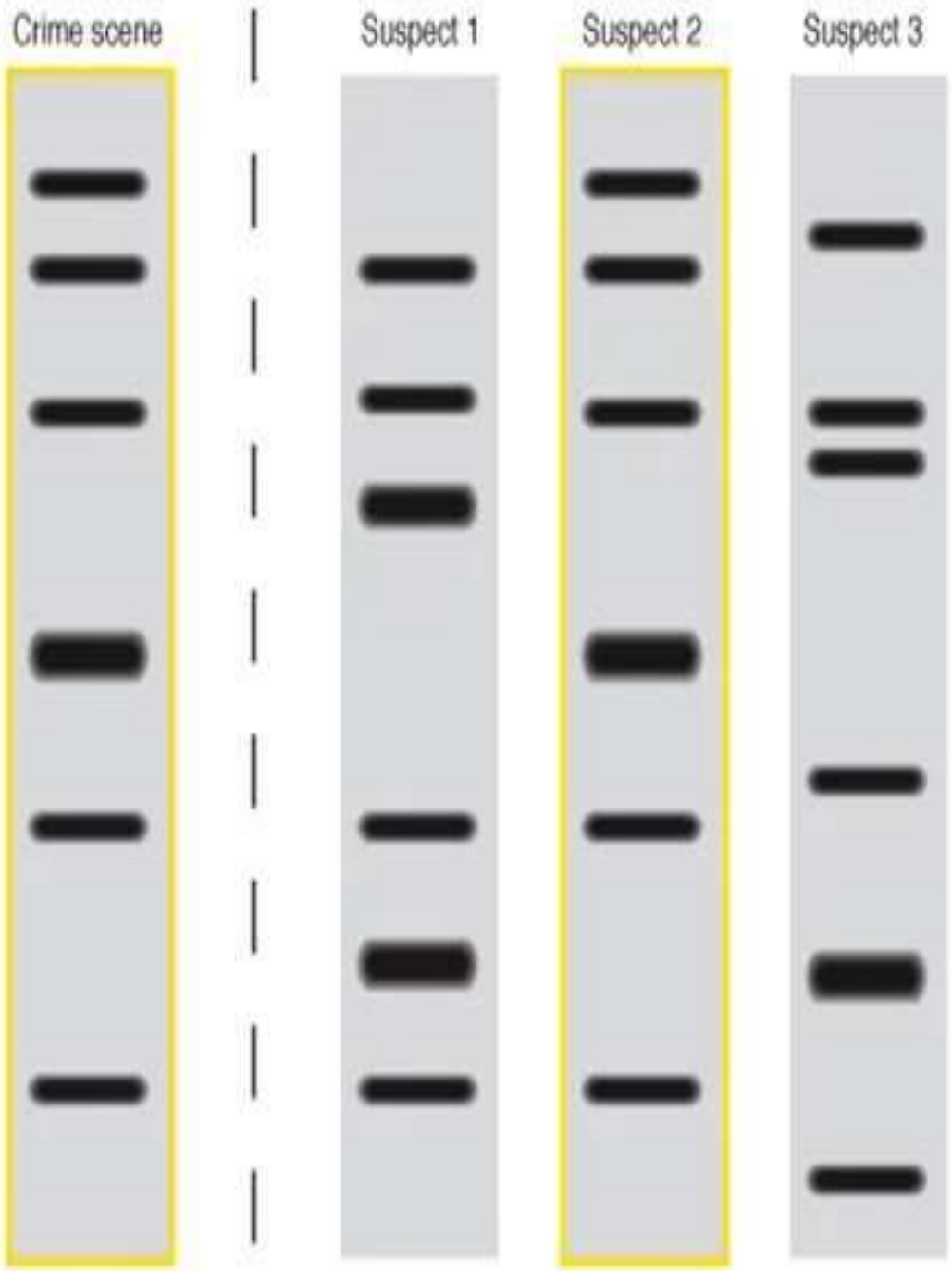
This technique of transferring and hybridizing DNA onto nitrocellulose membrane is known as southern blotting, a most widely used DNA detection technique by molecular biologists.

After the hybridization with the radioactive probes, the **X- ray film** is developed form the southern blotting and only the areas where the radioactive probe binds will show up on the film.

Now these bands when **compared with the other known sample**s, will give the final result of the DNA fingerprinting.




