Advance Laboratory Techniques
Lecture 4

DNA Fingerprinting

Conventional fingerprint of an individual comes from finger tip and unique for an individual, but it can be changed by surgery. There is another type of fingerprint unique to an individual called DNA fingerprint. This remains same in all body parts, tissues and cells as well as cannot be altered by any known methods. Thus, DNA fingerprint method is becoming primary method for identifying an individual.

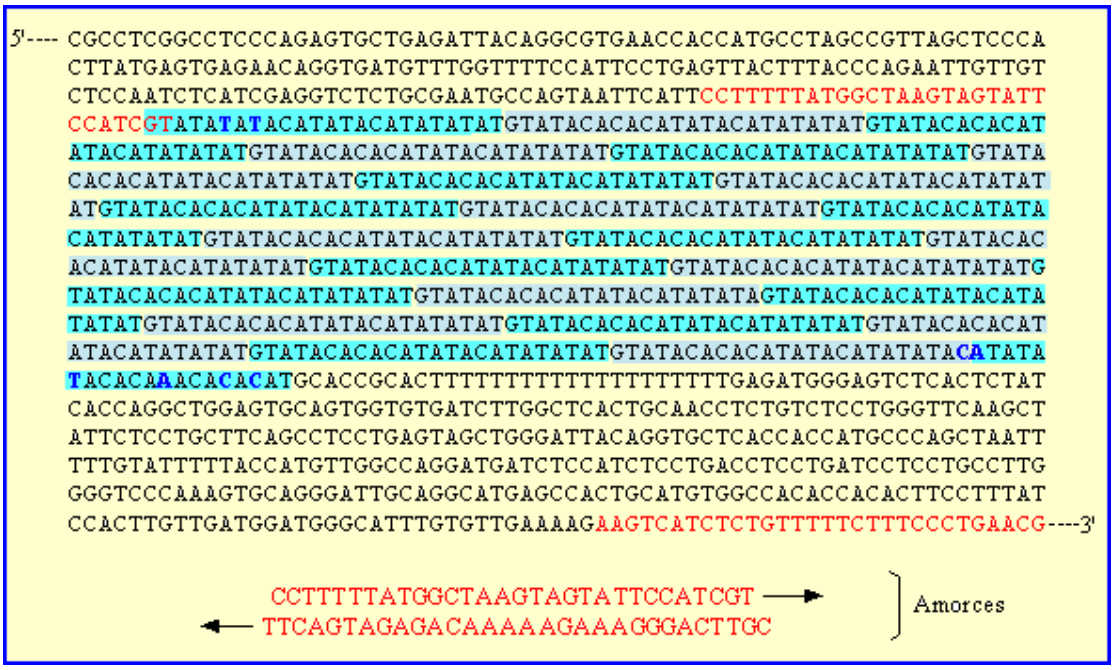
The DNA of every human being on the planet is 99.9% same. However, 0.1% of DNA is unique to the individual that makes all the difference. These differences are a consequence of mutations during evolution. As single change in nucleotide may make a few more cleavage site of a given nucleotide or might 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 difference in cleavage site position. This is the basics of DNA fingerprinting. DNA Fingerprinting is used by scientists to distinguish between individuals of the same species using only samples of their DNA. The process of DNA fingerprinting was invented by Sir Alec Jeffrey at the University of Leicester in 1985.

The DNA profiling of each individual is unique because of the diverse in polymorphic regions present in genome of every individual. These polymorphic regions used for identification are the non-coding regions of the genome. The polymorphic regions of the DNA do not code for proteins and which make-up 95% of our genetic DNA. Hence these regions are therefore called the ―junk DNA. Although these ―junk DNA regions do not code for proteins, they are involved in regulating gene expression, they help in reading of other genes that code for protein and are a large portion of the chromosome structure. The junk DNA regions are made-up of length polymorphisms, which show variations in the physical length of the DNA molecule. In DNA profile the length of the polymorphisms in the non-coding areas is measured as it varies with each individual. These polymorphisms are identical repeat sequences that are present in non- coding DNA region. 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.

