

CHAPTER 6
MOLECULAR BASIS OF
INHERITANCE
DNA Fingerprinting

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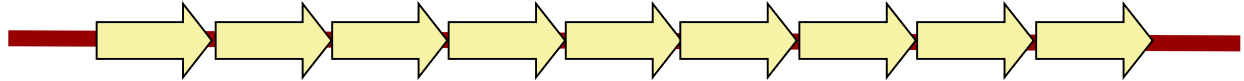
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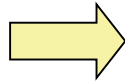
Key Terms

Repeat : Repeat Unit = Polymer : Monomer

Repeat



Repeat Unit



Repeat Length

No.of Repeat Units

What is DNA Polymorphism

Genetic variation, in the form of multiple alleles of many genes, exists in most natural populations of organisms. We have called such genetic differences between individuals *DNA markers*; they are also called *DNA polymorphisms*.

(The term *polymorphism* literally means “multiple forms.”)

The probability of such variation to be observed in noncoding DNA sequence would be higher as mutations in these sequences may not have any immediate effect/impact in an individual's reproductive ability. These mutations keep on accumulating generation after generation, and form one of the basis of variability/polymorphism.

Simple Sequence Repeats or Tandemly Repeated DNA

- Blocks of perfect or slightly imperfect tandemly repeated DNA sequences.
- Individual arrays can occur at a few or many different chromosomal locations.