1 Extraction
DNA sample
2 Restriction enzymes
3 Electrophoresis
long DNA fragments
short DNA fragments
4 Transfer to membrane
5 Incubation with labelled probes
6 X-ray
DNA fingerprint


Crime scene
Suspect 1
Suspect 2
Suspect 3

Advantages

The RFLP is considered to be more accurate than the PCR, mainly because the size of the sample used more, use of a fresh DNA sample, and no amplification contamination.

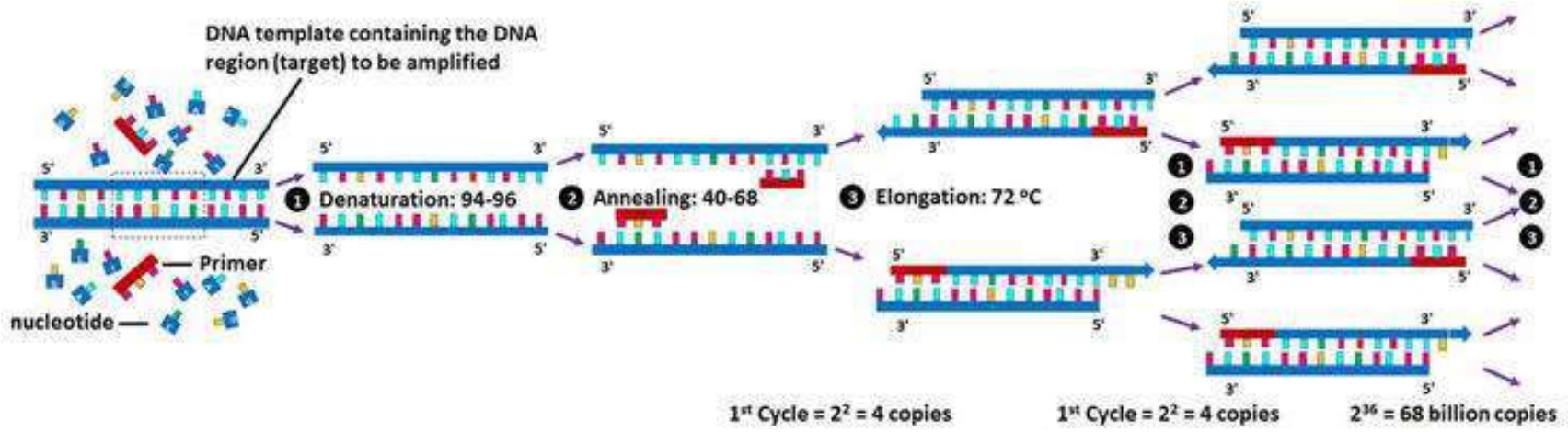
Limitation

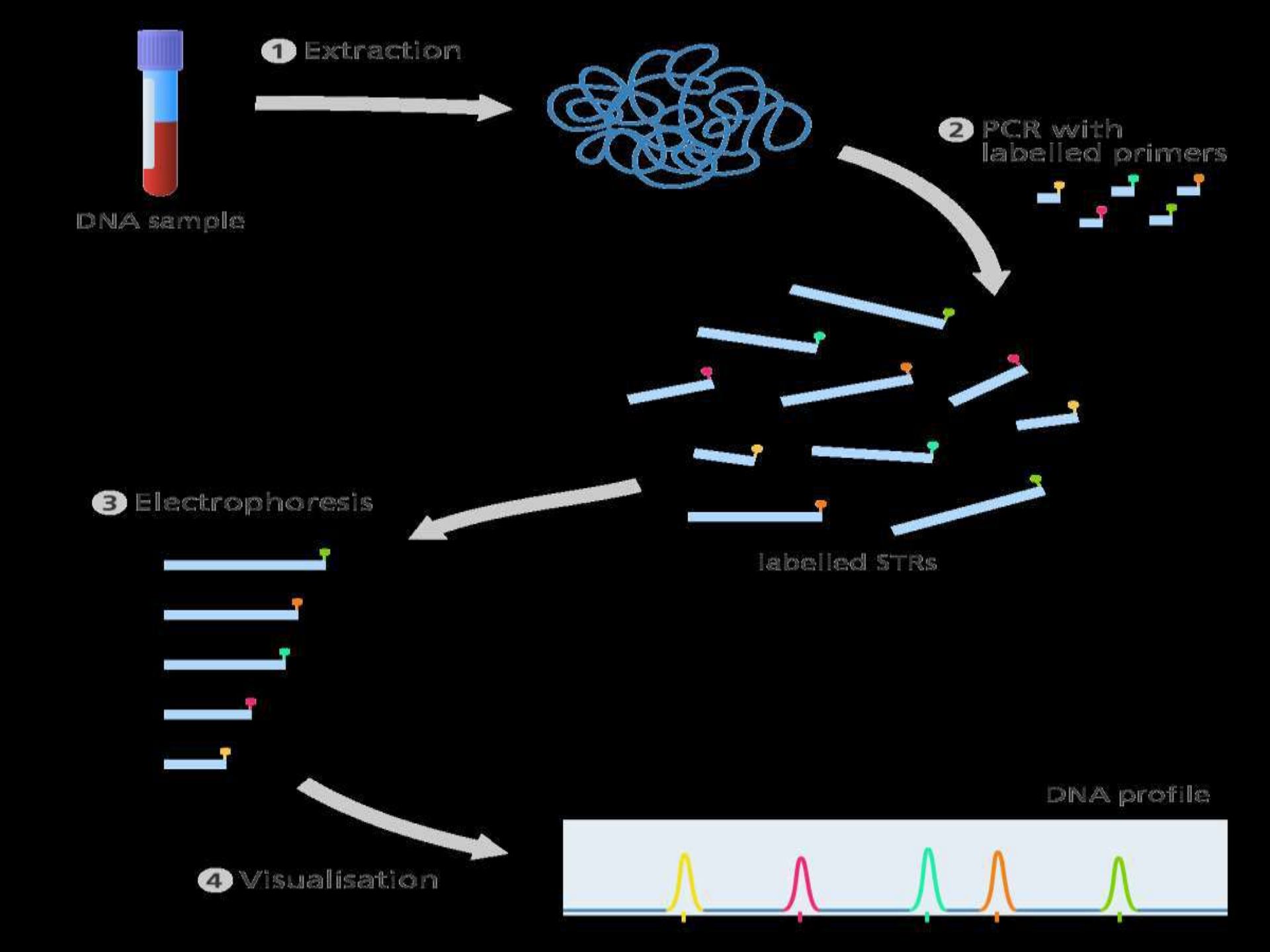
The RFLP, however, require longer time period to complete the analysis and is costly.

B. Polymerase Chain Reaction (PCR) amplification of short tandem repeats (STRs)

Thousands of copies of a particular variable region are amplified by **PCR** which forms the basis of this detection.

