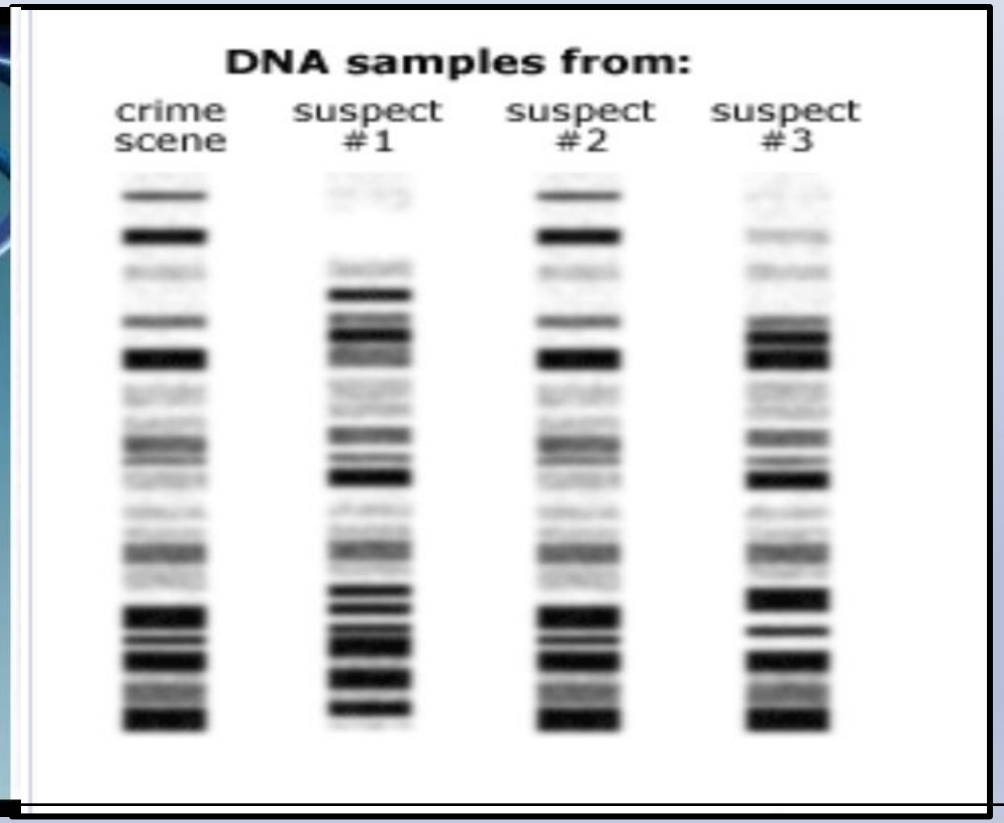
DNA Fingerprinting

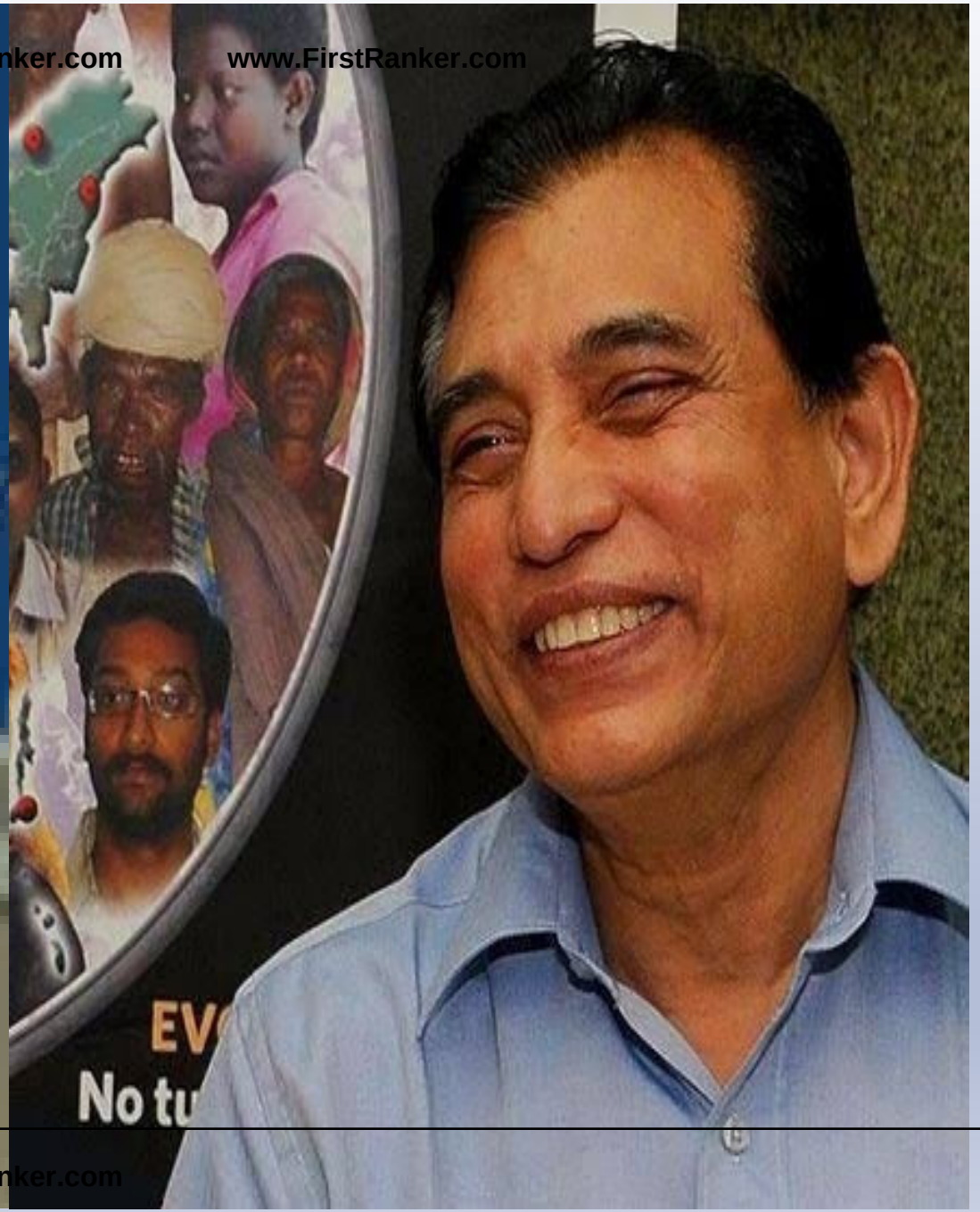


Introduction

- **DNA fingerprinting** is science of identifying a person based on fact that each individual has unique DNA, like unique finger prints. Exception being twins and fact that part of this DNA uniqueness been contributed from parent.
- DNA fingerprinting is known as DNA profiling, genetic fingerprinting, DNA typing.
- It has been successfully used worldwide in solving many crime and paternity cases.
- DNA Barcoding - DNA analysis intended to identify a species, rather than an individual.

Introduction

- DNA fingerprinting discovered by Professor Alec Jeffreys at Leicester University in1984 discovery by while studying genetic variations in myoglobin.
- In India DNA fingerprinting was started by Dr. V K Kashyap and Dr. Lalji Singh.
- Dr. Lalji Singh: the "Father of Indian DNA fingerprinting"



- Colin Pitchfork was the first criminal caught based on DNA fingerprinting evidence.

- He was arrested in 1986 for the rape and murder of two girls and was sentenced in 1988.

Character of DNA

- DNA can tolerate wide range of temperature, pH and other factors.
- DNA can be denatured easily but denaturing doesn't change its property-specificity(length/ weight), VNTR`s which is used in DNA fingerprinting.
- This property can be altered if target DNA`s length is altered by DNase, bacteria, fungi, UV rays.
- DNA mixed with detergents, oil, gasoline and other adulterants does loose its typing characteristics

Various Methods for DNA fingerprinting

- Restriction Fragment Length Polymorphism (RFLP)
- Amplified Fragment Length Polymorphism (AFLP)
- Random Amplification Polymorphic DNA
- Single Sequence Repeats
- Inter Simple Sequence Repeats
- *Ligase chain reaction:* Method of DNA amplification

Various Methods for DNA fingerprinting

- *DNA sequencing:* Biochemical methods for determining the order of the nucleotide bases in a DNA oligonucleotide
- Polymerase chain reaction(PCR).- Mainly used for amplification of target DNA sequence (when test sample has small quantity of DNA).
- Most commonly used are- RFLP & PCR

RFLP vs PCR

Features	RFLP	PCR
Amount of DNA sample required	Large (300-500 ng), e.g. Collecting sample for Paternity test	Small (25 ng) (because small quantity been amplified). e.g. limited sample from crime scene
DNA degradation	Useless when degradation is present (because small quantity of target DNA left)	Useful (because small quantity of target DNA been amplified)
Time required	More	Less

RFLP vs PCR		
Features	RFLP	PCR
Decomposed sample	Not useful (because small quantity of target DNA left)	Useful (because small quantity of target DNA been amplified)
Sensitivity	Less	More(because small quantity been amplified)
Tedious	More	Less
Labor intensive	More	Less
Sensitivity to contamination	Less sensitive	More sensitive
Result of the test	Non-discrete	Discrete (binary ‘yes/no’)

Specimen collection

❖ Samples Collected from Living Subjects:-

I. Blood (most common, preferred)- .

- ➢ 5ml venous blood in EDTA Tube
- ➢ mixed thoroughly without shaking
- ➢ Heparin not to be used as it interferes PCR
- ➢ Sample to be preserved at 2-8ºC (not frozen)

Specimen collection

❖ **Samples Collected from Living Subjects:-**

II. Buccal epithelial cells by sterile swabs:-

- easy to collect,
- swab tip should not be touch by hand,
- fresh swab immediately taken from pack should be stabbed to the subject`s mouth for 10 second.
- Sampled swab should be air dried before and packed in sealed container

Specimen collection

❖ Samples Collected from Living Subjects:-

III. Hair follicles with roots (plucked hair)-

- 10-20 plucked head hairs with roots,
- roots contain keratinocytes which contain nuclear DNA,
- Shaft may be source of mitochondrial DNA.
- Cut or naturally fall hair should be avoided, but may contain nucleated corneocyte (keratinocyte in last stage of differentiation) may be present

Specimen collection

❖ Samples Collected from Dead Bodies:-

- Postmortem material is inferior to live blood and tissue for DNA testing as DNA progressive undergo degradation by autolytic and bacterial enzymes.
- In relatively fresh dead bodies- unclotted 10 ml of blood in EDTA sterile tube is used. Buffers present along with EDTA inhibit the activity of nucleases so it is added advantage.

Specimen collection

❖ Samples Collected from Dead Bodies:-

- Clotted blood is not a good source of DNA as WBC settled down
- In intermediate postmortem intervals- Brain tissue is a good source of DNA.
- If decomposition is establishing- muscle, spleen, femur bone marrow and teeth (morals) are preferred.
- In advanced decomposition- Hard tissue (bone and vascular pulp of teeth) is the best source of DNA.

Specimen collection

- **From traces of organic matter/ DNA:-** Sample from these source are so little that often PCR is required before fingerprinting e.g.-
  - Blood stain on cloth, newspaper, wood or tiles
  - Semen stain on cloth, paper, floor
  - Hair- body/pubic
  - Fingernail scrapings
  - Saliva stain on cigarette buds/ licked envelope

DNA Storage

There are 4 broad strategies for long term preservation of DNA:-

- Room temperature on a dry solid matrix.
- -20°C
- -80°C
- -196°C (in liquid nitrogen)
- Blood and other biological liquid can be stored on FTA card after drying at room temperature

FTA Card

- FTA stands for Fast Technology for Analysis of nucleic acid.
- It chemically treated filter paper designed for the collection, preservation and shipment of biological samples at room temperature.
- Liquid sample containing DNA material made to absorb on FTA card can preserve DNA material long time, claimed over 50 years

FTA Card

- It has weak base, chelating agent, anionic surfactant or detergent and uric acid on cellulose base filter paper.
- Nucleic acid damage from nuclease, oxidation, UV light, microbes, fungus is reduced on FTA card.
- When analyzing nuclear material, a small piece containing sample is punched from card, it is washed and DNA material extracted is use for PCR or RFLP.

FTA Card

- Other benefits:-
  - No risk for spreading infectious agents due to the chemicals included.
  - Rapid isolation of pure DNA.
  - Transportation at room temperature.
  - Reduces potential of cross contamination.
  - Submitting samples via regular post
  - Low transportation costs.

DNA Fingerprinting

❖ **DNA extraction-**

- Before the DNA can be analyzed, it must be extracted from the cells and purified.
- The most common methods of DNA extraction include organic extraction (also called phenol chloroform extraction), Chelex extraction and solid phase extraction.
- Depending on quantity, extraction procedure is chosen.
- RFLP was preferred but if sample (DNA) is less then PCR is preferred

RFLP Principle

- Restriction fragment length polymorphism (RFLP) is a technique that uses variation in homologous DNA sequences(known as polymorphism) in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence.
- The term may refer to a polymorphism itself, as detected through the differing locations of restriction enzyme sites

RFLP Principle

- RFLP analysis was the first DNA profiling technique testing.
- RFLP analysis is basic however slow and cumbersome.
- It requires a large amount of sample DNA combined process of probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing and autoradiography can take up to a month to complete.