- No. of repeats varies among individuals
    - ™ VNTR (Variable Number of Tandem Repeats)
        - Minisatellites

- Microsatellites

- Minisatellites
  - ™ Repeat unit length – 6-100 bases
  - ™ Two to several hundred repeats at each minisatellite
  - ™ Thousands of different minisatellites scattered throughout the genome, but often clustered near the end of the chromosomes – the telomeres
- Microsatellites
  - ™ Repeat unit length: 1-7 bases
  - ™ 5 to 100 repeats at each microsatellite
  - ™ Thousands of different microsatellites, randomly scattered throughout genome
  - ™ Example of minisatellite

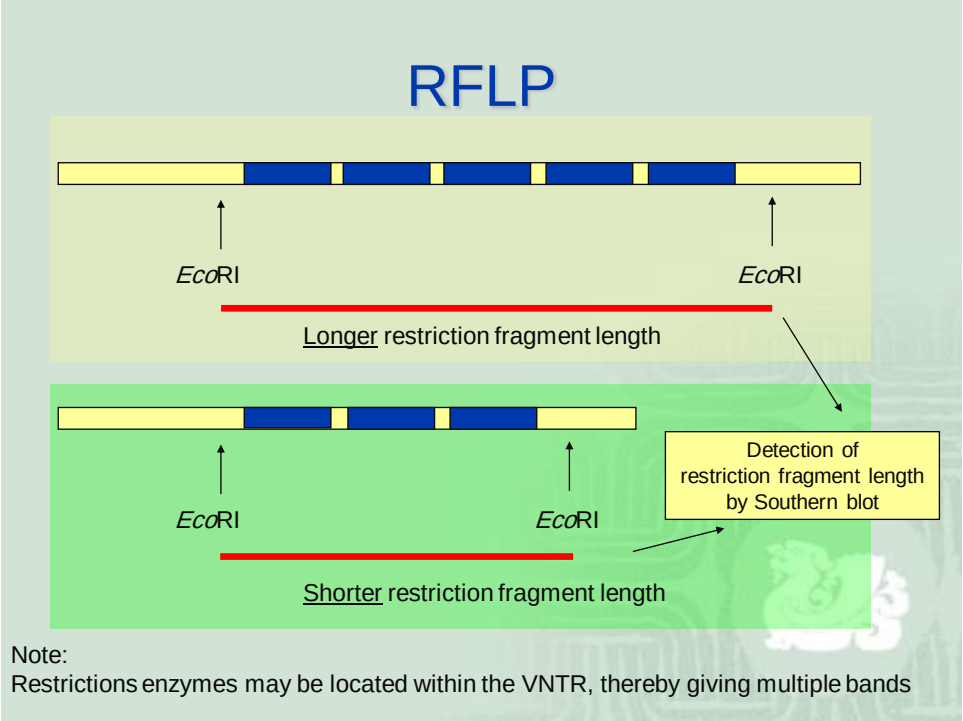


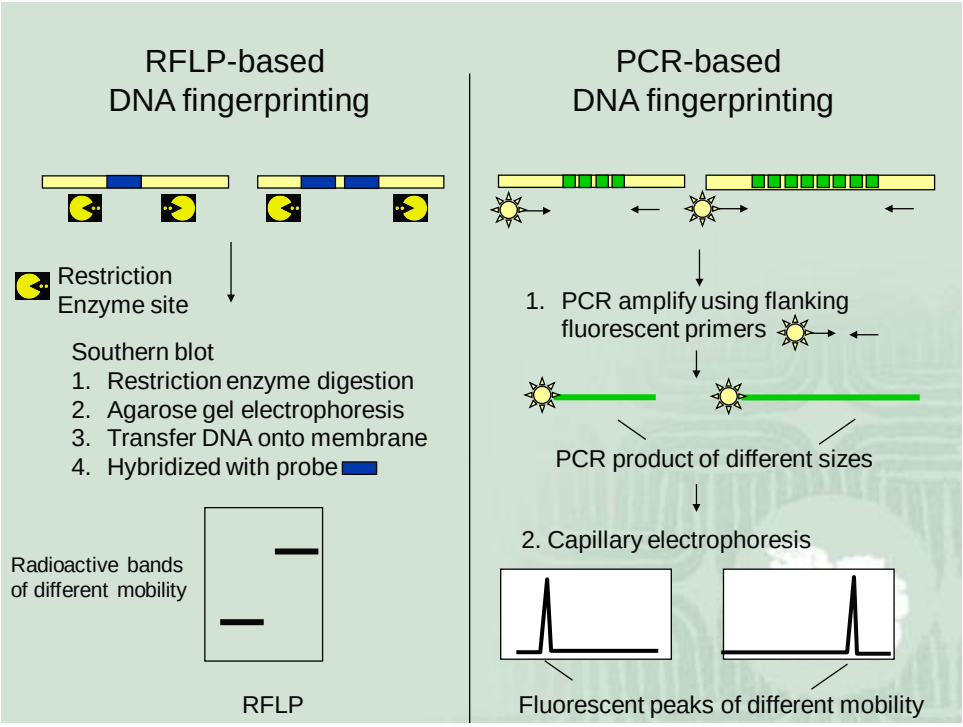
Examples of microsatellite

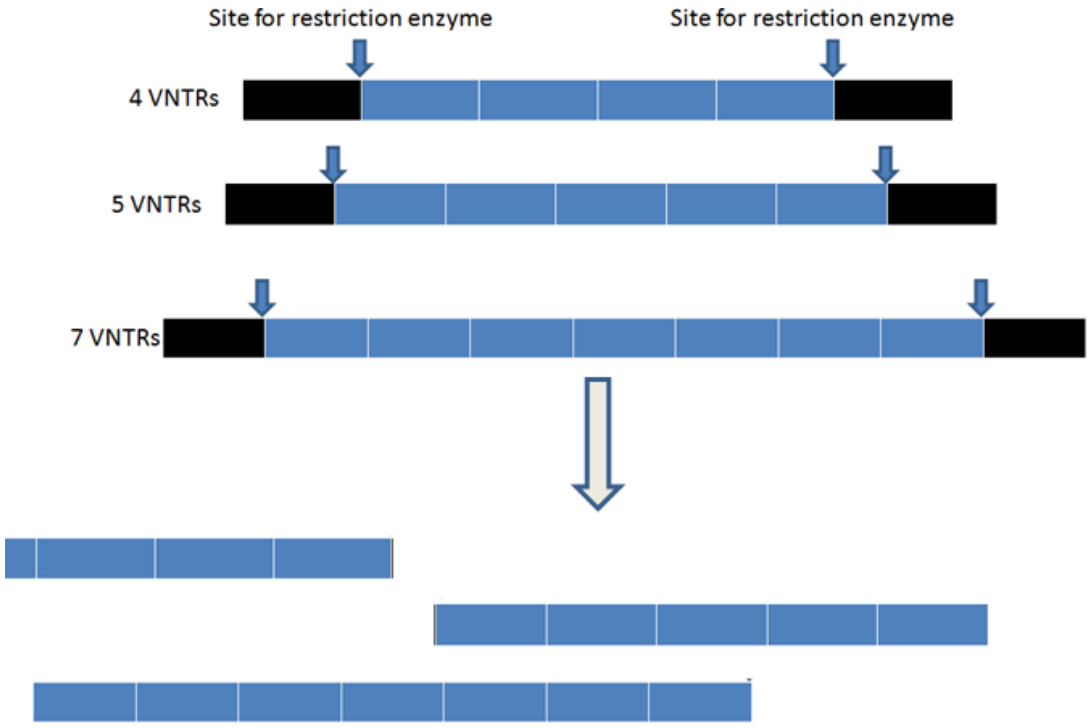
- CACACACACACACACACACACACACACACACACACACACACACACAC ACA
  - ™ $(CA)_{24}$

- AATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATGA ATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAA TGAATGAATGAATGAATGAATG

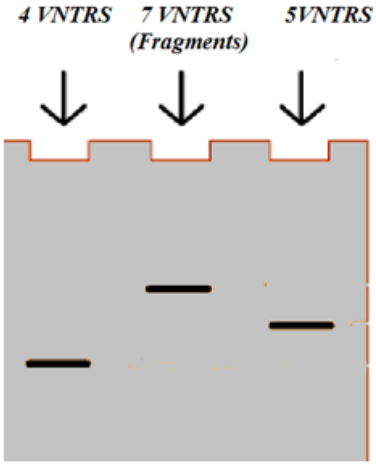
  ™ $(AATG)_{28}$







Different sizes of fragments after restriction digestion



Identification of band using VNTR probe (Radio activity labeled nucleotide sequence complimentary to VNTR)

Note: **VNTR sequences from different loci can be combined to create DNA fingerprint. Resulting patter of each individual is theoretically unique.**

We inherit a copy of chromosome, one from father and one from mother for each of the 23 pairs of chromosomes, which indicate that we carry two copies of each VNTR locus, just like we have two copies of genes donated by our parents. At a particular VNTR site if you have the same number of sequence repeats, you are called homozygous at that site; if you have a different number of repeats, you are said to be heterozygous‖. VNTR sequences from different loci can be combined to create DNA fingerprint. Resulting pattern of each individual is theoretically unique.

The two types of DNA fingerprinting tests: RFLP and PCR/STR. Restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) amplification of short tandem repeats (STRs) are two main DNA tests widely used for DNA fingerprinting. Other diagnostic methods exist, but they lack accuracy and precision.

Restriction fragment length polymorphism (RFLP):

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. The first step in this process is to isolate the DNA from the sample material to be tested. As mentioned, 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. 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 samples, will give the final result of the DNA fingerprinting.

Polymerase chain reaction (PCR) amplification of short tandem repeats (STRs)

PCR generates the repeated copies of a specific area of the DNA fragment. These areas are the alleles and are specific sequences of base pairs. 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. For the amplification of STRs using PCR, a short synthetic DNA, called primers are specially designed to attach to a highly conserved common non- variable 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.

