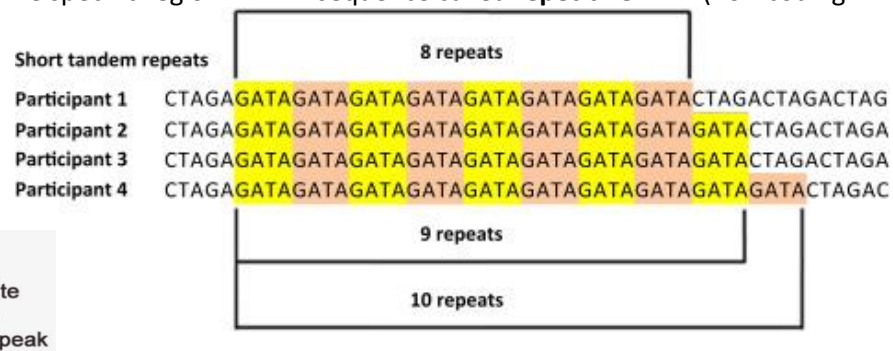
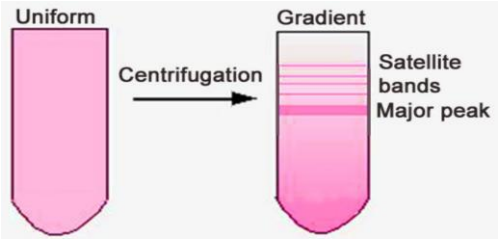
DNA Fingerprinting – Developed by **Alec Jeffreys (1985)**

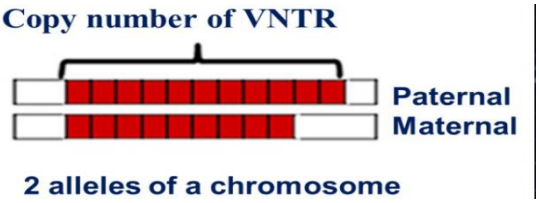
- It is the technique to identify the similarities and differences of DNA fragments of 2 individuals.
- It involve identifying differences in some specific region in DNA sequence called **repetitive DNA** (non-coding repetitive sequences).
- DNA separated from bulk genomic DNA durung density gradient centrifugation.
- Bulk DNA forms MAJOR PEAK.



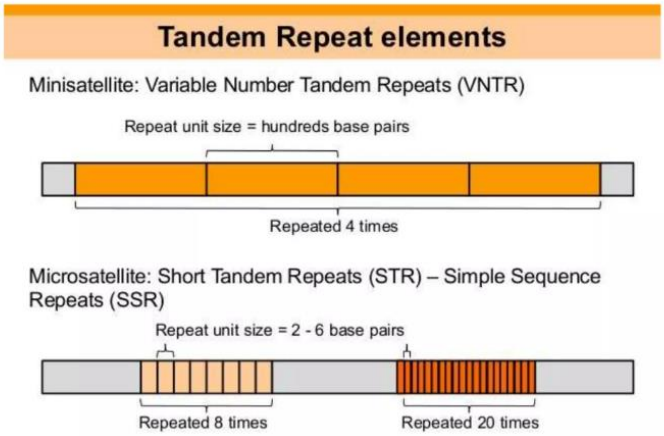


Satellite DNA- SMALL PEAK of repetitive DNA that shows a very high degree of polymorphism and is classified as :

Mini Satellite	Microsatellite
Upto 100 baispairs	2-6 baispairs
C & G base rich	T & A bases rich
Reffered as Variable Number Tandem Repeats (VNTR)	Reffered as ShortTandem Repeats



- In a person copy number varies in each chromosome.
- Two alleles of a chromosome contain different copy



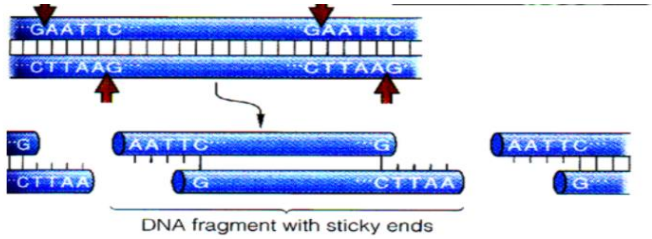
DNA Polymorphism: Any difference in nucleotide sequence (inheritable mutation) observed in population.
Mutation (higher in non-coding sequence as it don't affect an individual's reproductive ability) accumulate generation to generation

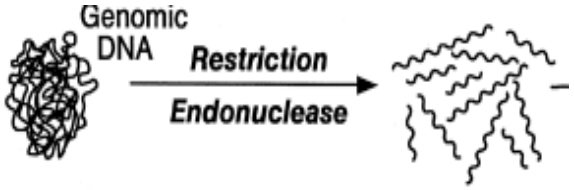
- Play great role in evolution & speciation.
- More than one allele with variation greater than 0.01 frequency.