STR with a known repeat sequence is amplified and separated using gel-electrophoresis. The distance migrated by the STR is examined.




1 Extraction
DNA sample
2 PCR with labelled primers
labelled STRs
3 Electrophoresis
4 Visualisation
DNA profile

For the amplification of STRs using PCR, a short synthetic DNA, called primers are specially designed to attach to a highly conserved common nonvariable region of DNA that flanks the variable region of the DNA.

By comparing the STR sequence size amplified by PCR with the other known samples, will give the final result of the DNA fingerprinting.

Advantages

- Small amount of specimen is sufficient for the test.
- Takes a shorter time to complete.
- Less costly.

Limitation

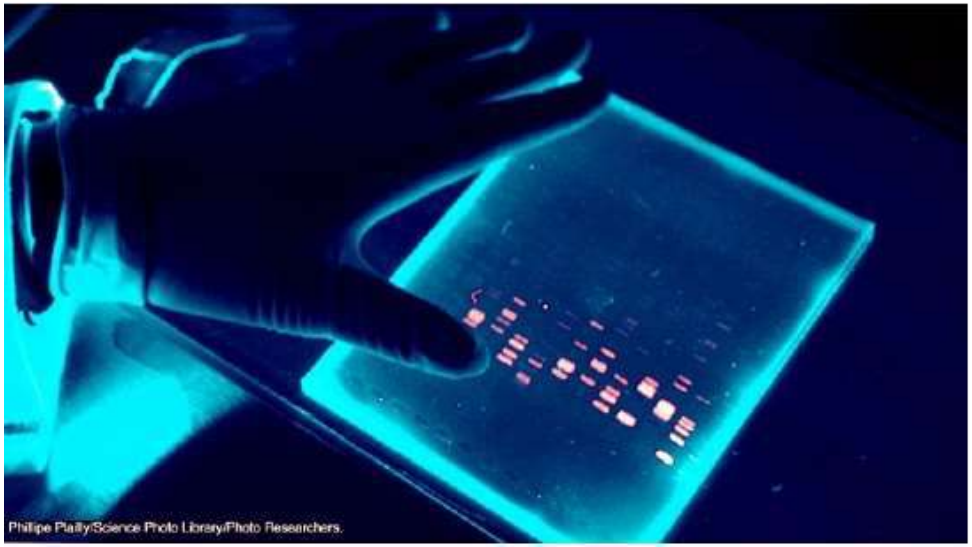
- Less accurate than RFLP.
- Possibility of amplification contamination.