Applications of DNA Fingerprinting:

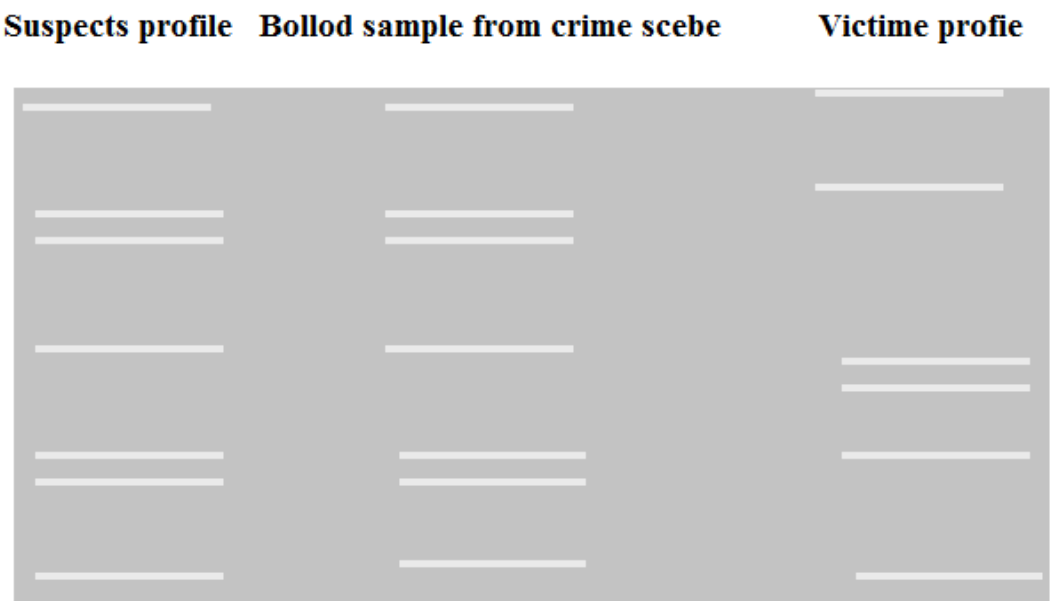
1. **In Forensics Science**: Forensic science can be defined as the intersection of law and science. The DNA profile of each individual is highly specific. The chances of two people having the exact DNA profile are 30,000 million to 1 (except for identical twins). Biological materials used for DNA profiling are: Blood, Hair, Saliva, Semen, Body tissue cells etc.

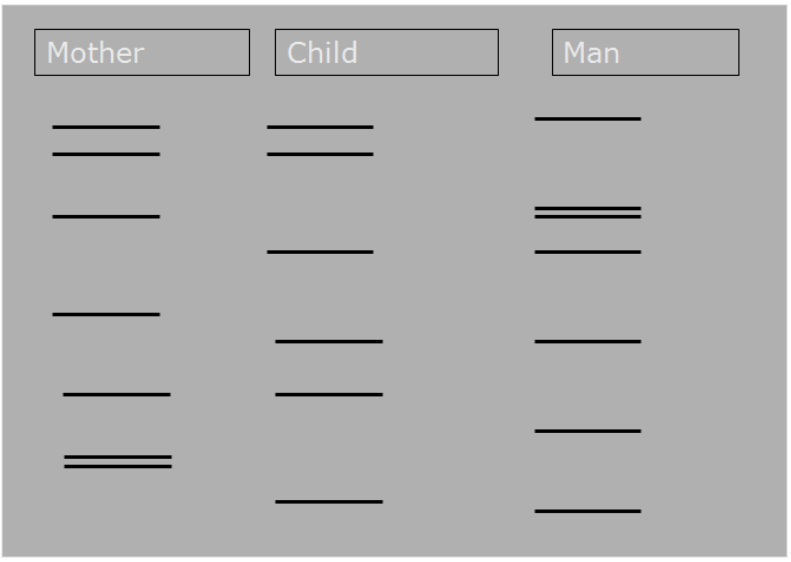
2. **Paternity and Maternity Determination:** A Person accedes to his or her VNTRs from his or her parents. Parent-child VNTR prototype analysis has been used to solve disputed cases.

3**. Personal Identification**: The concept of using DNA fingerprints as a sort of genetic bar code to pinpoint individuals has already been discussed above.

4. **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, familial Alzheimer's, sickle cell anemia, thalassemia, and many others.

5**. Detection of AIDS:** By comparing the band of HIV "RNA" (converted to DNA using RT-PCR) with the bands form by the man's blood, person suffering with AIDS can be identified.





- By comparing the DNA profile of a mother and her child it is possible to identify DNA fragments in the child which are absent from the mother and must therefore have been inherited from the biological father.