Basic technique for the detection of RFLPs

- RFLP is being discussed to understand basic principal-
- Sample DNA is fragmented by restriction enzyme (endonuclease) which breaks DNA at specific sites (where specific sequence is recognized by enzyme) process called as restriction digest.
- The specific DNA fragments thus produced are then separated in sequence (sequence according to their length/weight) on agarose gel by process gel electrophoresis.

Basic technique for the detection of RFLPs

- Sequenced DNA fragment bands on agarose gel are then transferred to a membrane- Southern blot technique
- Now membrane contain sequential band of DNA fragment as on agar gel.
- Membrane is then soaked in solution containing specific labelling probe (designed to hybridize with complementary target DNA fragments- target DNA)

Basic technique for the detection of RFLPs

- After soaking Membrane is then wash.
- If target (complementary) DNA fragment is present on membrane then probe hybridizes(sticks) to membrane and will not wash away,
- If hybridization of probes does not occur then probe will washes away.
- Traces probe hybridized to trace complementary DNA fragment on membrane can be detected by florescent/ enzymatic or radioactive property.

VNTR AND STR

Variable Number of Tandem Repeats (VNTR)

AGTTCGCGTGA AGTTCGCGTGA AGTTCGCGTGA

AGTTCGCGTGA AGTTCGCGTGA

Repeat sequence length: 10-100 base pairs/repeat

Short Tandem Repeats (STR)

ATGCC ATGCC ATGCC ATGCC ATGCC

Repeat sequence length 2-9 base pairs/repeat

Variable number tandem repeats, or VNTRs represent specific locations on a chromosome in which tandem repeats of 9-80 or more bases repeat a different number of times between individuals and also variation in length. Each variant acts as an inherited allele, allowing them to be used for personal or parental identification. VNTRS are readily analyzed using the RFLP approach and a probe specific to a VNTR locus. Their analysis is useful in Genetics and DNA fingerprinting for comparative analysis in forensics.

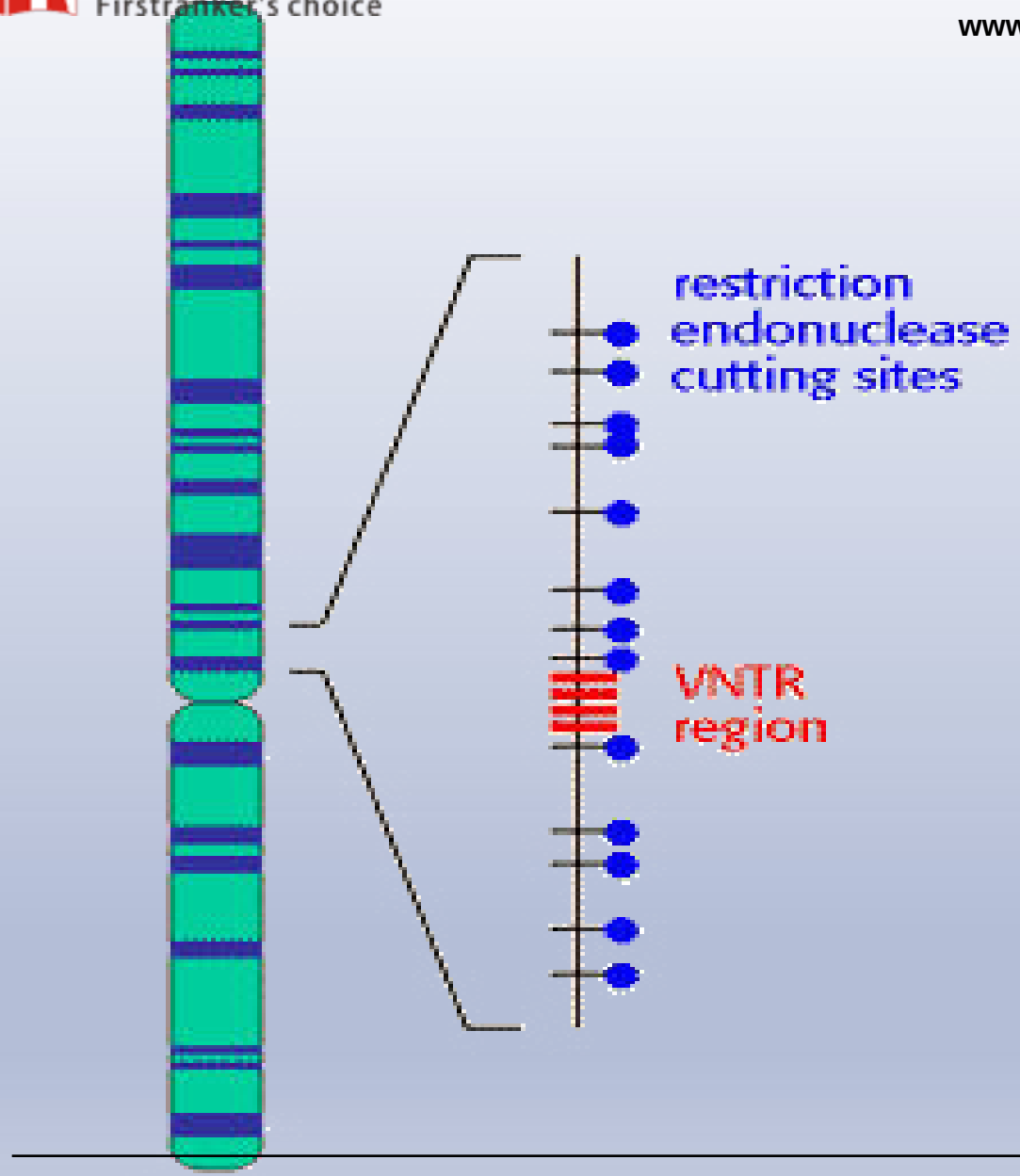
Currently the most popular method of DNA fingerprinting is done by using Short term Repeats (STRs or Microsatelite) which have repeat sequences of only 2-5 base pairs . Since the length of DNA fragment being analyzed is short enough can be amplified by polymerase chain reaction (PCR)

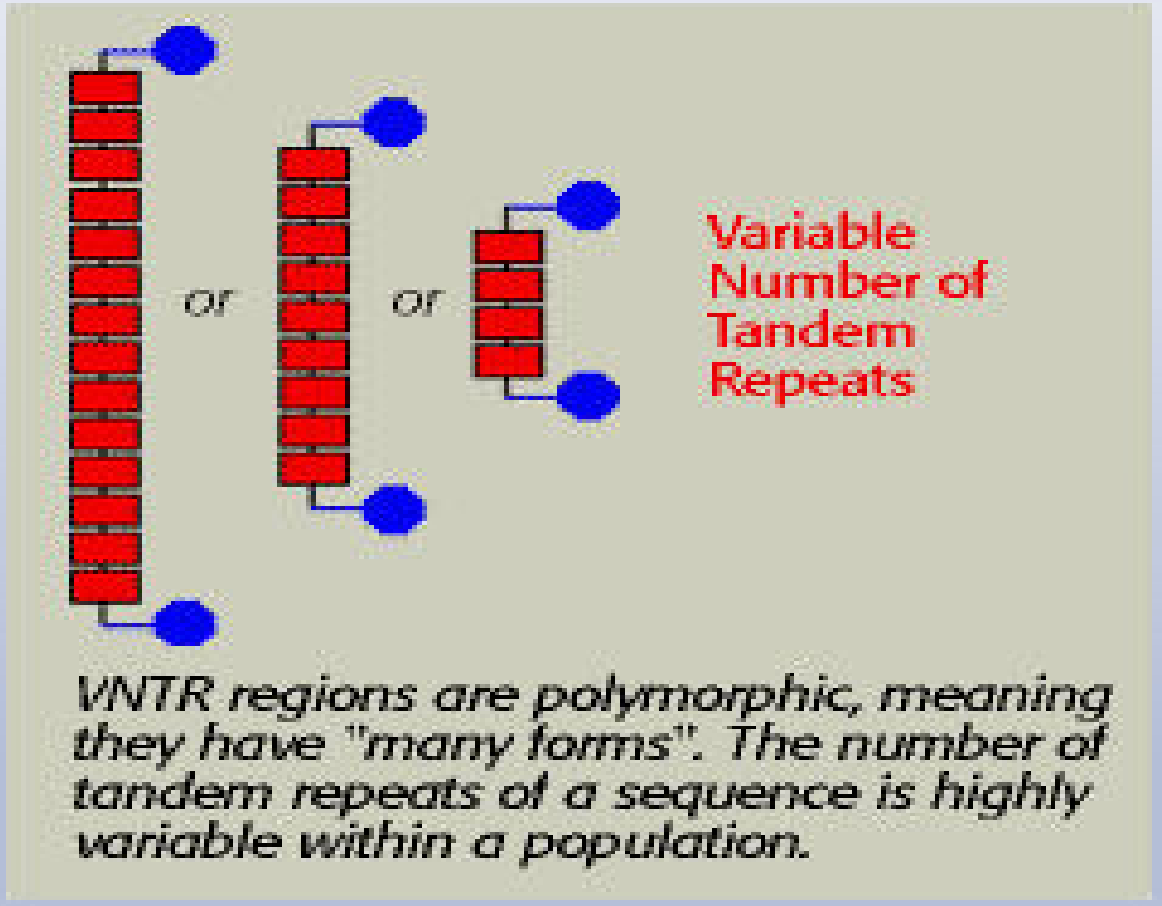
RFLP- VNTR

- A restriction fragment length polymorphism is said to occur when the length of a detected fragment varies between individuals, it indicating non-identical sequence homologies.
- Each fragment length is considered an allele(polymorphism)

VNTR

- In the schematic above, the rectangular blocks represent each of the repeated DNA sequences at a particular VNTR location
- The repeats are in tandem i.e. they are clustered together and oriented in the same direction.
- Flanking regions are segments of non-repetitive sequence (shown here as thin lines), allowing the VNTR blocks to be extracted with restriction enzymes(endo nuclease) and analyzed.



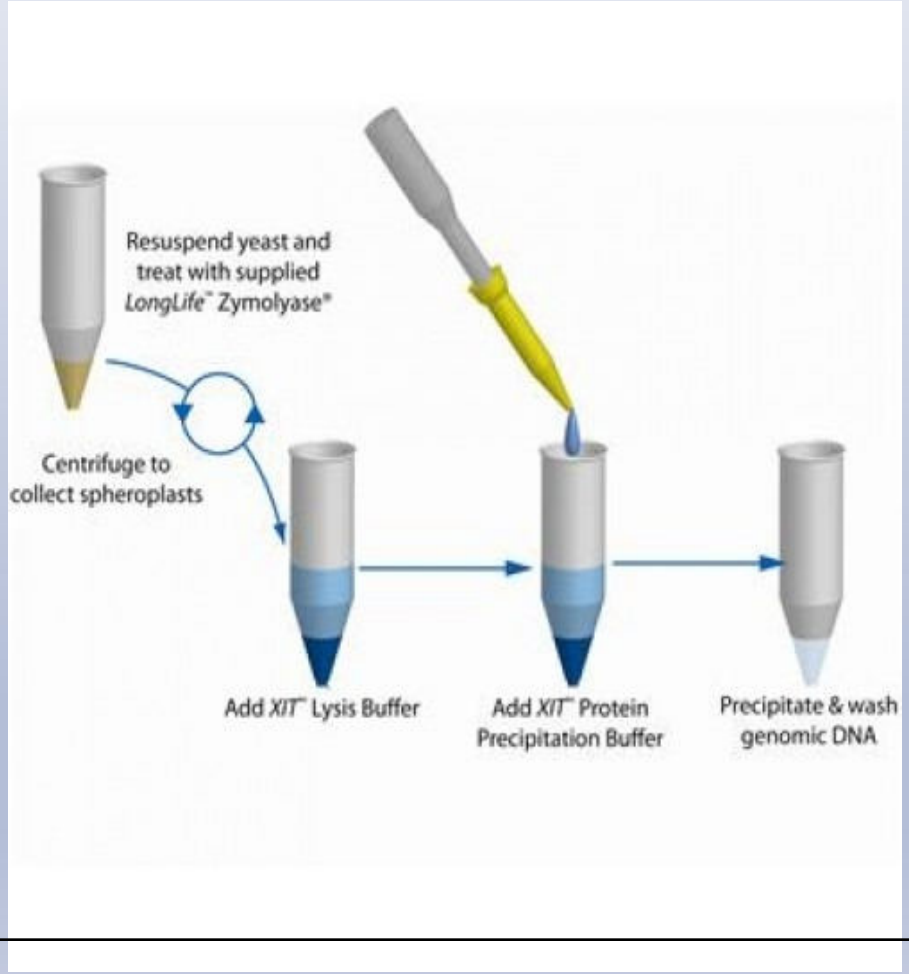


Polymorphism VNTR

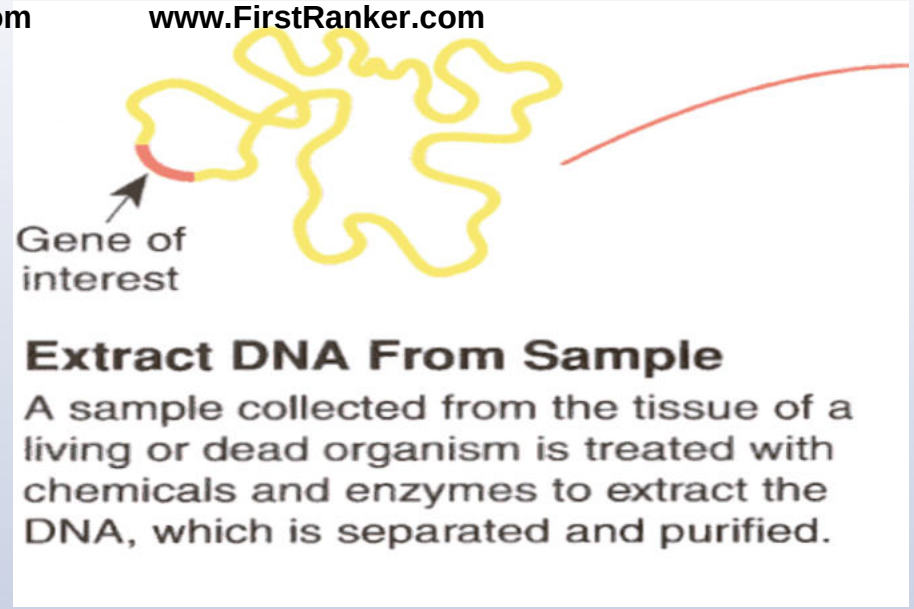
- The exact number and size of fragments produced by a specific restriction enzyme(endonuclease) varies from individual to individual, i.e. they are individualistic in nature and establish nearly 100% identity.