Applications of DNA Fingerprinting

•DNA Fingerprinting is used by scientists to distinguish between individuals of the same species using only samples of their DNA. It is a primary method for identifying an individual.

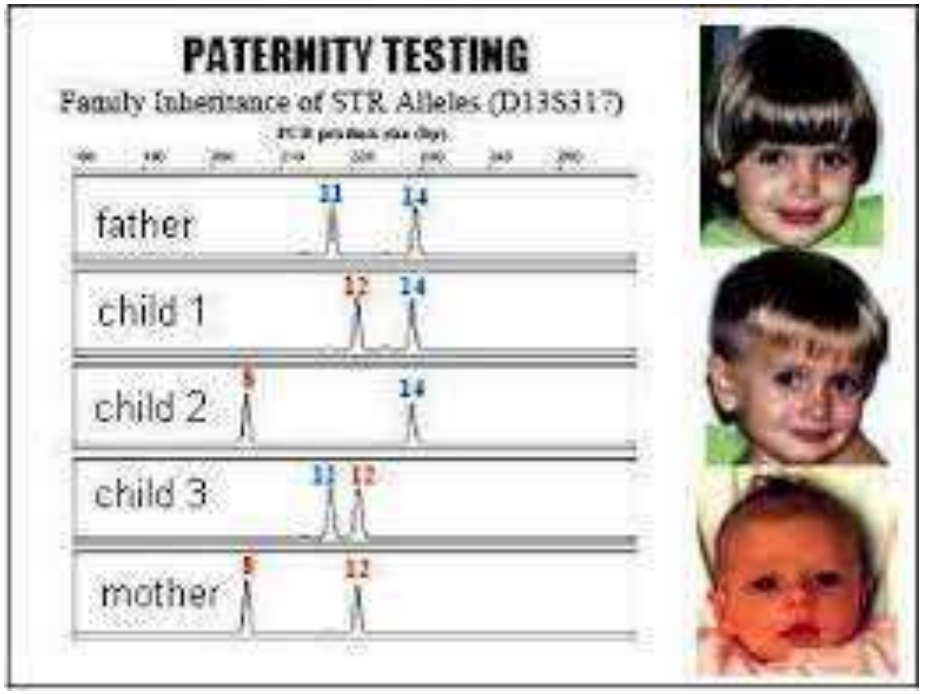
•Forensic Science:

Biological materials used for DNA profiling are: Blood, Hair, Saliva, Semen, Body tissue cells etc. DNA isolated from the evidence sample can be compared through VNTR (Variable number of tandem repeats) prototype. It is useful in solving crimes like murder and rape.



•**Paternity and Maternity Determination:**

A Person accedes to his or her VNTRs from his or her parents. Parent-child VNTR prototype analysis has been used to solve disputed cases. This information can also be used in inheritance cases, immigration cases.