eg

gtccat**CAGCAGCAGCAGCAGCAGCAG**tgctg

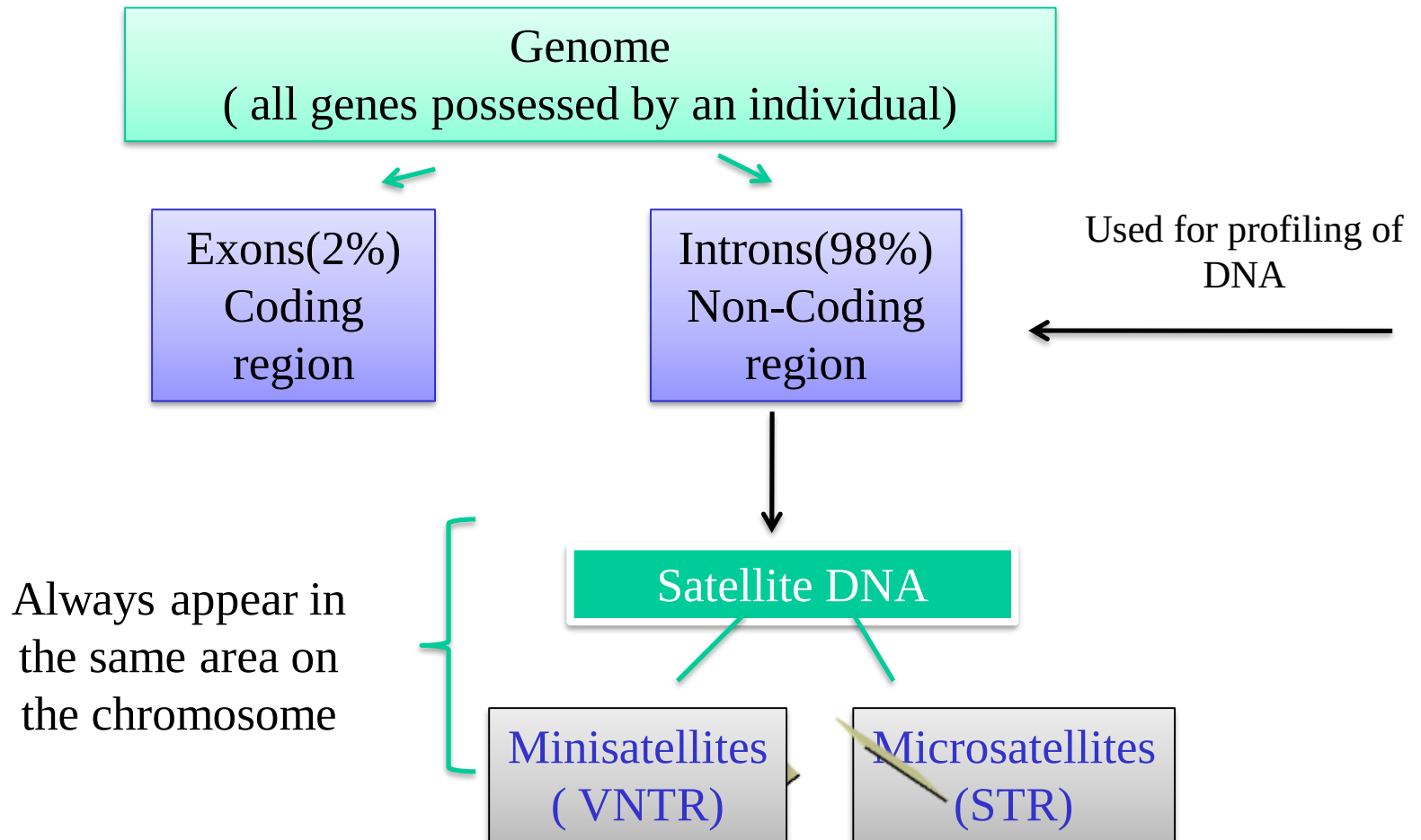
tgtact**GTGTGTGTGTGTGTGT**actcg

Simple sequence length polymorphism(SSLP)

Array of repeat sequences displaying length variations.They are multiallelic and of 2 types

1. Minisatellites or VNTRs (Variable number tandem repeats)
repeat unit is few tens of nucleotide are found more towards end of chromosome, and are not evenly spread throughout the genome.
2. Microsatellites or STRs (Short tandem repeats)
repeats are much shorter, di-, tri-,tetra.and are much more used.

Their length polymorphisms are typed usually by PCR
the fragments are shorter in length, analyzed by gel electrophoresis.



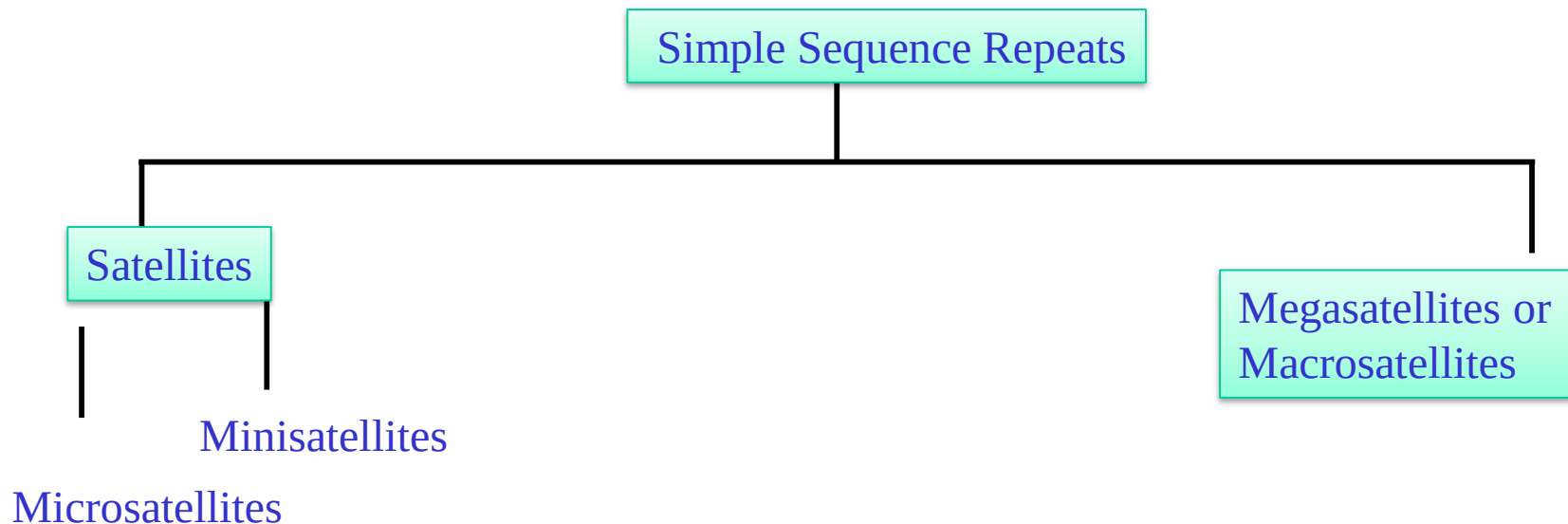
•Diff individual have diff. no. of repeats.
therefore diff .satellite patterns