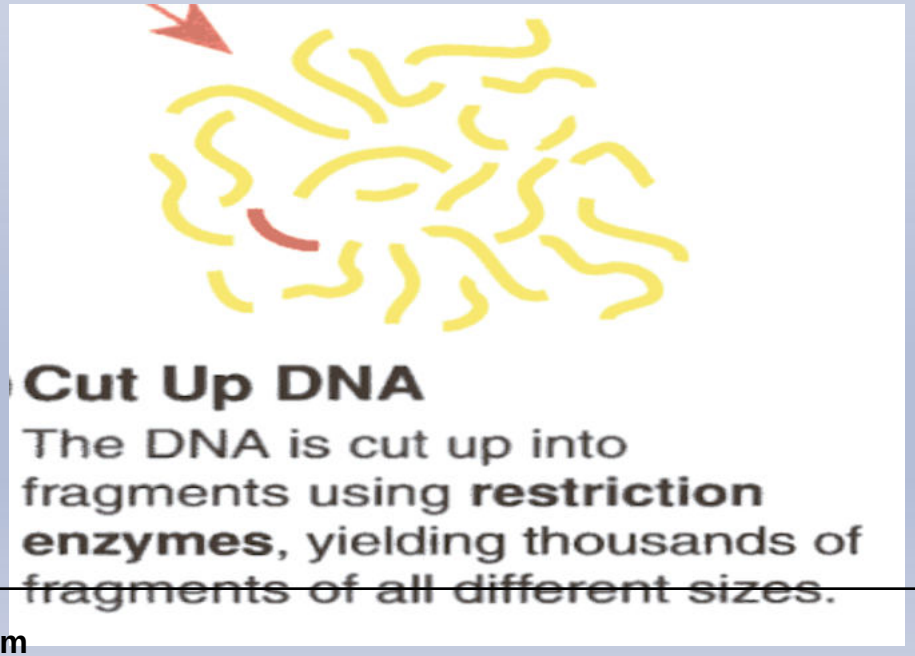
Procedure

I. **Isolation/extraction of DNA:** DNA must be recovered from the cells or tissues of the body. Only a small amount of tissue—blood, hair or skin —is needed. For example, the amount of DNA found at the root of one hair is usually sufficient



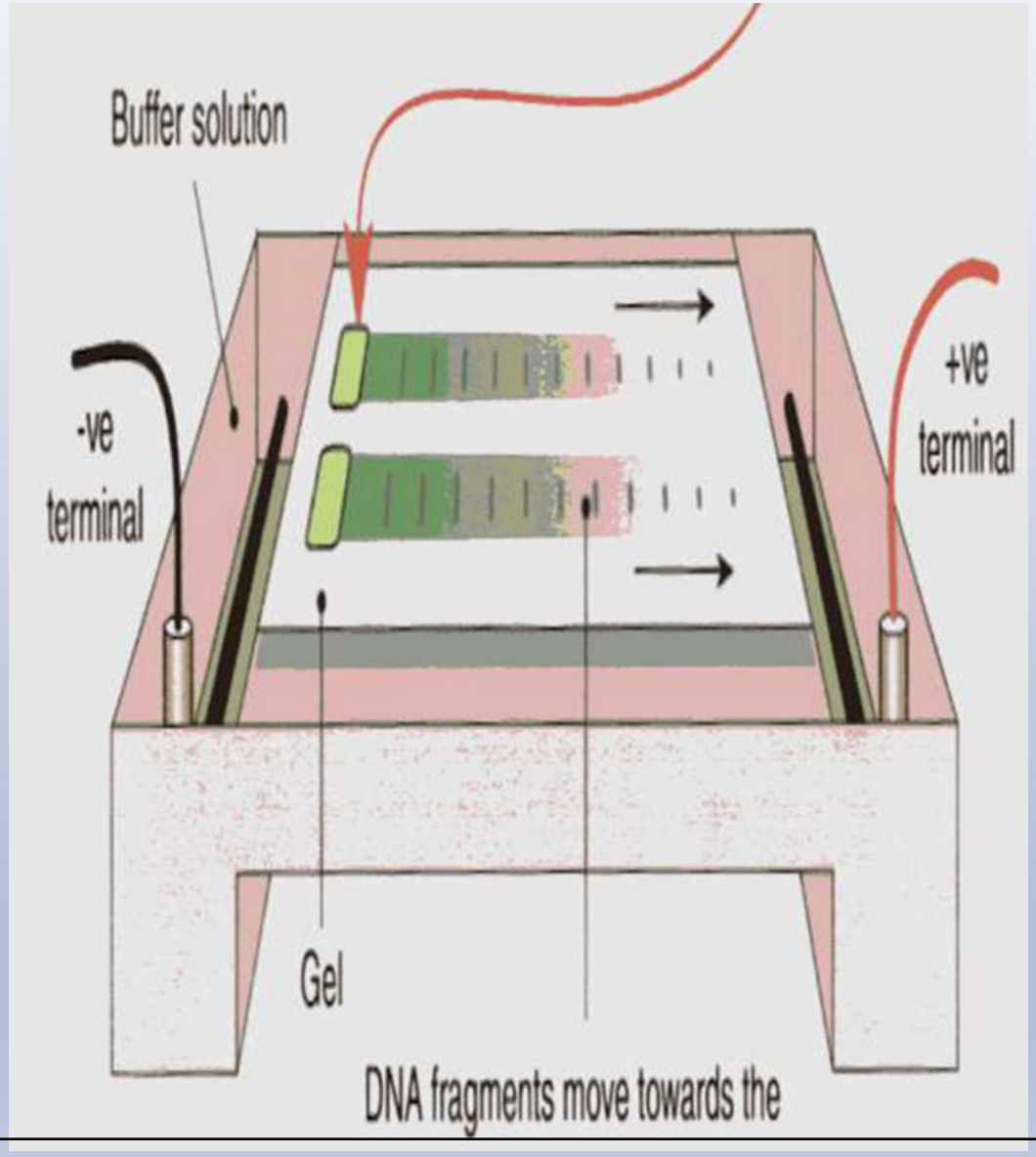
II. **Cutting and sizing:** Special enzymes called *restriction enzymes(endo nuclease)* cut the DNA at specific site. For example, an enzyme EcoR1 cuts DNA only at sequence GAATTC.

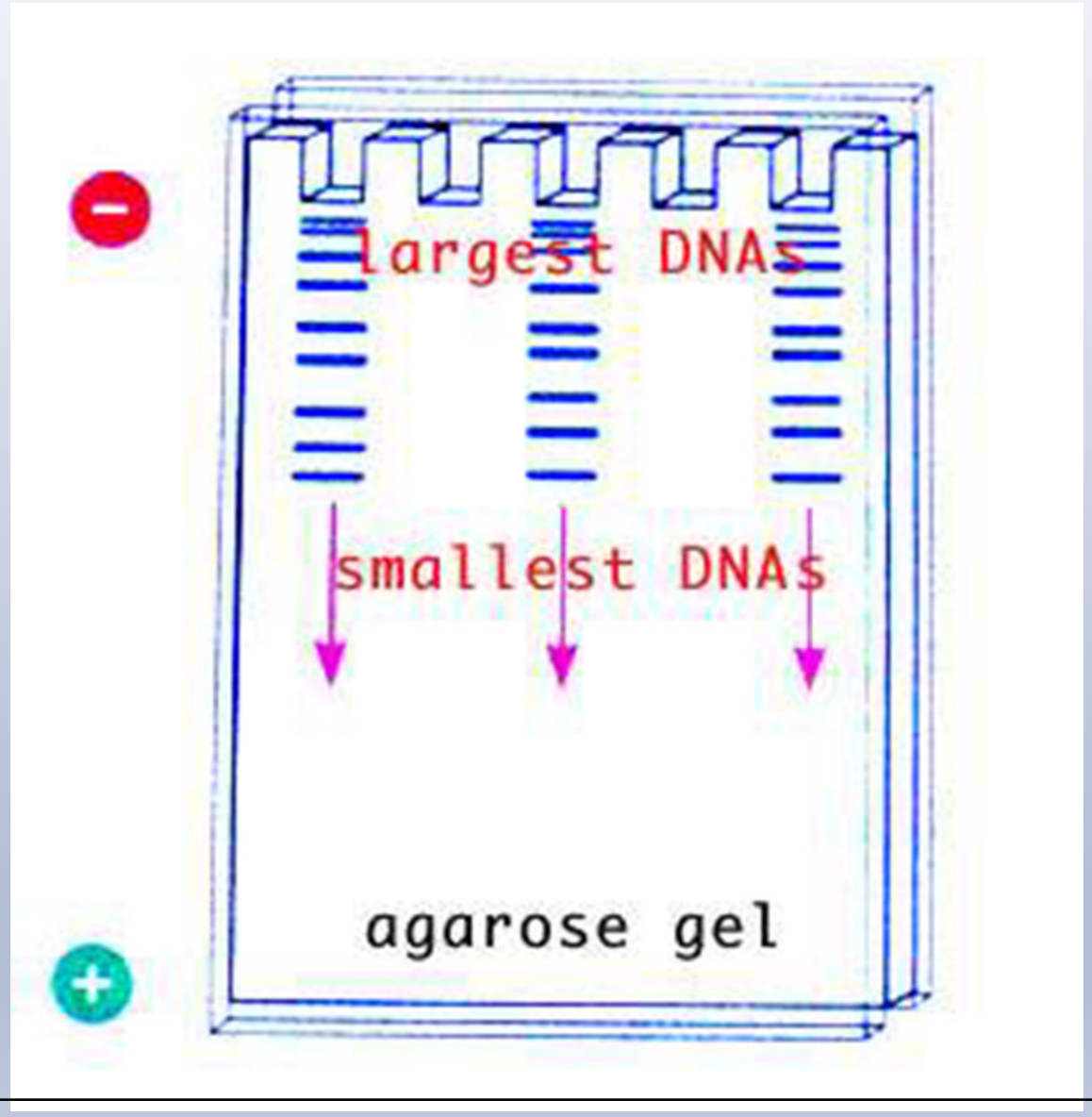




III. Sorting By Gel Electrophoresis

- Sorting VNTR fragments are separated on the basis of size using a process called gel electrophoresis.
- DNA fragments are injected into wells and an electric current is applied along the gel.
- DNA is negatively charged so it is attracted to the positive end of the gel.
- The shorter DNA fragments move faster than the longer fragments. Thus DNA is separated on basis of size.