- **Personal Identification:**

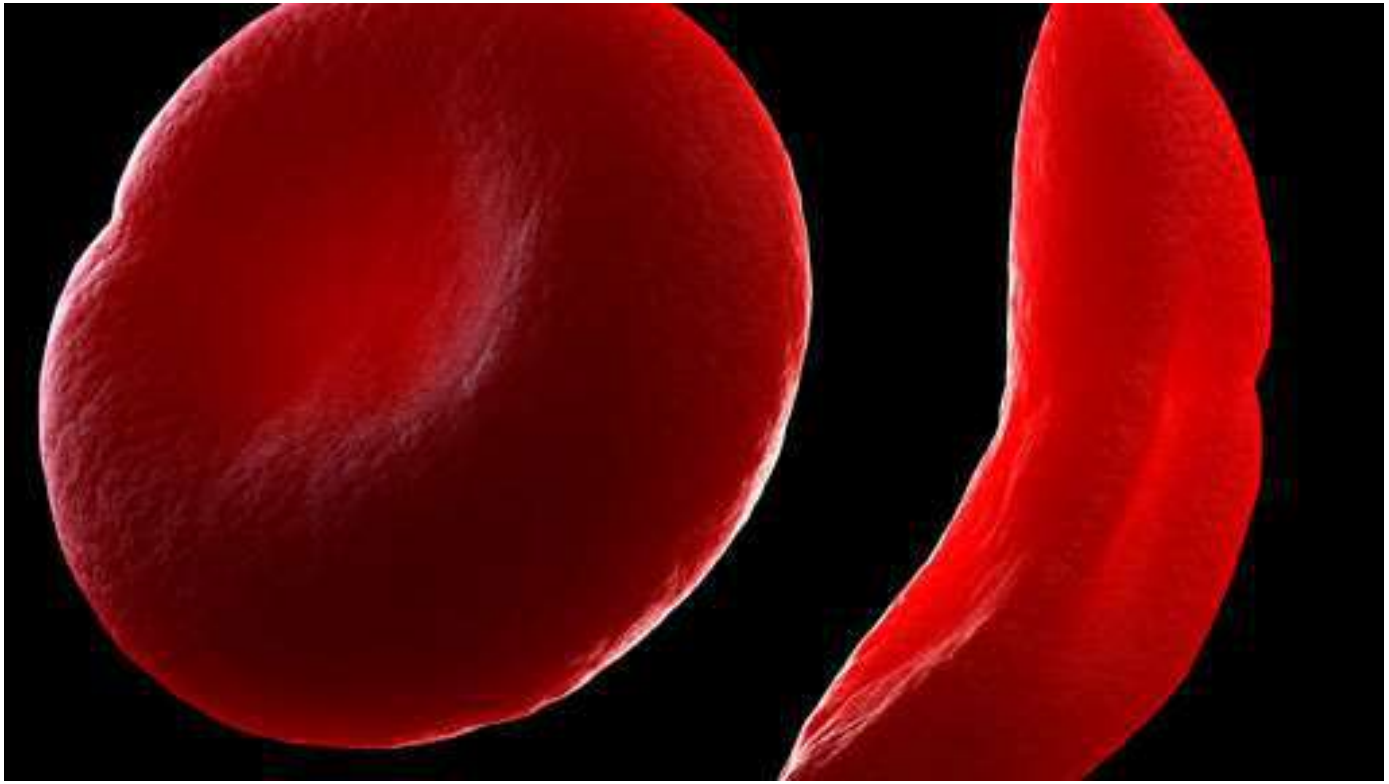
It utilizes the concept of using DNA fingerprints as a sort of genetic bar code to pinpoint individuals.

PERSONAL IDENTIFICATION
HOW IS DNA USED TO IDENTIFY INDIVIDUALS ?

- No individual is exactly like any other genetically, except for identical twins.
- Biology has used this fact to develop a powerful tool called DNA Fingerprinting for use in identifying individuals.
- DNA fingerprinting analyzes sections of DNA that may have little or no function but that vary widely from one individual to another.