STR- Short Tandem repeats

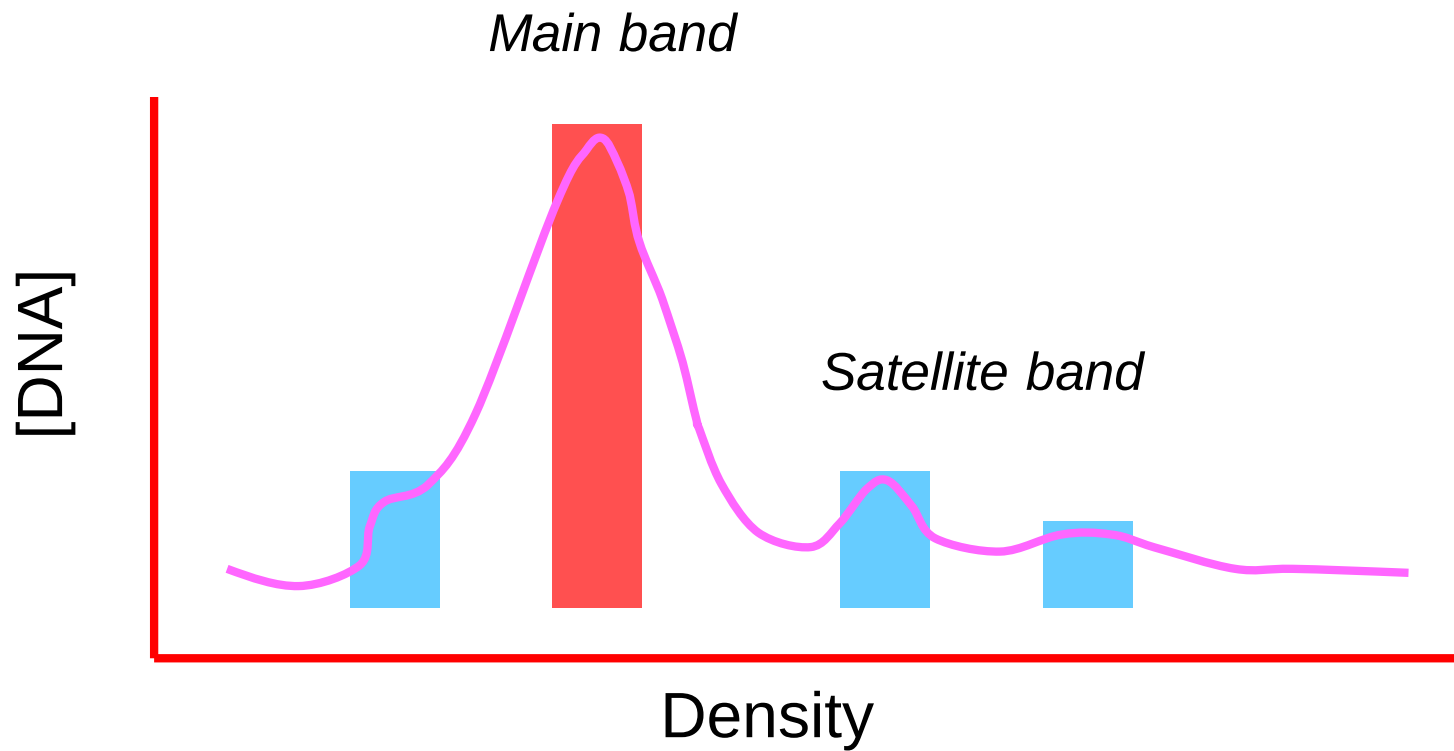
VNTR- Variable number tandem repeats

Classification of SSRs

Depending on the average size of the repeat units, SSRs can be classified into 4 categories:



Satellite DNA

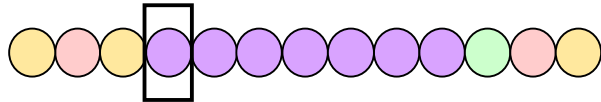


Microsatellite Repeats

- SSRs with short arrays of repeat units of 1-5 bases.
- Dispersed throughout genome.