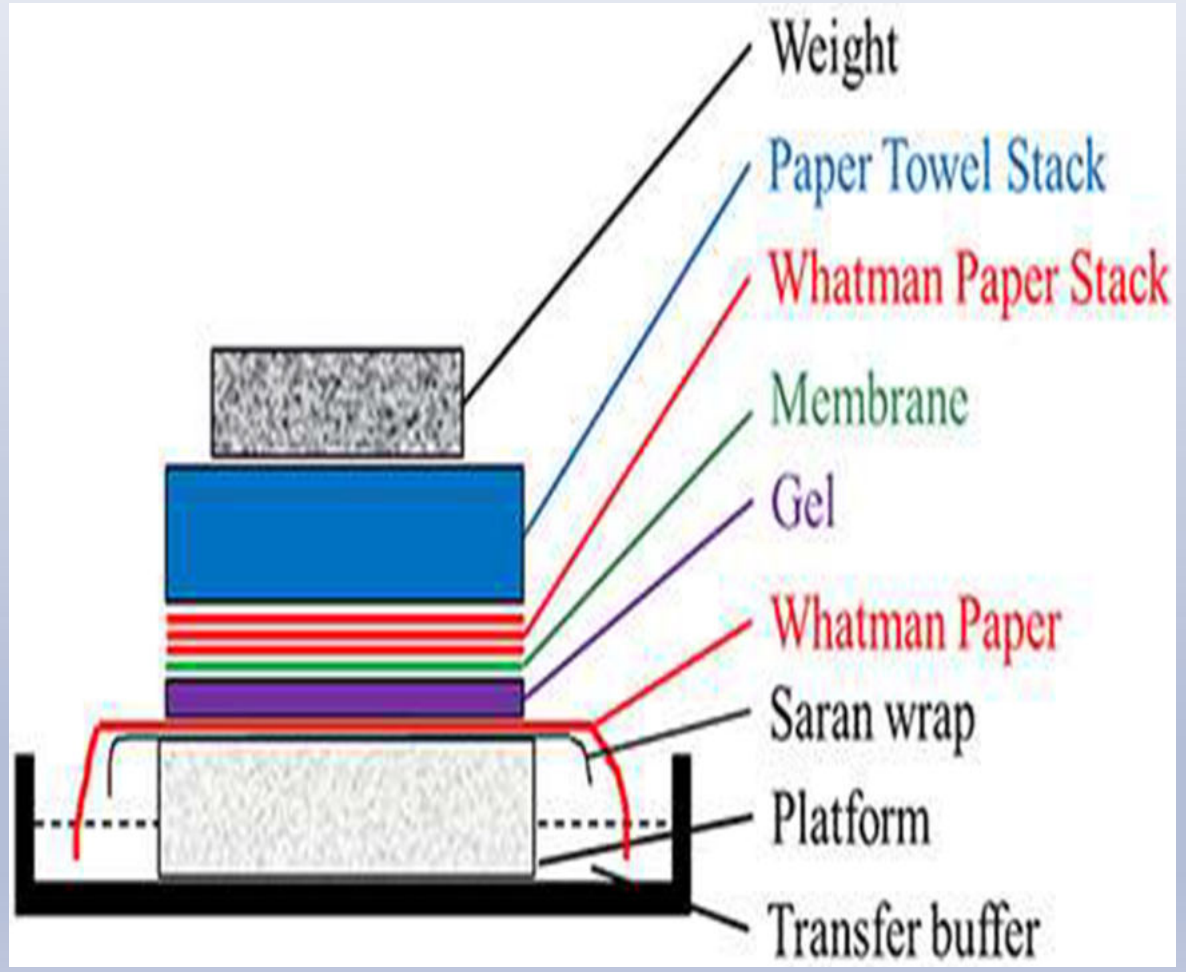


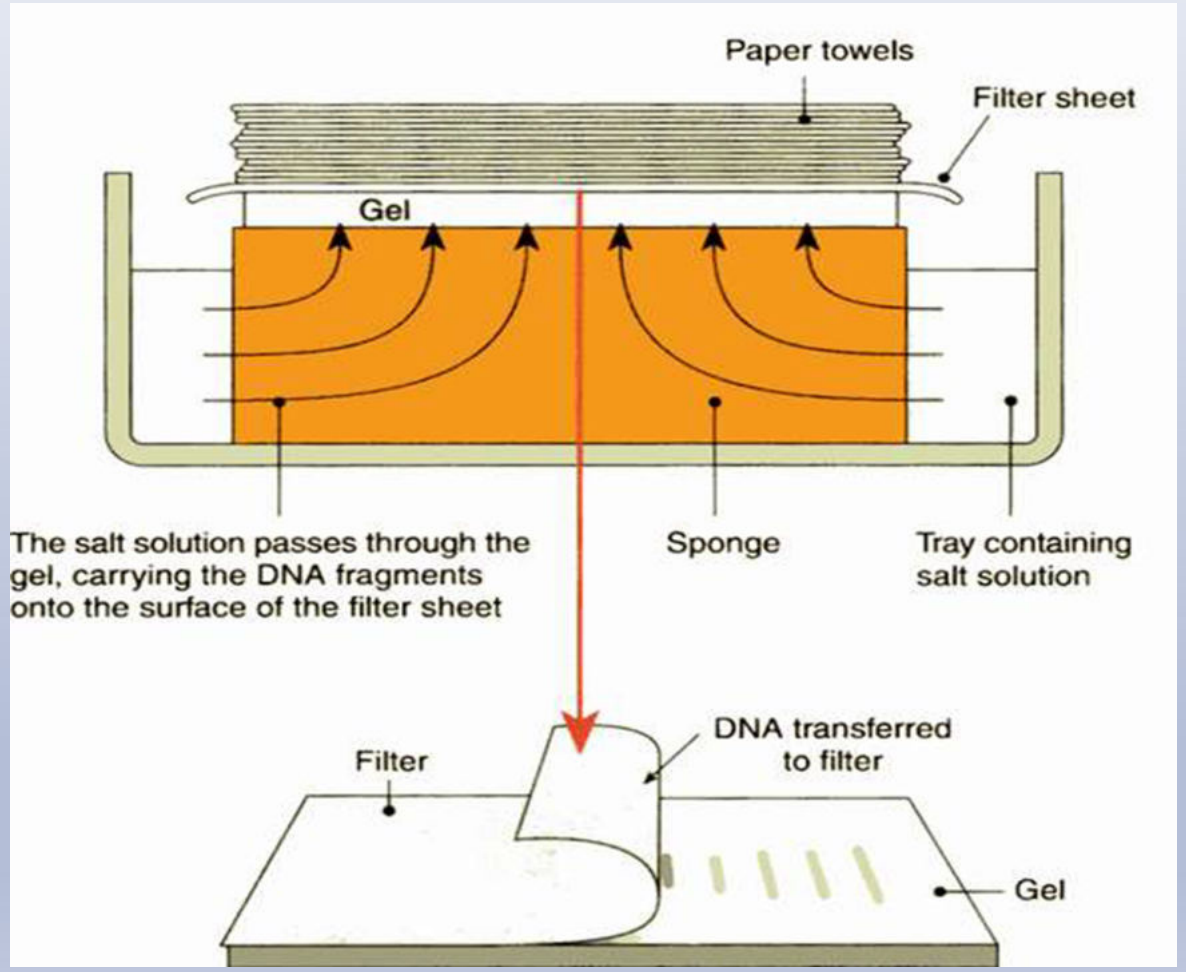


IV. Transfer of DNA to nylon (Southern blotting)

- Specific DNA can be identify if it can be labelled / tagged/ hybridize(with a complimentary genetic probe.)
- It is not possible in gel, so DNA is denatured and then transferred to nitrocellulose or nylon membrane, which picks DNA like a blotter picks up ink by capillary action, it is then fixed by heating
- The resulting blot formed is essentially a replica of the gel. This is called *Southern blot* named after Dr Edward Southern

Southern blot

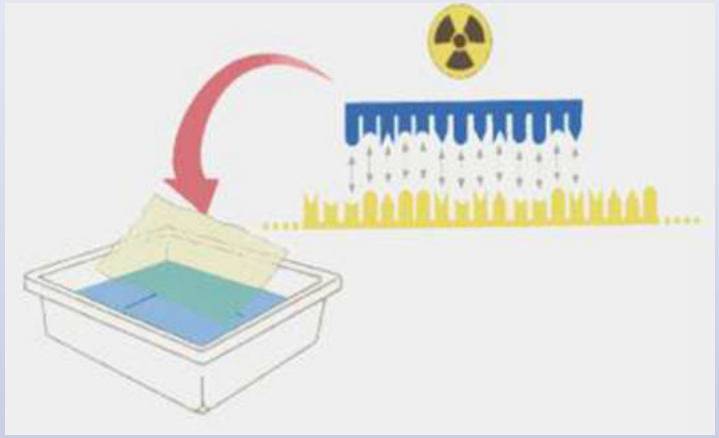


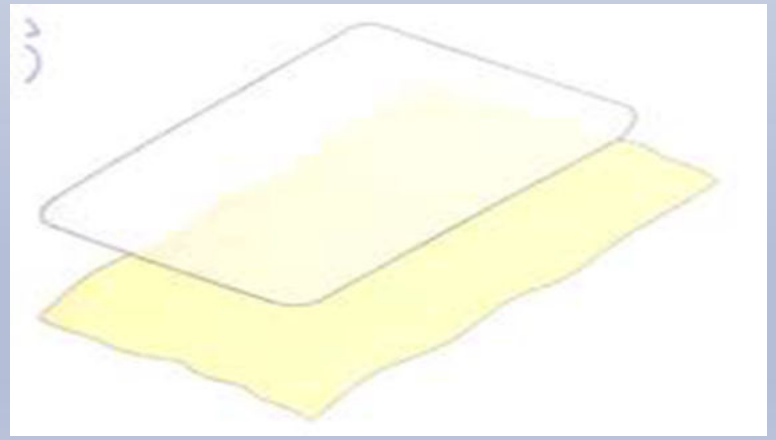


V. Hybridization

- Adding known radioactive DNA probes (short sequence probe, complimentary to the region of DNA which one wishes to detect) to the nylon sheet leads to fragment location.
- The nylon membrane is immersed in a solution that contains DNA probe impregnated with radioactive P32. Each probe typically sticks in only one or two specific/complementary sequences on the nylon sheet. This process is termed as *hybridization*

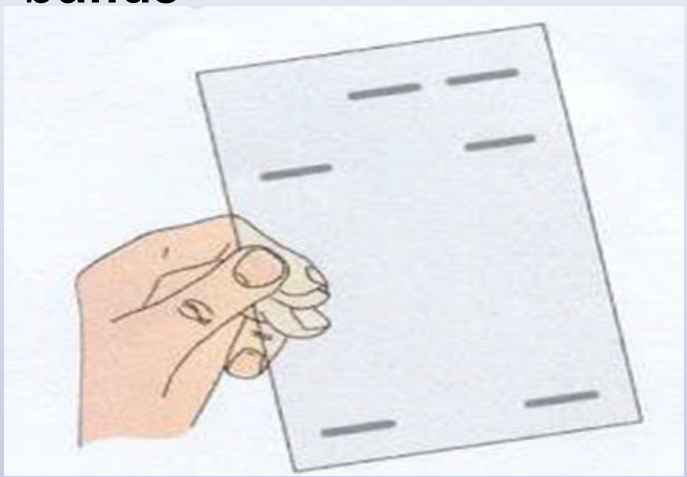
Radioactive probe in solution binds to DNA