Sourthern blotting Technique

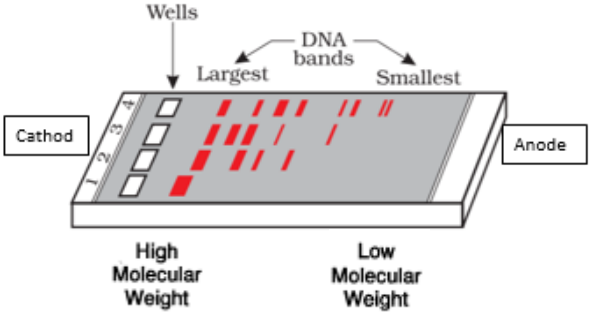
1. **Isolation of DNA** - DNA is taken from the cell **(** Samples– Any cell, blood, stains, semen, saliva, hair root,bonr , skin etc) and purified via chemical processing and centrifugation. Followed by Amplification using PCR.

2. **Digestion of DNA** – cut into fragments **by restriction endonucleases** (molecular scissor).

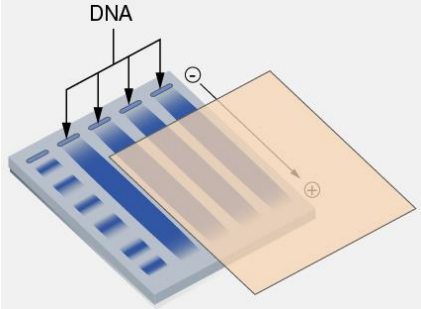




3. **Separation of DNA fragments by gel (Agrose) Electrophoresis**. -When electricity supplied the DNA fragments being negatively charged move from cathode (-ve) towards Anode (+ve). Small fragments moves faster than larger one.



4. **Transferring (blotting) separated DNA fragments to synthetic membranes, such as nitrocellulose or nylon.**



5. **Hybridization is done using a labeled VNTR probe.**
   Nitrocellulose paper is transferred into radioactive labelled VNTR probe (single stranded nucleotides fragments) that binds to complementary sequence to form Hybrid DNA.

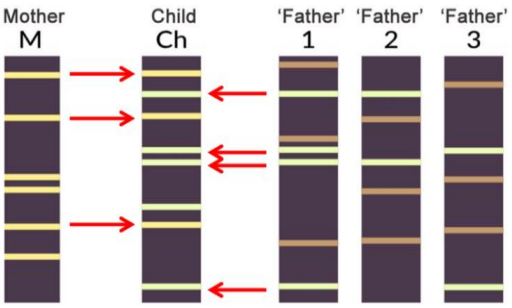
6. **Detection of hybridized DNA fragments by autoradiography**

   Hybridised DNA is photographed to X-ray film that gives an image in the form of light and dark bands of different sizes

- VNTR varies in size from 0.1 to 20kb.
- DNA fingerprint differ in every individual except Monozygotic Twins.
- Sensitivity of technique can be increased by Polymerase Chain Reaction (PCR).

Applications of DNA Fingerprinting

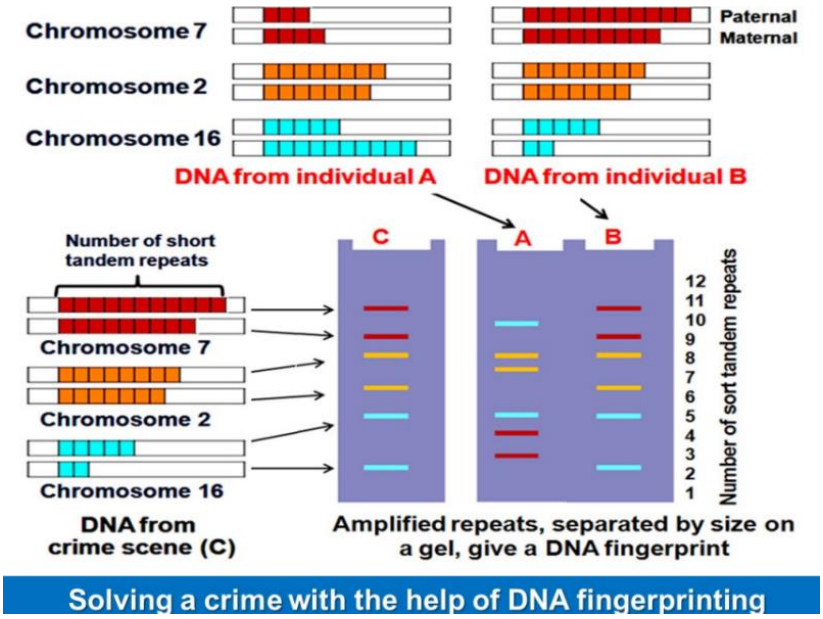
1. Test of paternity.
2. Identification of the criminals in unknown cases based on tissue samples e.g. rape, murder, etc.
3. Population diversity determination or phylogenic status of animals
4. Determination of genetic diseases.



Solving paternity using DNA fingerprinting

Here, person 1 is likely to be the father of child

5.



Solving a crime with the help of DNA fingerprinting