Examples:

Mononucleotide repeats:



- A & T are very common
- Accounts for about 10Mb or 0.3% of the human genome.

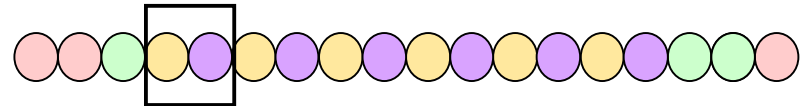
eg ctga**AAAAAAAAAA**atgc

Dinucleotide repeats:

- Arrays of CA/TG repeats are very common.
- Accounting for 0.5% of the genome.
- Highly polymorphic.

aact**CACACACACACA**tgc

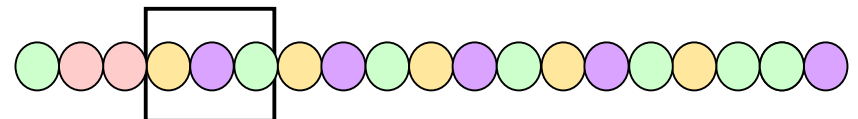
ttga**GTGTGTGTGTGT**acg



Trinucleotide repeats:

- Comparatively rare.
- Highly polymorphic.
- Involved in some neurodegenerative disorders.eg

tgag**CAGCAGCAGCAGCAG**atcg



Minisatellite Repeats

- Moderately sized arrays of repeat units of 6 to 64 bases.
- Located at or close to telomeres of all the chromosomes.
- Normally not transcribed.

Example of minisatellite repeats:

Telomeric DNA

DNA Fingerprinting

- The first method for using DNA analysis to identify individuals was developed in the mid-1980s by Sir Alec Jeffreys of Leicester University.
- This technique was not based on any of the types of polymorphic site listed above, but on a different kind of variation in the human genome called a **hypervariable dispersed repetitive sequence**.
- **As the name** indicates, this is a repeated sequence that occurs at various places (“dispersed”) in the human genome. The key feature of these sequences is that their genomic positions are variable: they are located at different positions in the genomes of different people.

DNA Fingerprinting

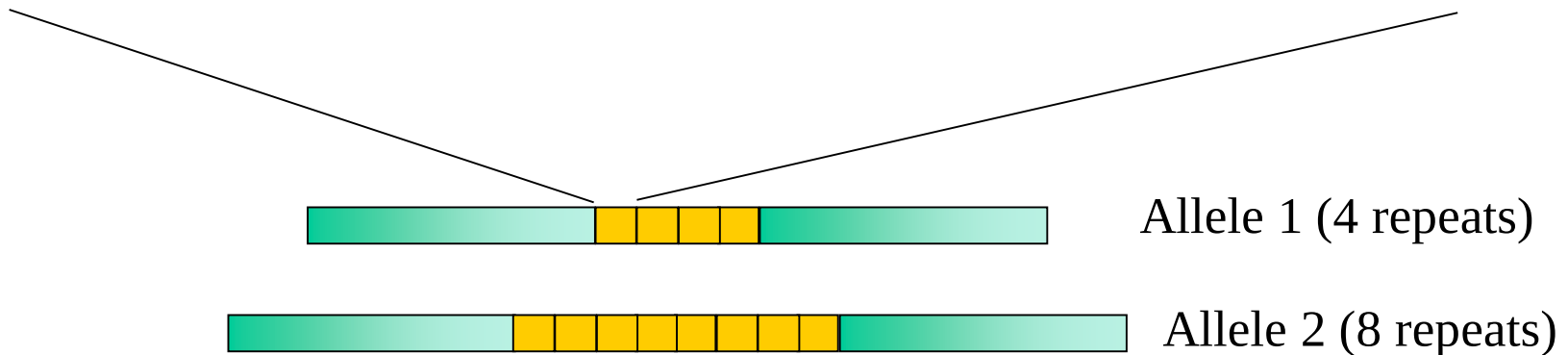
Minisatellites

- ⇒ Highly variable between individuals.
- ⇒ Original DNA fingerprinting technique
- ⇒ Relies on stretches of tandemly repeated sequences (usually 15 - 100bp)
- ⇒ also called variable number tandem repeats (VNTRs)
- ⇒ alleles show high variability in numbers of repeats