X-ray film

Revealing a pattern of bands



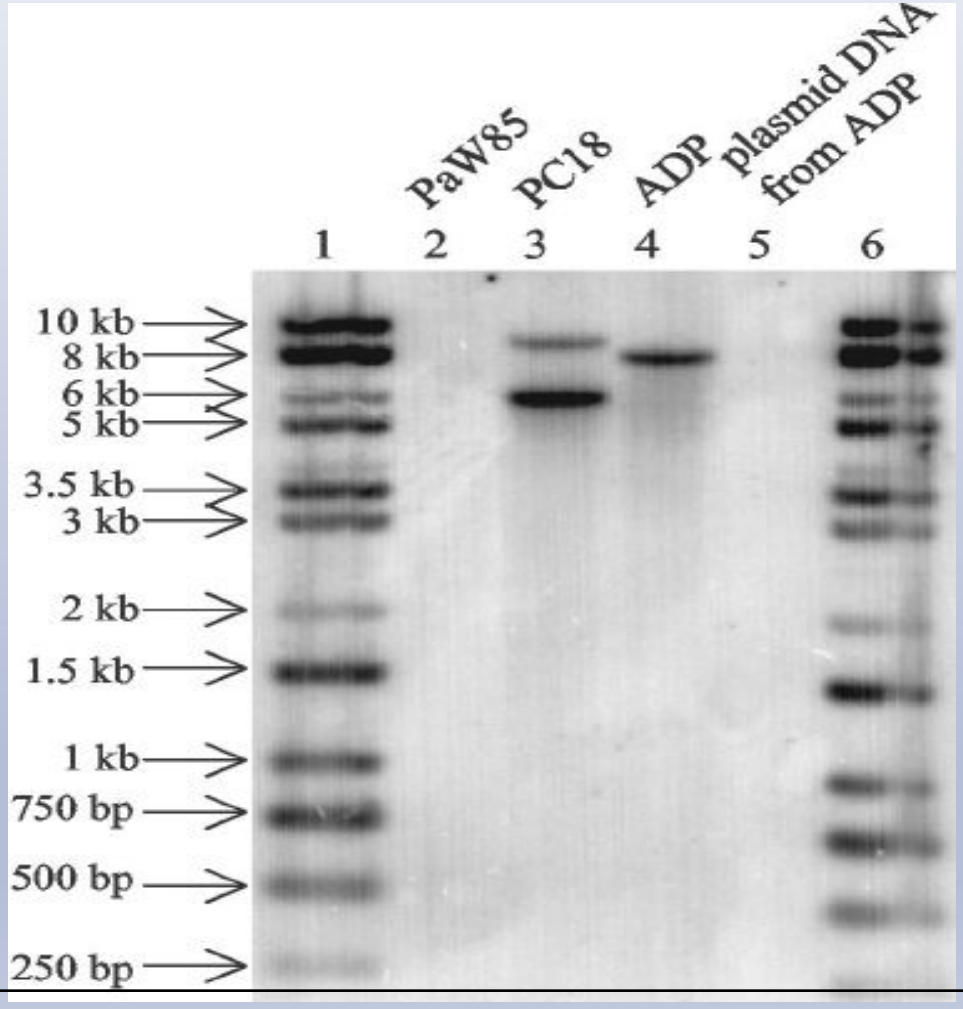
VI. Washing

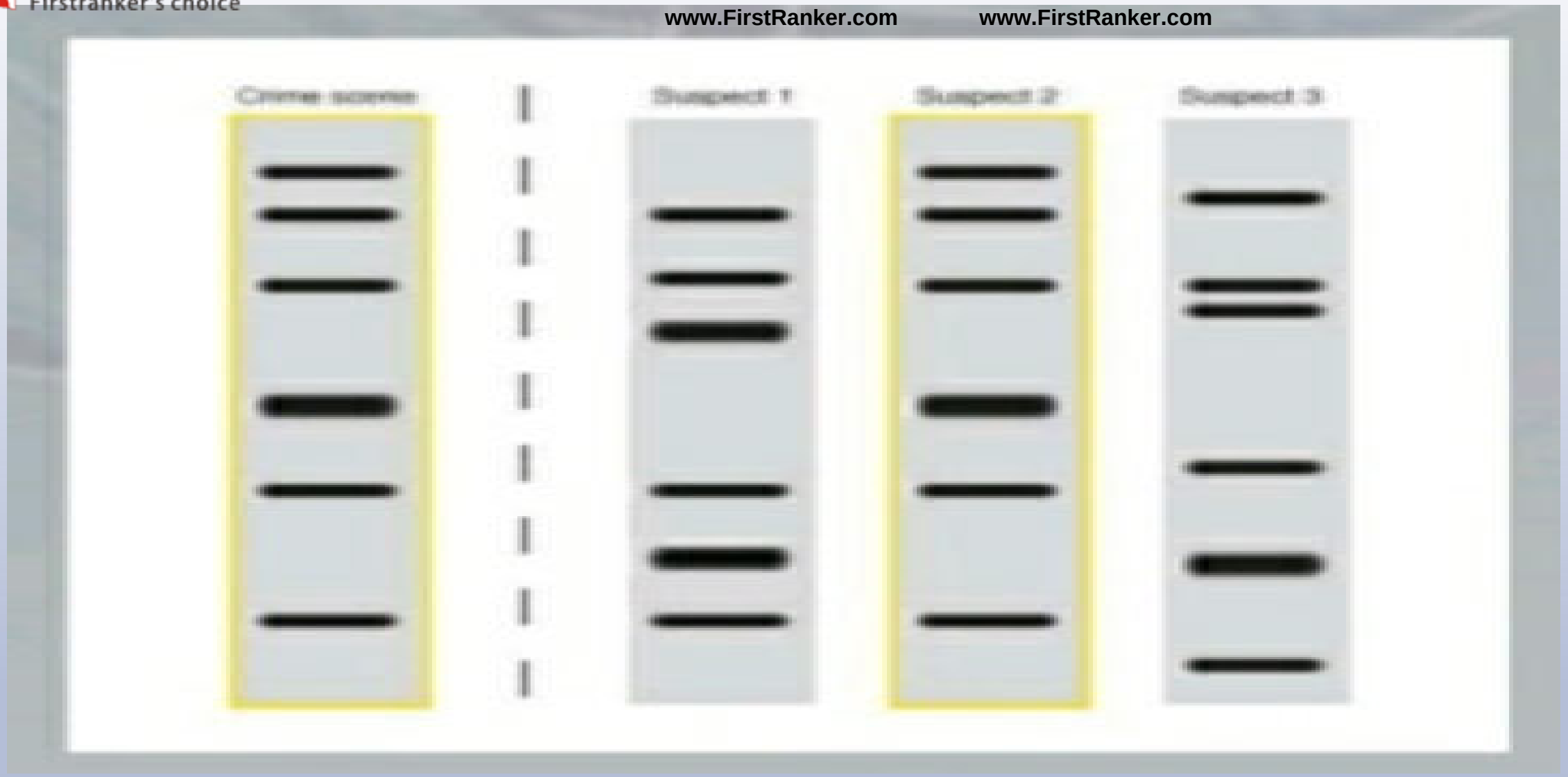
The membrane is washed to remove excess or unbound probe and exposed to an X-ray film.

- Spots on the X-ray film correspond to the locations of the fragments in the gel that are complimentary to the probe (*autoradiography*).
- Nowadays, many radioactive probes are detected by chemical luminescence which is analyzed by computer scanners

VII. DNA fingerprint- autoradiograph

The final print is known as an autoradiograph or 'DNA fingerprint' which appears as lines on the film.





Result Matching

- Two-step process:- It determine if two samples arose from one source.
- First- DNA-banding pattern of test sample is compared with known DNA sample visually. If matching not found result is declared, if matching is observed then bands are forwarded for second step.
- Second step – matching verified by computer assisted allele sizing. Match should fall within 2.5% of each other, if not found it should be considered ‘nonmatching.’

Result Matching

- If the DNA-banding pattern of a sample cannot be positively determined due to technical problems, the results should be considered ‘inconclusive Now a days highly polymorphic single locus genes such as the VNTR genes are utilized instead of multilocus DNA analysis
- Due to the large number of distinguishable alleles in most populations, it is possible to establish a ‘*DNA signature*' for almost any individual

Limitations of RFLP

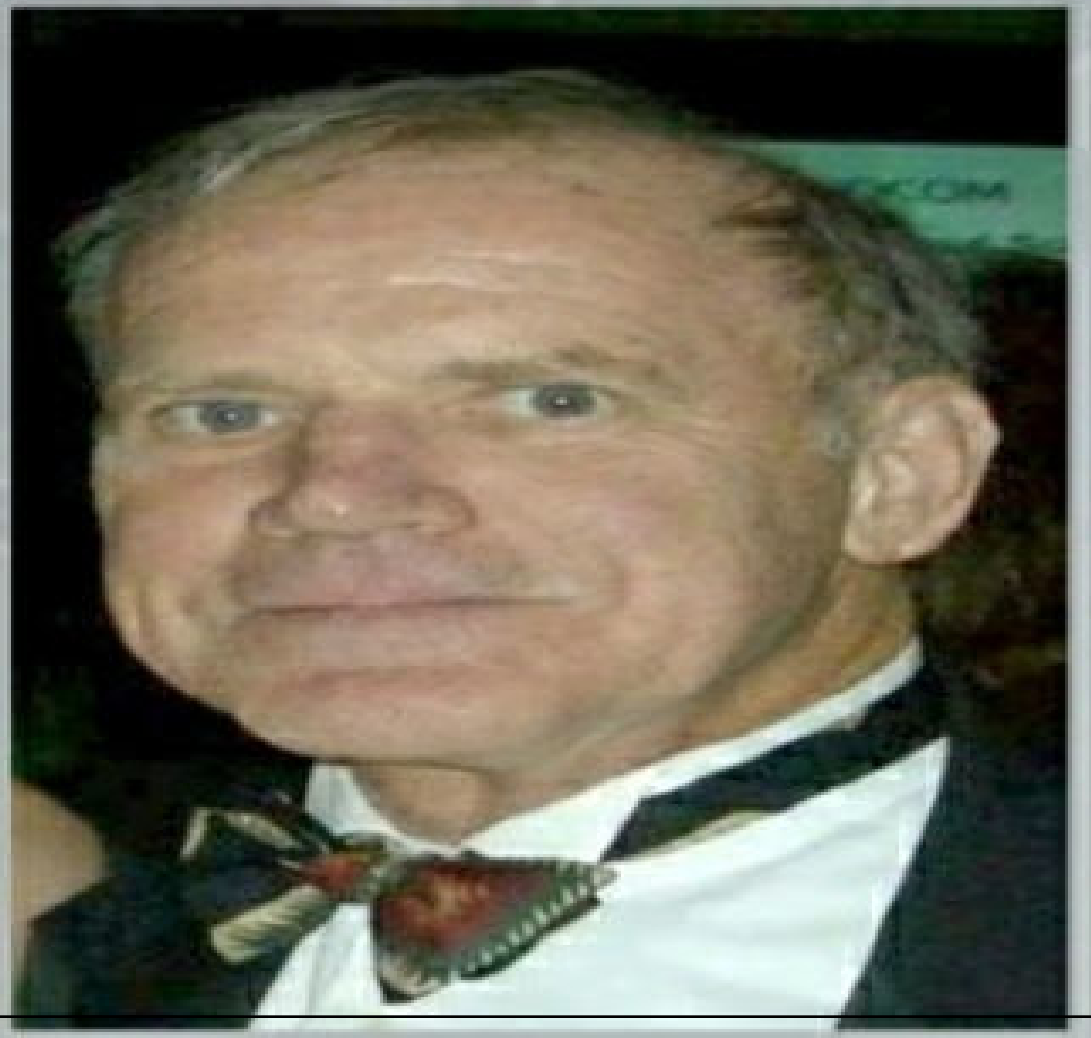
- Slow- It takes few weeks to perform.
- Cumbersome- For every probing, the membrane is stripped off the previous probe and rehybridized and autoradiography performed again.
- Requires a large amount of sample DNA
- It is not useful where DNA is degraded—limits use in cadaveric tissue.

PCR

- PCR is technique for amplifying(increase in number of copies) of particular DNA fragments in sample in vitro, so that it can be readily analyzed.
- PCR itself does not accomplish DNA typing, but increases the amount of DNA(**DNA amplification)** available for typing.
- Developed by Karry Mullis of the Cetus Corporation in 1983.

POLYMERASE CHAIN REACTION

- Dr. Karry Mullis invented this technique in 1983.
- He got noble prize for this famous invention.



Reaction requires:-

- Heat resistant DNA polymerase (Taq polymerase)- enzyme which attaches nucleotides on the growing strand of DNA and thus helps to expand it.
- Template DNA – target DNA segment which is required to be amplified(increase in number of copies).
- DNA Primers – it is short sequences of nucleotides which bind at the end of the template DNA segment. It is specific according to target template DNA

Reaction requires:-

- Deoxynucleoside triphosphates - equal amounts of dATPs, dTTPs, dCTPs, dGTPs
- Other chemical in buffer like Mg, pH control etc.

Process

- Chemicals are mixed in sterile condition in thermal cycler (PCR Machine) and run for certain cycles(20-30).
- A cycle in thermal cycler has 3 stages at three temperatures:-

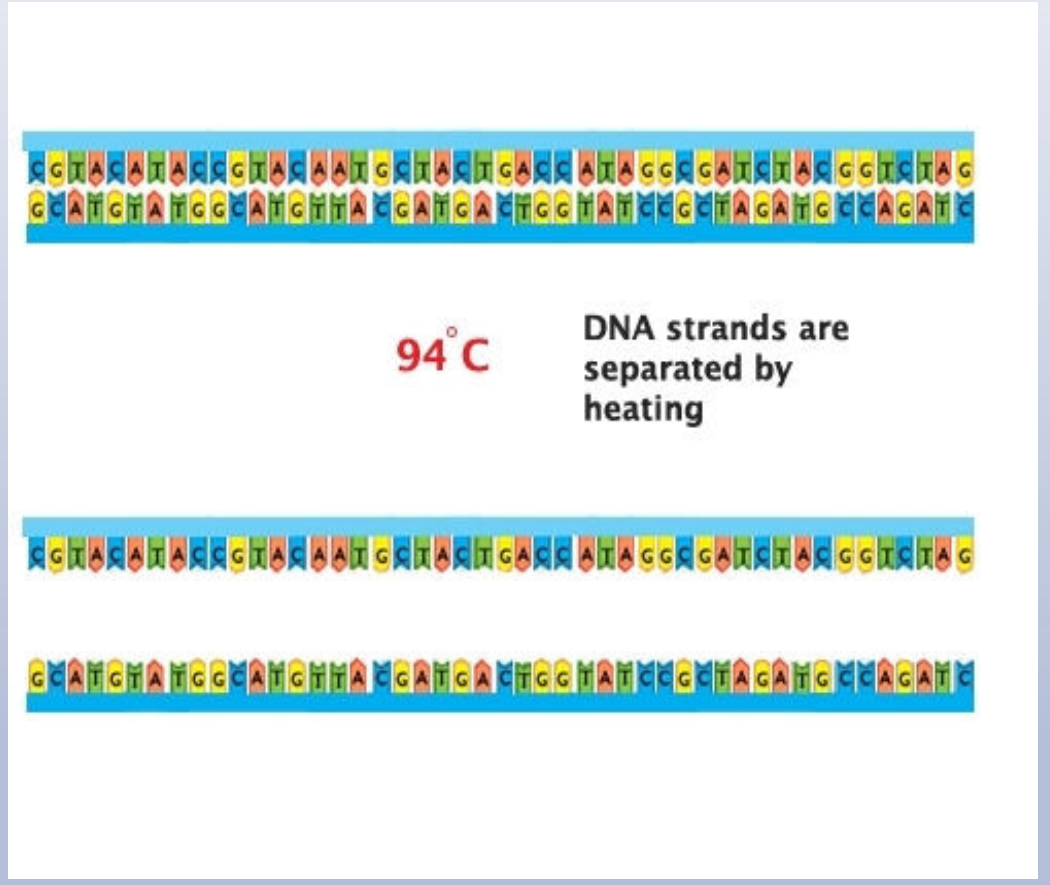
I. **Denaturation:-**Heat samples to **94°C** for a minute or so

II. **Annealing:** cooling **50 or 60°C**

III. **Extension:-** Maintain temperature at **72°C** for a minute or two

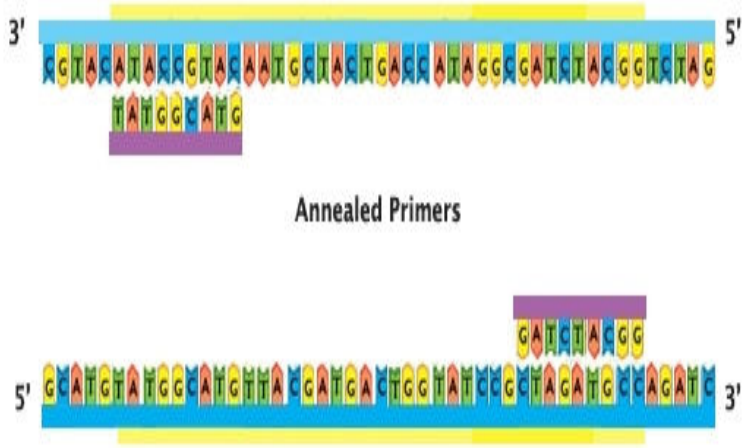
I. Denaturation

- Heating samples to **94°C** for a minute or so - double stranded DNA dissociates to two single strands.