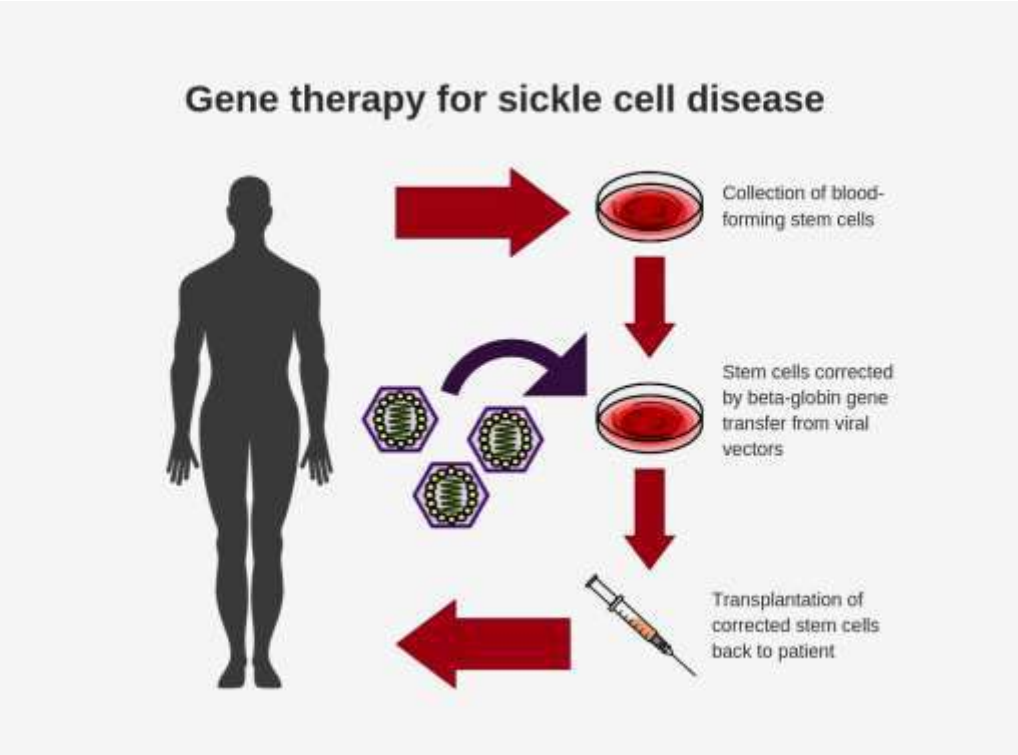
- **Diagnosis of Inherited Disorders:**

It is also useful in diagnosing inherited disorders in both prenatal and newborn babies. These disorders may include cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia, and many others.



•Development of Cures for Inherited Disorders:

By studying the DNA fingerprints of relatives who have a history of some particular disorder, DNA prototypes associated with the disease can be ascertained.

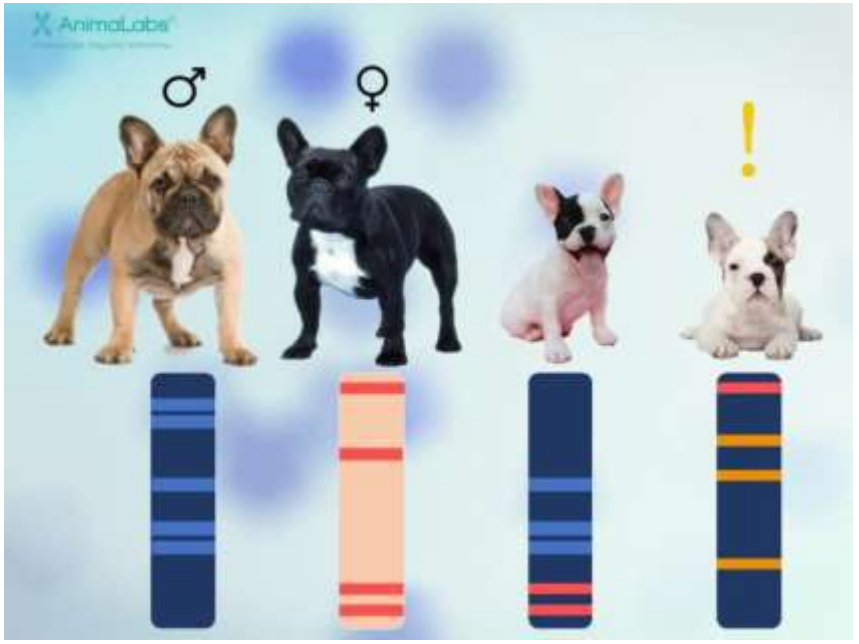


•**Detection of AIDS:**

By comparing the band of HIV “RNA” (converted to DNA using RTPCR) with the bands form by the man’s blood, person suffering with AIDS can be identified.

•**Breeding Program:**

Breeders conventionally use the phenotype to evaluate the genotype of a plant or an animal.As it is difficult to make out homozygous or heterozygous dominance from appearance, the DNA fingerprinting allows a fastidious and precise determination of genotype. It is basically useful in breeding race horses and hunting dogs.



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THANKYOU