Minisatellites

Discovered by Sir Alex Jeffreys in 1985.
e.g. 33 bp repeat found in human myoglobin gene

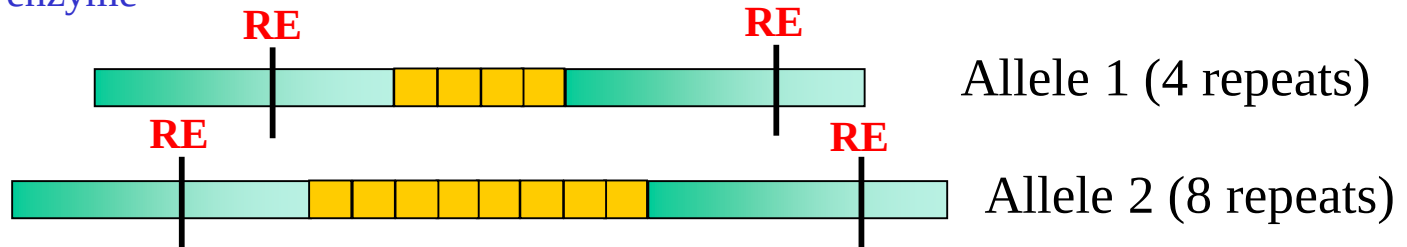
CTAAAGCTGGAGGTGGGGCAGGAAGGACCGAGGT



Many different alleles exist, and may be homozygous or heterozygous

Minisatellites typing by gel electrophoresis

RE restriction enzyme

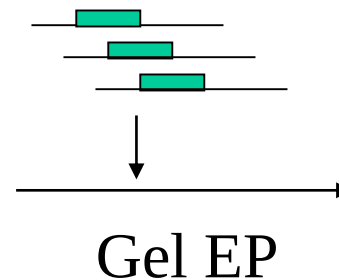


⇒ Take DNA from cells, & digest with restriction enzyme that cuts outside the repeated sequences.

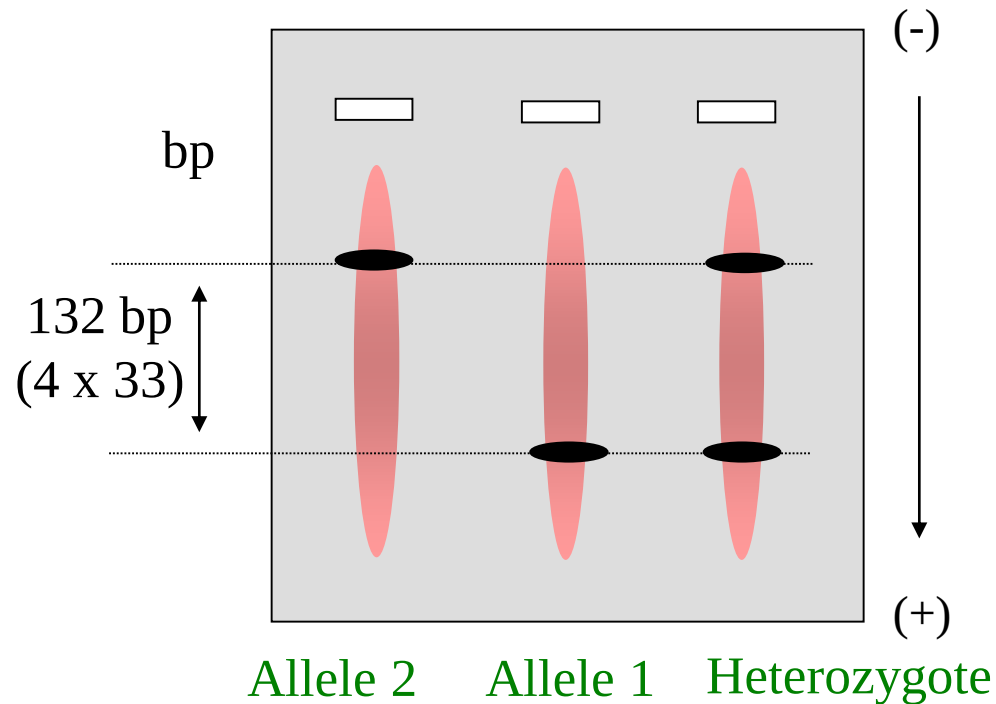
⇒ Run DNA through agarose gel electrophoresis, denature, and blot with DNA probe derived from the repeats