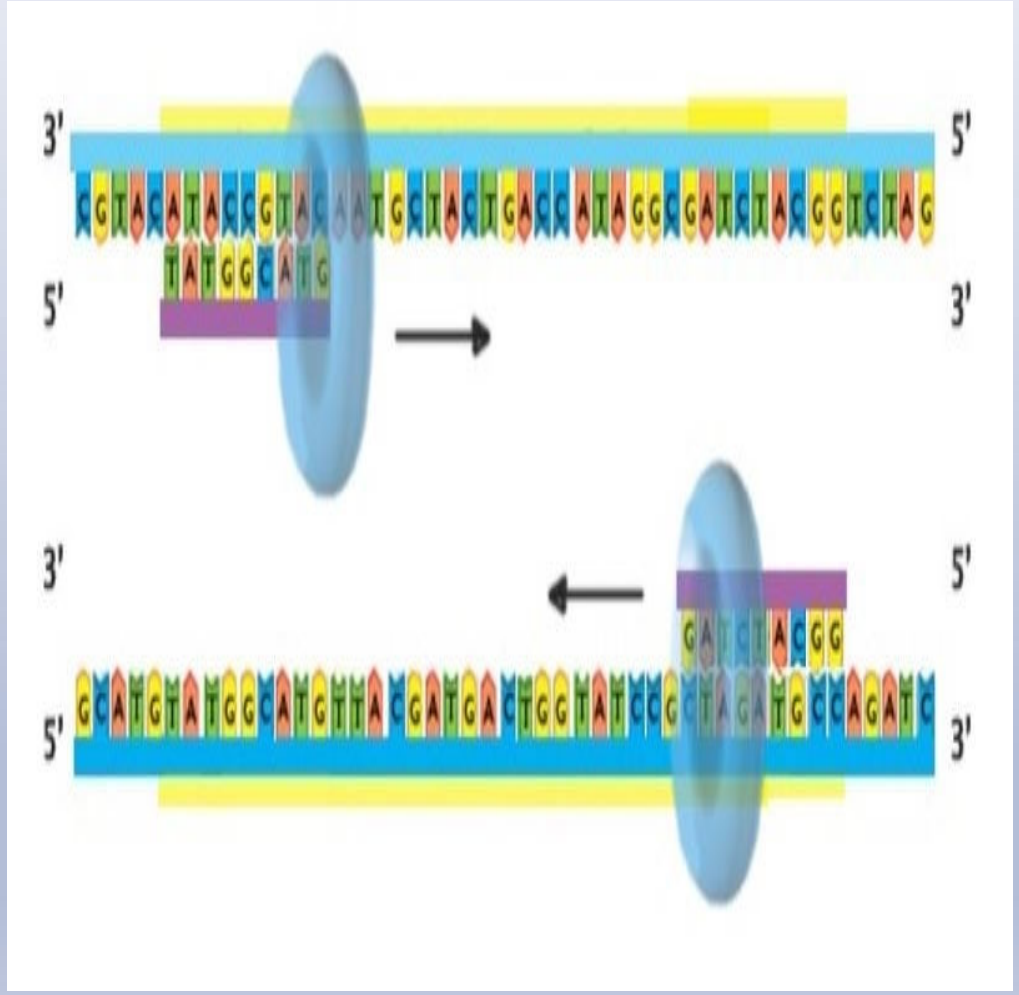
II. Annealing

- Drop temperature to around **50 or 60°C**- it will cause the primers to pair up with the single-stranded template (annealing).
- On the small length of double-stranded DNA (the joined primer and template), the polymerase attaches and starts copying the template.

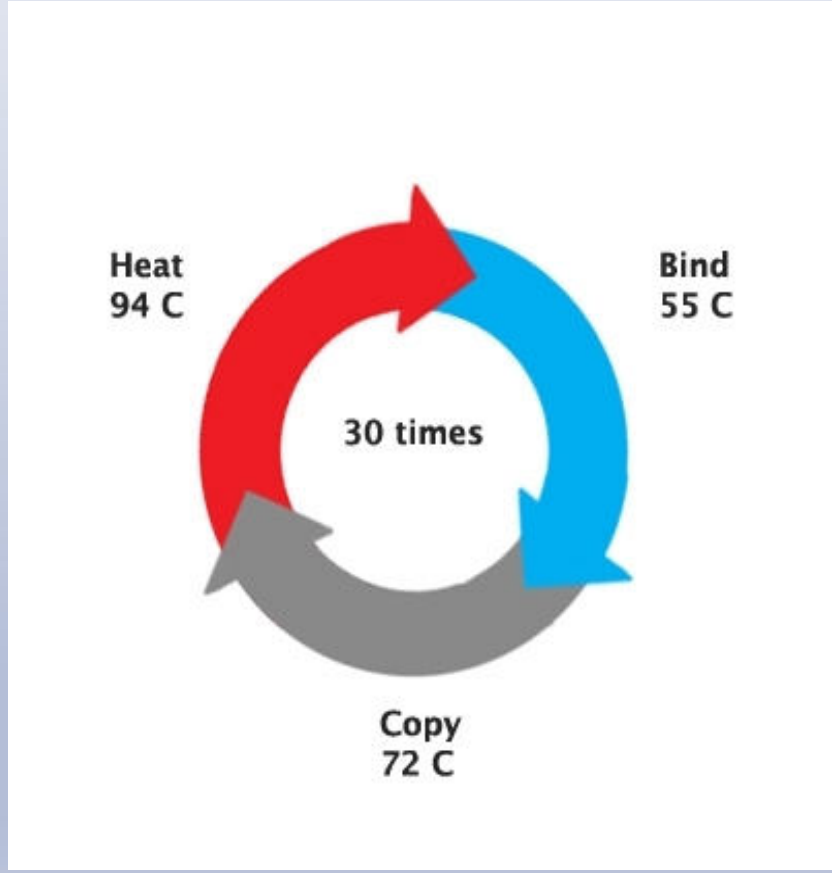


III. Extension/ Elongation

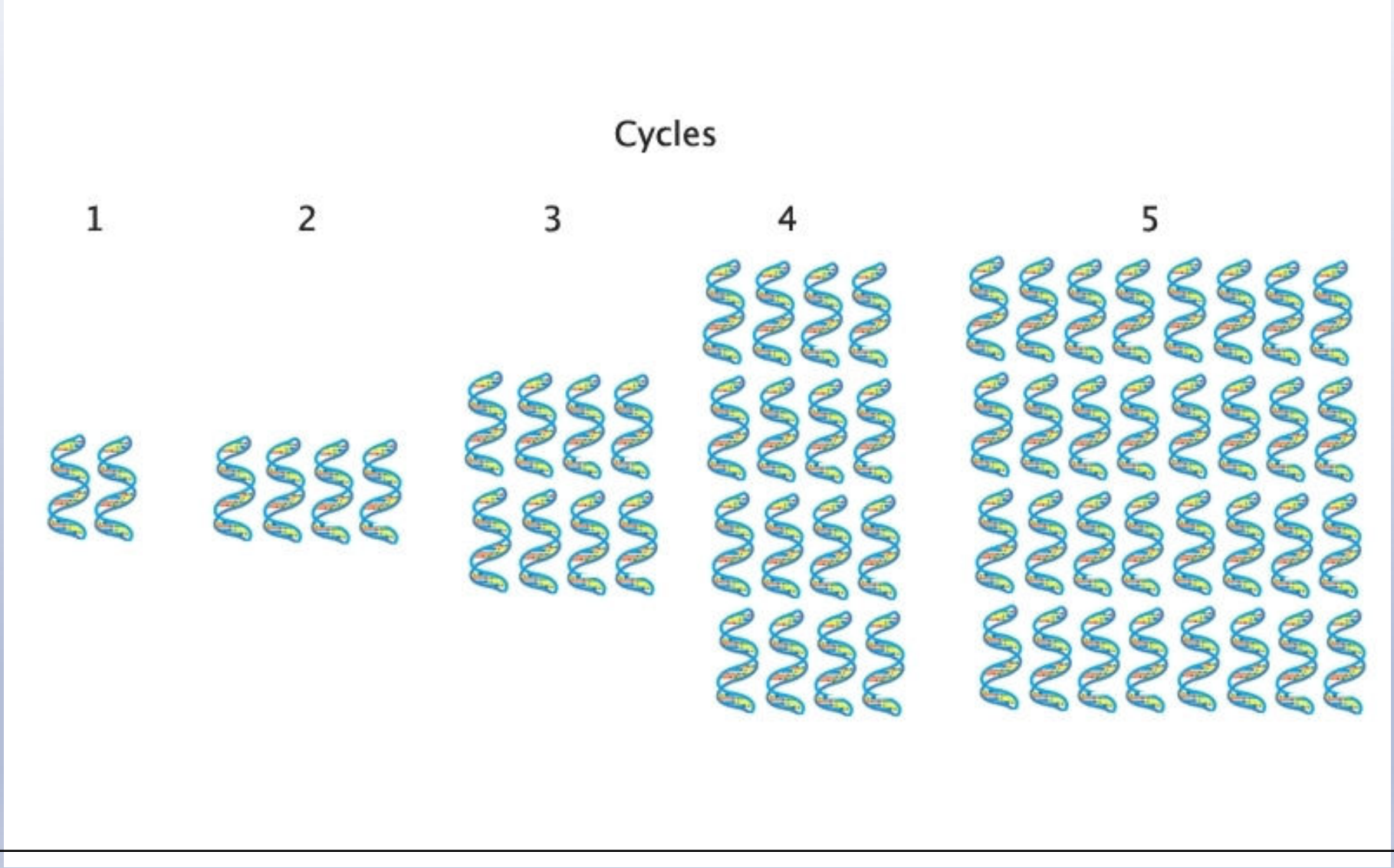
- Maintain temperature at **72°C** for a minute or two- it allow the polymerase to elongate the new DNA strands which are complementary to the template where primers are attached making a double stranded DNA.