↓
PCR

The sensitivity of the technique has been increased by use of polymerase chain reaction



Mini-satellites analysed by restriction digestion and Southern Blotting (probe based on the repeated sequence)

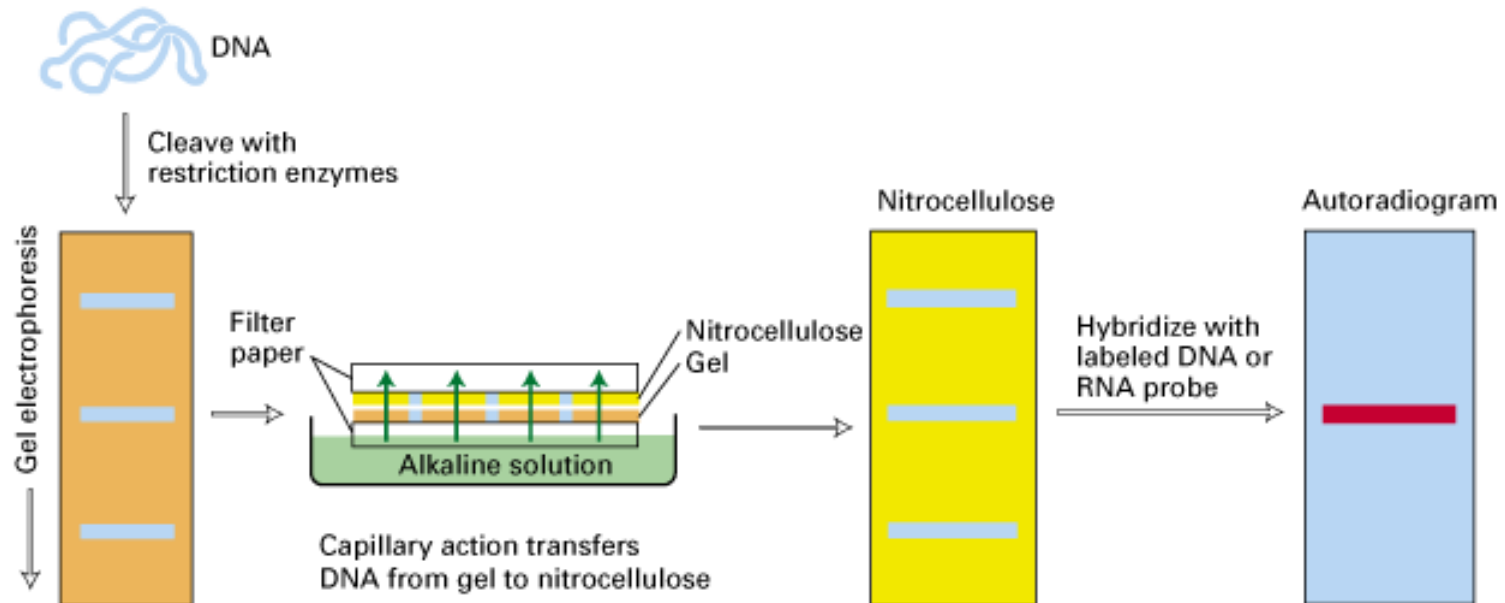


Minisatellites

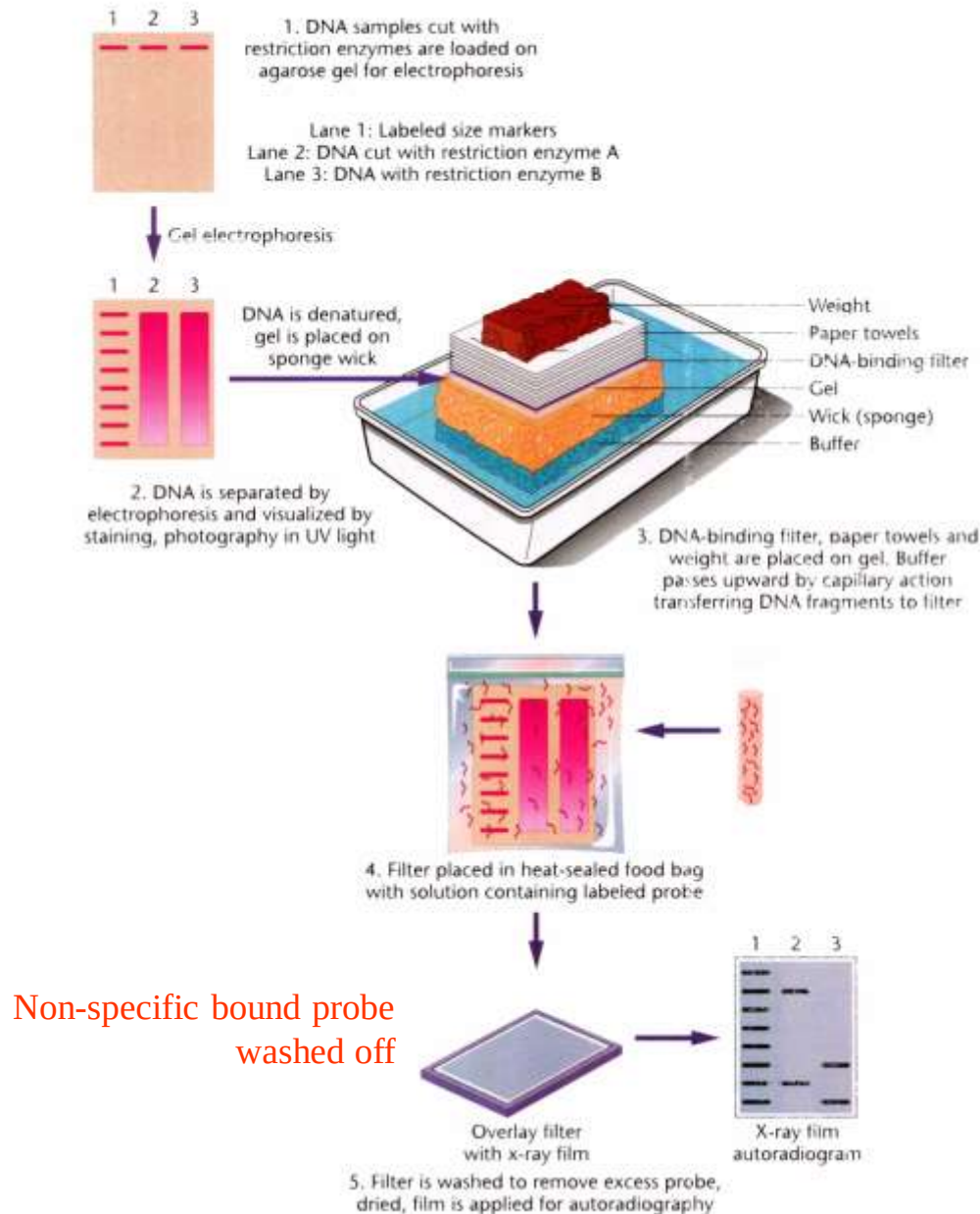
Genetic fingerprinting by hybridization probing

Southern blot

- Developed by E. M. Southern
- Southern blotting detects specific DNA fragments (can identify specific restriction fragments in a complex mixture of fragments)
- To prepare a fingerprint, a sample of DNA is digested with a restriction endonuclease, the fragments separated by agarose gel electrophoresis, and a Southern blot prepared



Southern Blotting Technique



- Hybridization to the blot of a labeled probe containing the repeat sequence reveals a series of bands, each one representing a restriction fragment that contains the repeat .
- Because the insertion sites of the repeat sequence are variable, the same procedure carried out with a DNA sample from a second person will give a different pattern of bands.
- These are the genetic fingerprints for those individuals.