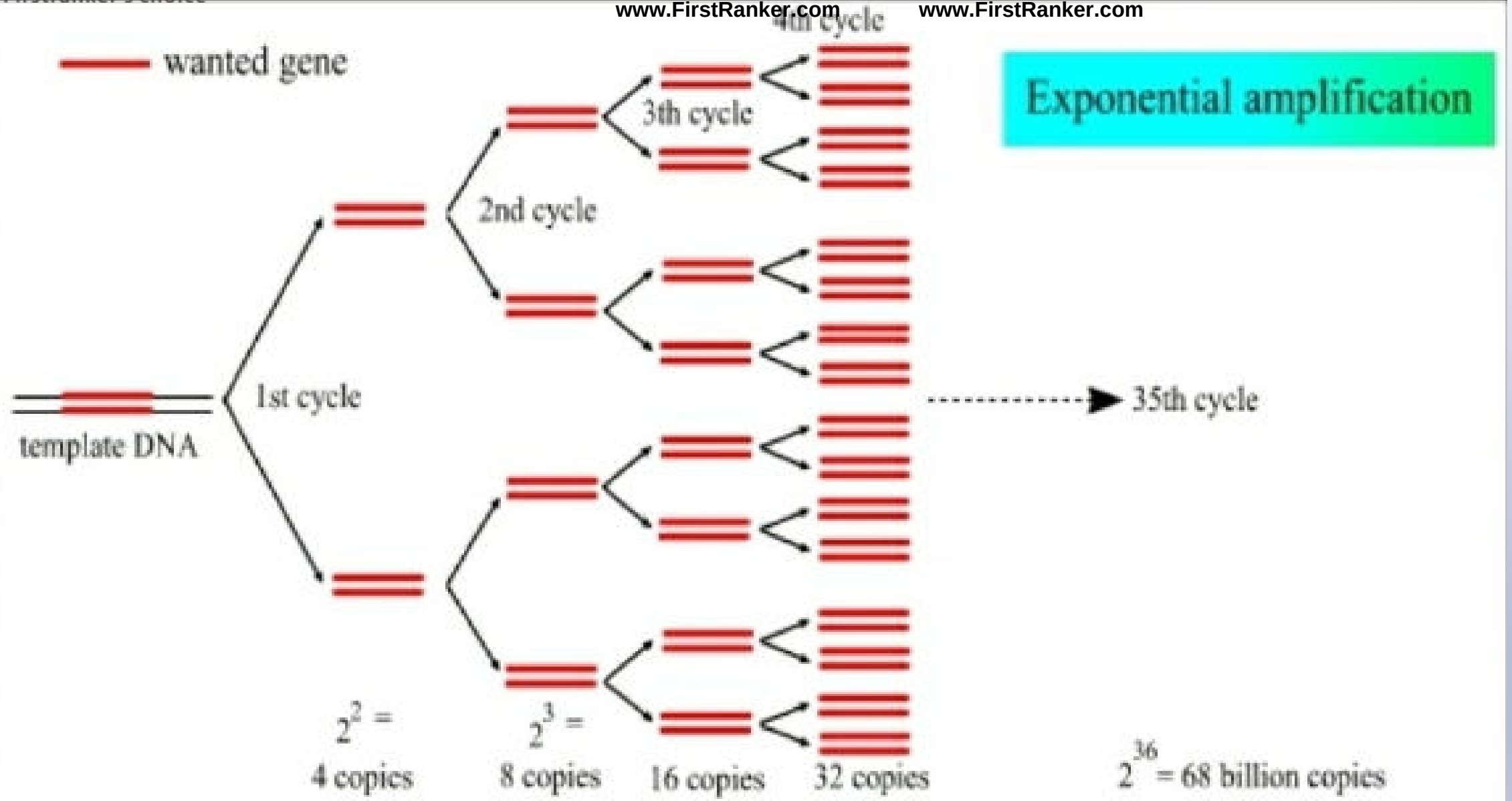


- In thermal cycler the process is completely automated.-The time and temperature can be programmed for repetitive cycles.
- Usually 20-30 cycles runs.



- Each separated strand can serve as a template for synthesis as long as Deoxynucleoside triphosphates and primer is provided for each strand as new primer binding sites are duplicated on each cycle.
- PCR amplification is logarithmic, meaning the number of copies of the target DNA doubled every cycle.
- After 20 cycles, this exponential process yields 2^{20} copies of target sequence





Other amplification based methods

- Dot blots- series of DNA probes to detect and record target such as for HLA DQ, DR at chromosome 6q in pattern of colored dots Amplified fragment length polymorphism (AmpFLP)
- Utilizing mitochondrial DNA.
- STR analysis is less susceptible to DNA degradation than other AmpFLPs. The chance of misidentification in this procedure is one in several billion.

Applications of DNA Fingerprinting

- Establishing identity of person in-
  - Linking suspect to biological evidence- blood, semen, hair etc. found at scene of crime.
  - Early identification of criminal by matching genetic material evidence found at crime scene from data base of criminal`s gene data bank e.g. CODIS 13 data bank.
  - Accidents/ Mass disaster- by DNA pattern matching with relatives or old confirmed material/ DNA band ladder print.

Applications of DNA Fingerprinting

- Skeletal remains/ Mutilated bodies- by DNA pattern matching with relatives or old confirmed material/ DNA band ladder print.
- Estimating close relatives,.
- Matching of missing, unknown deceased.
- Archeological, Immigration cases
- Record keeping for future identification.(eg. Soldiers in US Army)

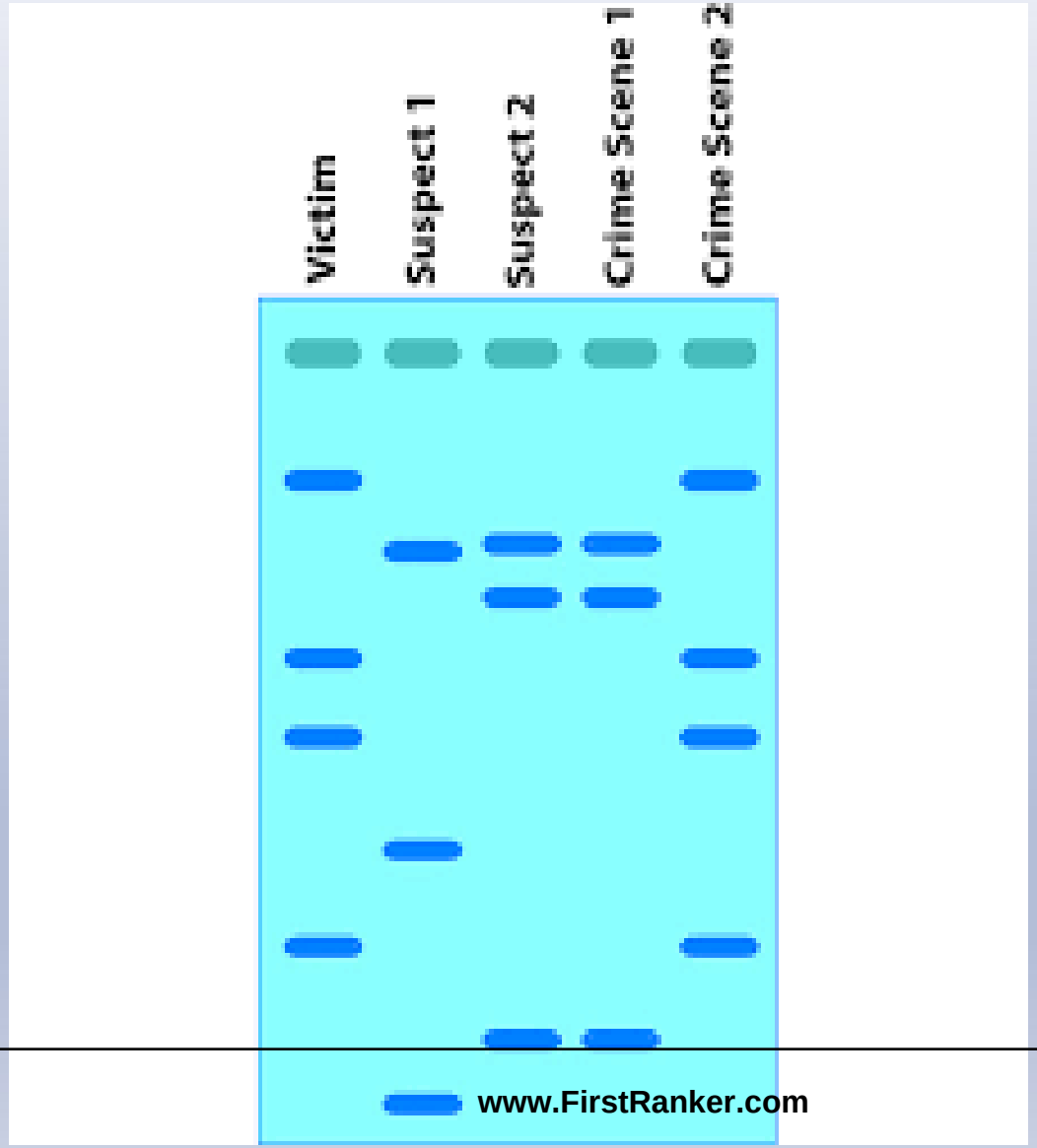
Applications of DNA Fingerprinting

- Establishing paternity.
- Race, gender identification of unknown
- Diagnosis of inherited disorders:- like cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia and thalassemia. Genetic counselors use DNA fingerprint information to help prospective parents understand the risk of having an affected child.

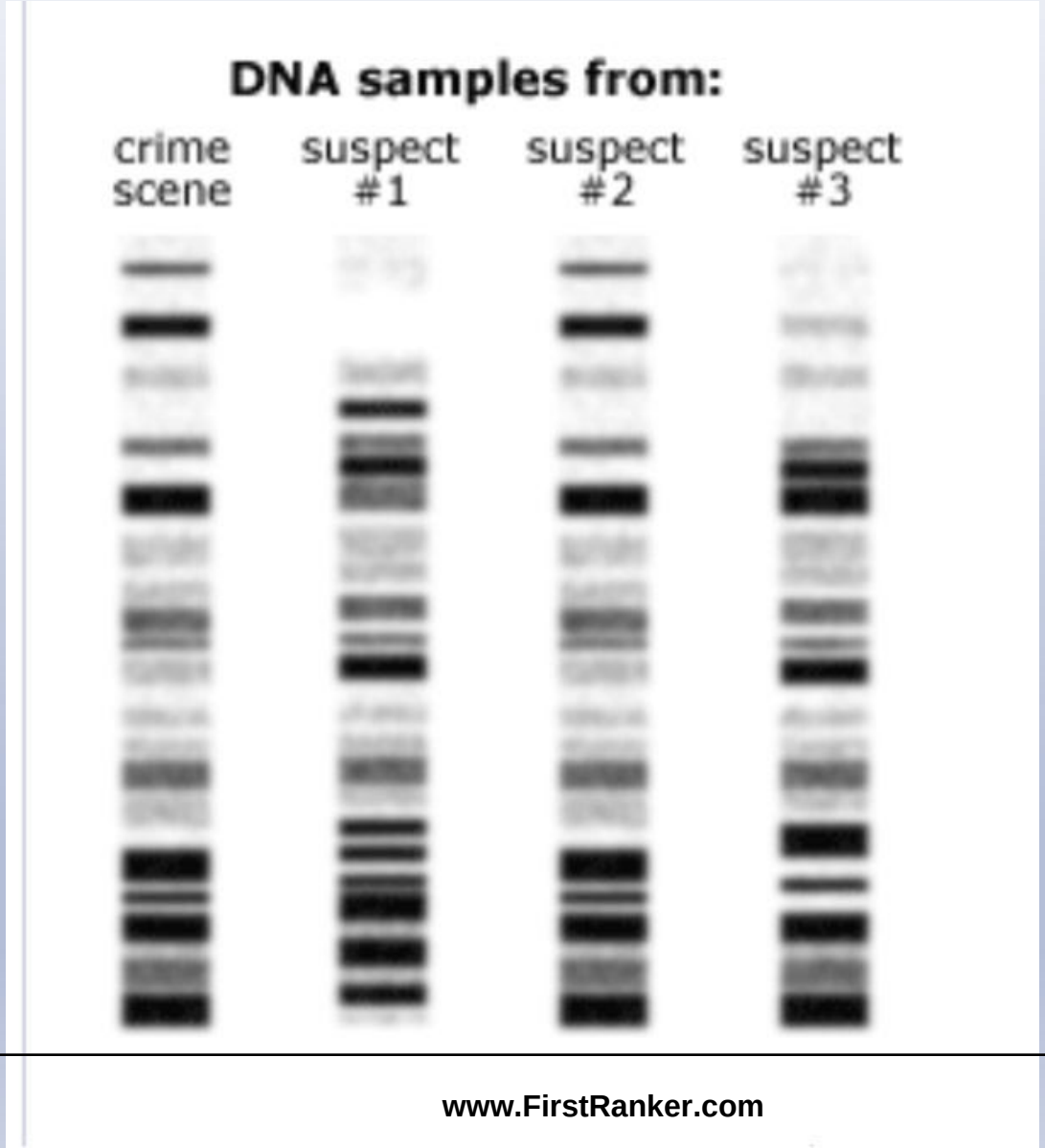
Applications of DNA Fingerprinting

- Developing cures for inherited disorders
- Protecting genome of endangered species.
- Pedigree analysis of pets