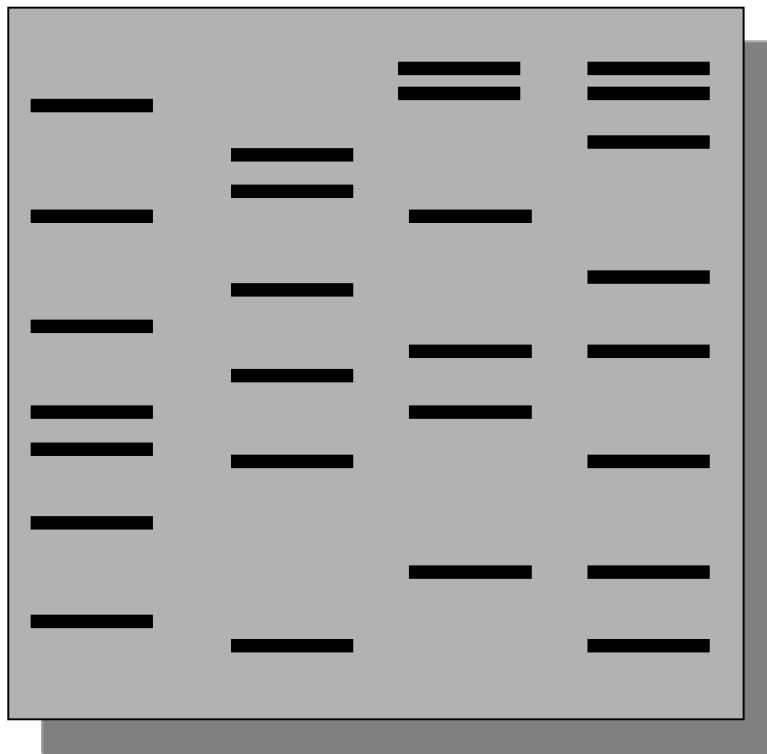
Why Genotyping ?

- Research in Molecular Genetics
 - Population Genetics
 - Epidemiological Studies
- Gene Mapping Studies
- Paternity identification
- Molecular Diagnosis
- Forensics

Application: Paternity Testing

Father 1? Father 2? Mother Offspring

Direction
of
migration
↓

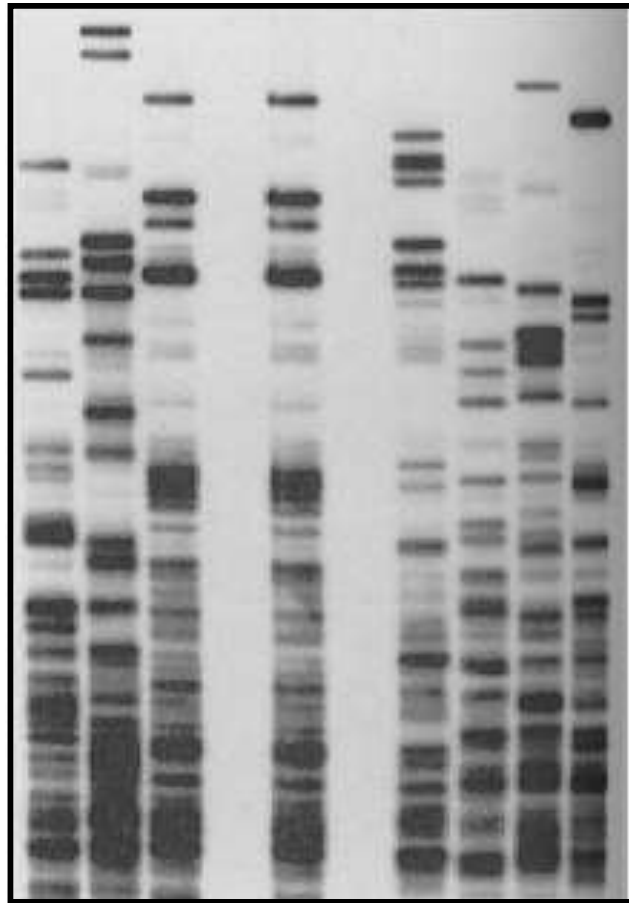


Parentage
Determination

M = Mother
C = Child
F1 = possible
father 1
F2 = possible
father 2



Forensic Analysis



Who left the bloodstain
at the crime scene ?

SUSPECT 3

Common Types of DNA Variants and Analysis Methods

- Minisatellites-RFLP
- Microsatellites-PCR based
- SNPs- RFLP, PCR based
- Insertions-RFLP, PCR based
- Deletions-RFLP, PCR based

SOURCES

Stryer-Biochemistry

Gene cloning and DNA analysis – T. A. Brown

NCERT