Anlysis of sample from crime scene

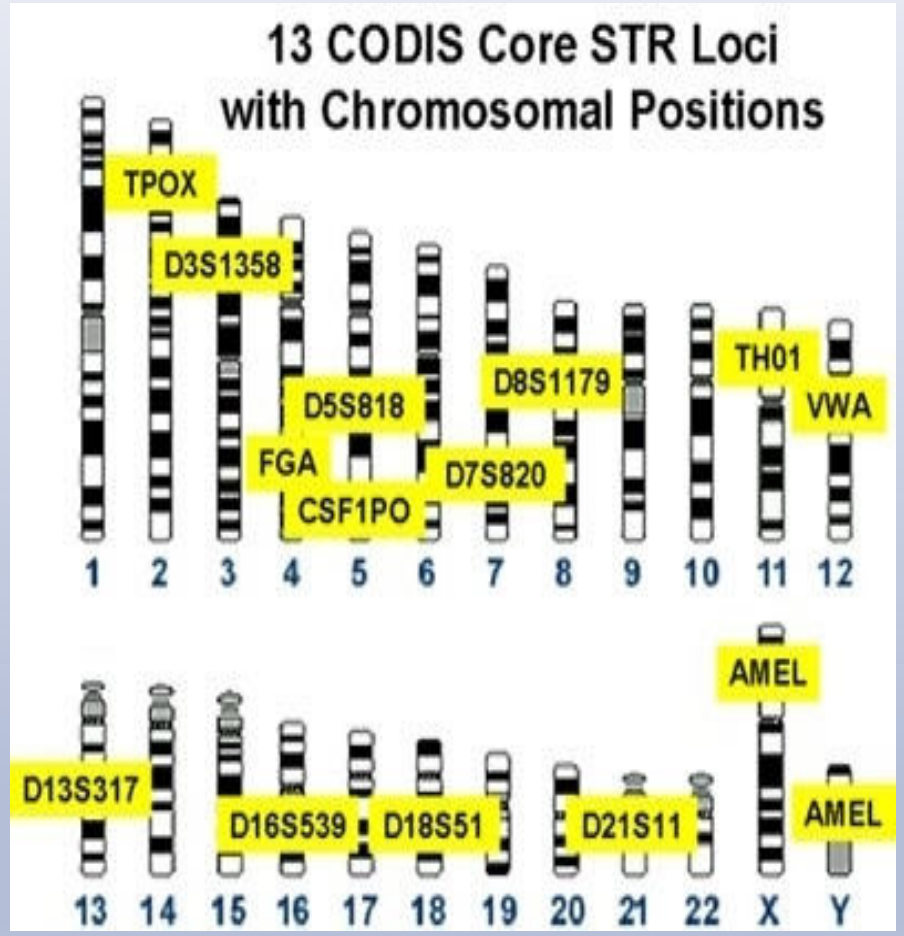


Anlysis of sample from crime scene



CODIS 13

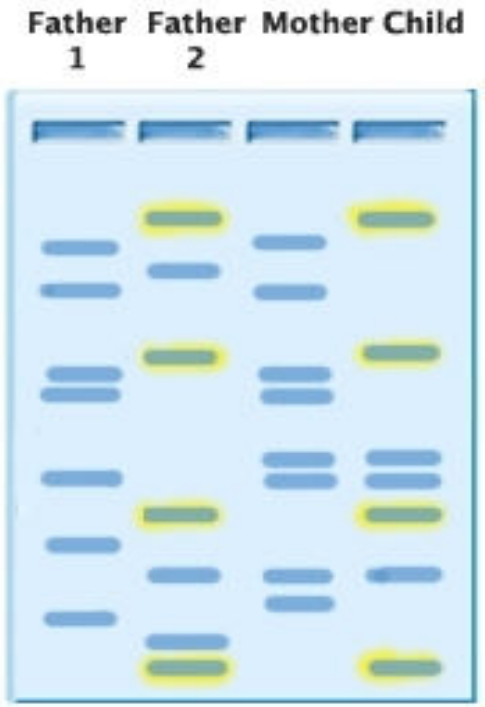
- CODIS is a DNA database funded by the FBI that stores DNA profiles of convicted felons.
- Use 13 markers plus one to determine gender.
- If any two samples have matching genotypes at all 13 CODIS loci, it is a certainty same individual (or an identical twin).

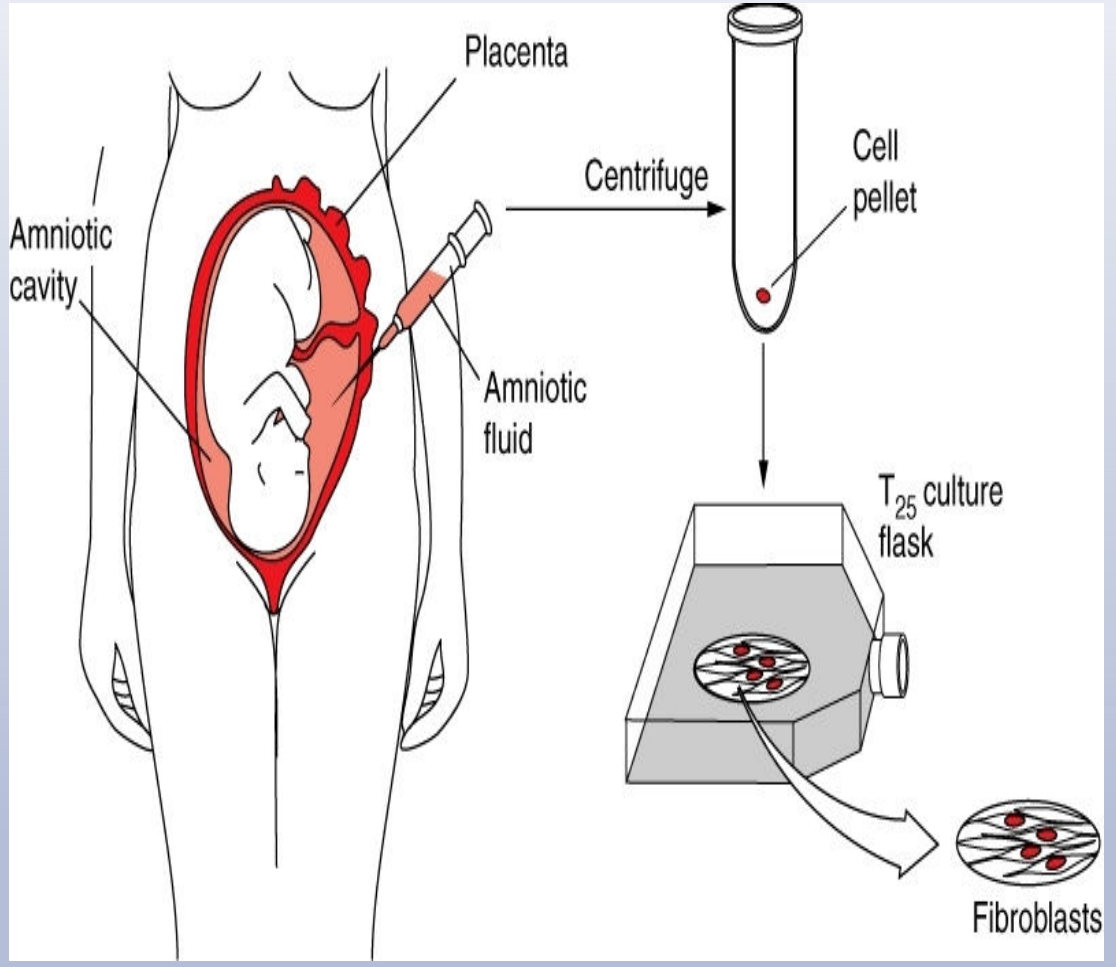


Paternity Testing

- This is possible because child receive its DNA(except mitochondrial DNA) only from mother or father, that means that any bands a child has must also be present one parent or the other.
- In the given example (see next slide) the highlighted bands that did not come from the mother, must have come from the father. Father 2 matches every band with the child that did not come from the mother.

Paternity Testing

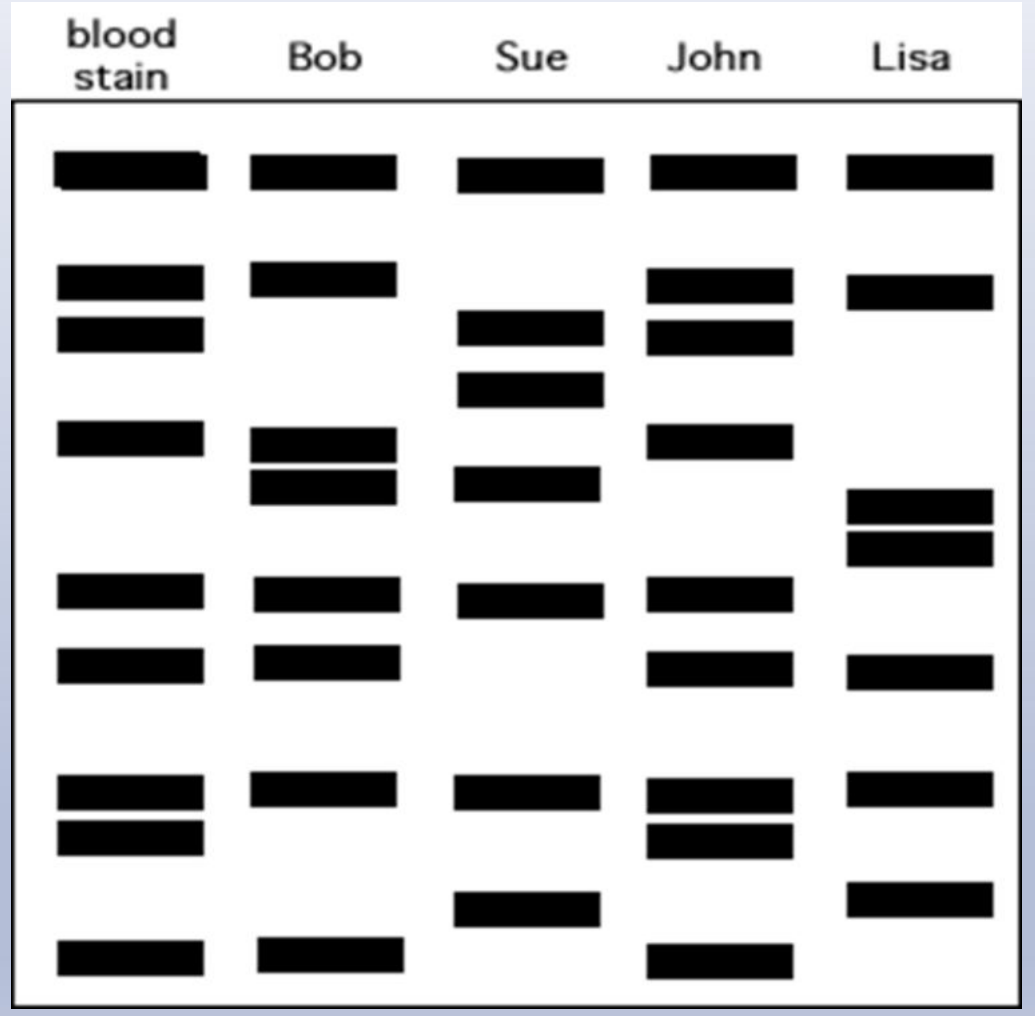




Paternity identification

Q.-Identification of person from suspects whose blood found at incident spot

A.- John



Limitation of DNA fingerprinting

DNA fingerprinting is controversial in a number of areas

- **Too sensitive-** a tiny amount of contaminant DNA in a sample may become amplified- leading to an erroneous conclusion.
- **Time constraints.-** The process is lengthy, with each of four or five loci exposed sequentially, it usually takes 10 weeks.

Limitation of DNA fingerprinting

- **Touch DNA:-** People leave microscopic level genetic material everywhere they go. High sensitive DNA fingerprint technique can detect DNA as less as 20 cells.

  It is relatively common for innocent person`s DNA to be present on near by area where he has not visited or on weapon which he might just touched much time befoe. this could erroneously tag person as criminal.

Limitation of DNA fingerprinting

- **Accuracy of the results:**
  - **Non uniqueness of DNA fingerprinting:** DNA segments(instead of complete DNA strands) are 'fingerprinted', target segment may not be unique
  - Laboratories that may not follow uniform testing standards and quality controls Result may alter.
  - Technical problems- Ambiguity in interpretation of the bands from misinterpretation, actual shifts, degradation, missing bands, extra bands.

Limitation of DNA fingerprinting

- Forensic specimens are often contaminated, making the extraction of pure DNA difficult, chance of false positive or false negative are high
- **Cost**- expensive.
- Unlike fingerprints DNA profile cannot be enlarged and shown in the court of law

Limitation of DNA fingerprinting

- **Ethical issues and privacy:-**

  Many countries are creating genetic information database as-

  - FBI has created Combined DNA Index System (CODIS 13) databank for criminals.
  - US is also making database for its soldiers.
  - Similar database is present in UK also.

  These database contains DNA fingerprint of from convicted criminals and from evidence found at crime scenes. misuse of the database, such as identifying individuals with stigmatizing illnesses such as AIDS.

Mitochondrial DNA (mtDNA)

- It small circular genome located in the mitochondria,
- It can be recovered from damaged, degraded or very small biological samples.
- Cells contain hundreds of copies of mtDNA genomes, as compared to two copies of the DNA located in the nucleus.
- So increases chances of recovering sufficient mtDNA where nuclear DNA lacking

Mitochondrial DNA (mtDNA)

- mtDNA is maternally inherited therefore-
  - Individual's mother, siblings, as well as all other maternally-related family members will have identical mtDNA sequences. So comparisons can be made from any maternal relative, • *Nucleic Acid Sequence Based Amplification* (NASBA), is used.
  - mtDNA is not useful in paternity suit.
  - mtDNA can`t be as specific as nuclear DNA

Famous cases

- Colin Pitchfork was the first criminal caught based on DNA fingerprinting evidence. He was arrested in 1986 for the rape and murder of two girls and was sentenced in 1988.
- In 2002 Elizabeth Hurley used DNA Fingerprinting to prove that Steve Bing was the father of her child Damien.

Famous cases

- O.J. Simpson was cleared of a double murder charge in 1994 which relied heavily on DNA evidence.
- The Bill Clinton– Monica Lewinsky scandal.
- Tandoor Kand Delhi

EXAMPLES

- In 2002 Elizabeth Hurley used DNA profiling to prove that Steve Bing was the father

of her child Damien