UNIT 4

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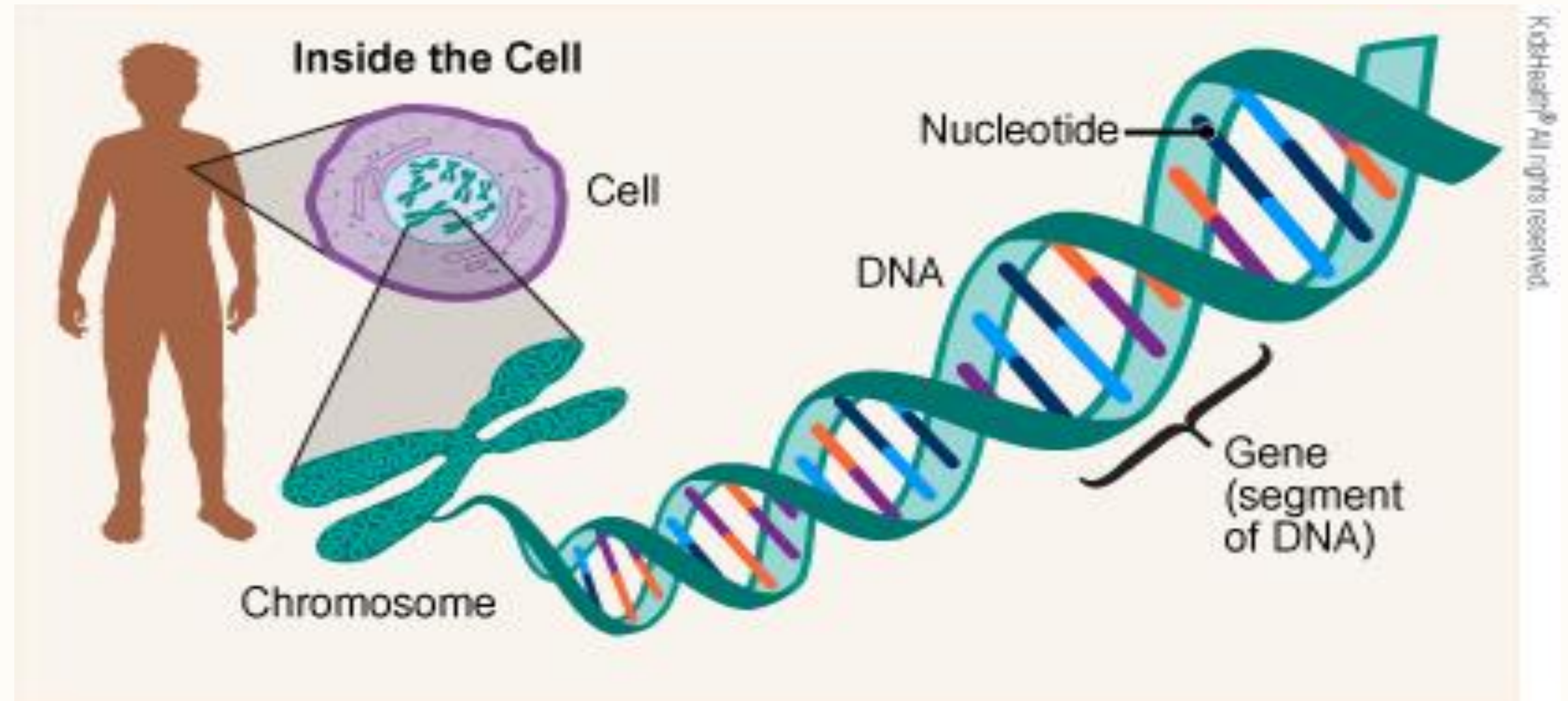
INTRODUCTION

- DNA profiling is also called as DNA fingerprinting or DNA typing
- The application of DNA technology to forensic medicine is the most remarkable recent advances in forensic identification. .
- In DNA profiling, DNA extracted from the sample is analyzed. DNA profiles are unique to each individual except in monozygotic twins.
- Alec Jeffreys in 1984 discovered unique application of RFLP technology to personal identification and labeled it as DNA fingerprinting.
- The chances that DNA profiles in two individuals are similar are about 1 in 30 billion to 300 billion, i.e. half the population of world.

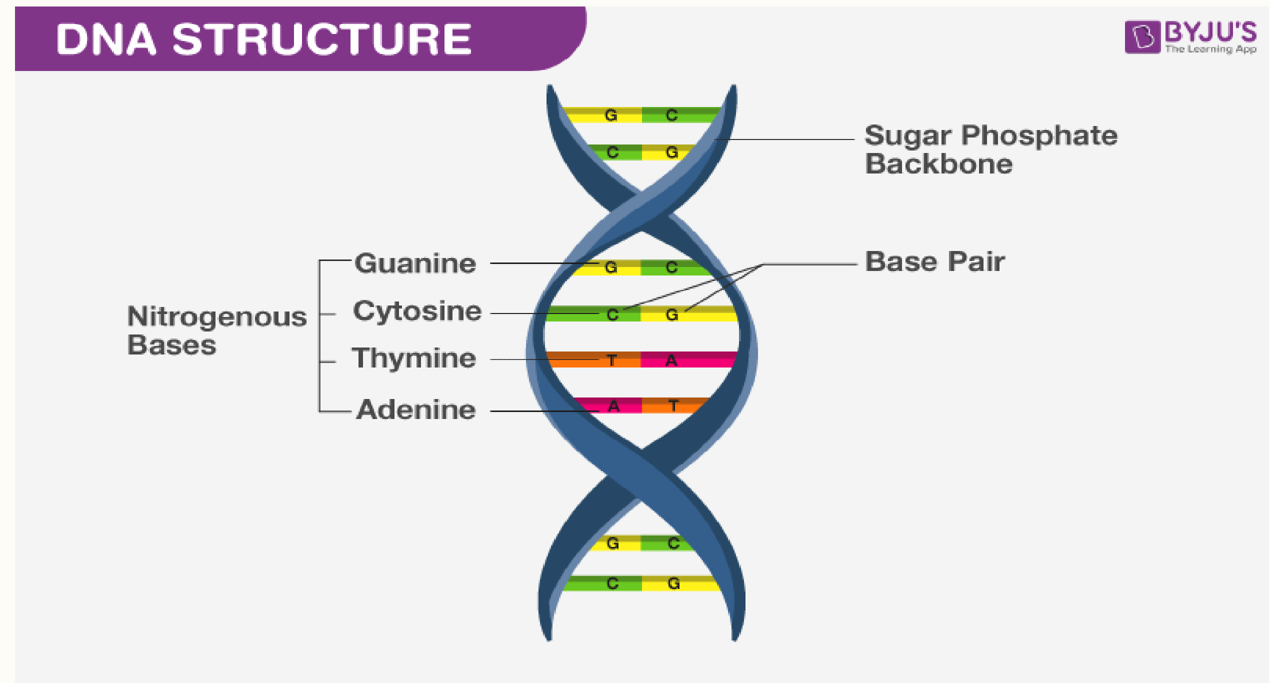
BASIC CONSIDERATION

Nucleus is present in all eukaryotic cells. The nucleus is made up of large part of the chromosomes. Each chromosome is made up of two complementary strands of deoxyribonucleic acid



DNA is a long polymer of nucleotides. Each nucleotide consists of phosphate, deoxyribose and one of the four bases that consist of adenine (A), thymine (T), guanine (G), and cytosine (C).

The complementary bases are joined by hydrogen bonds: A-T, C-G



Technique of DNA profiling

There are four methods of analysis as:

1. RFLP technique called as Restriction Fragment Length Polymorphism

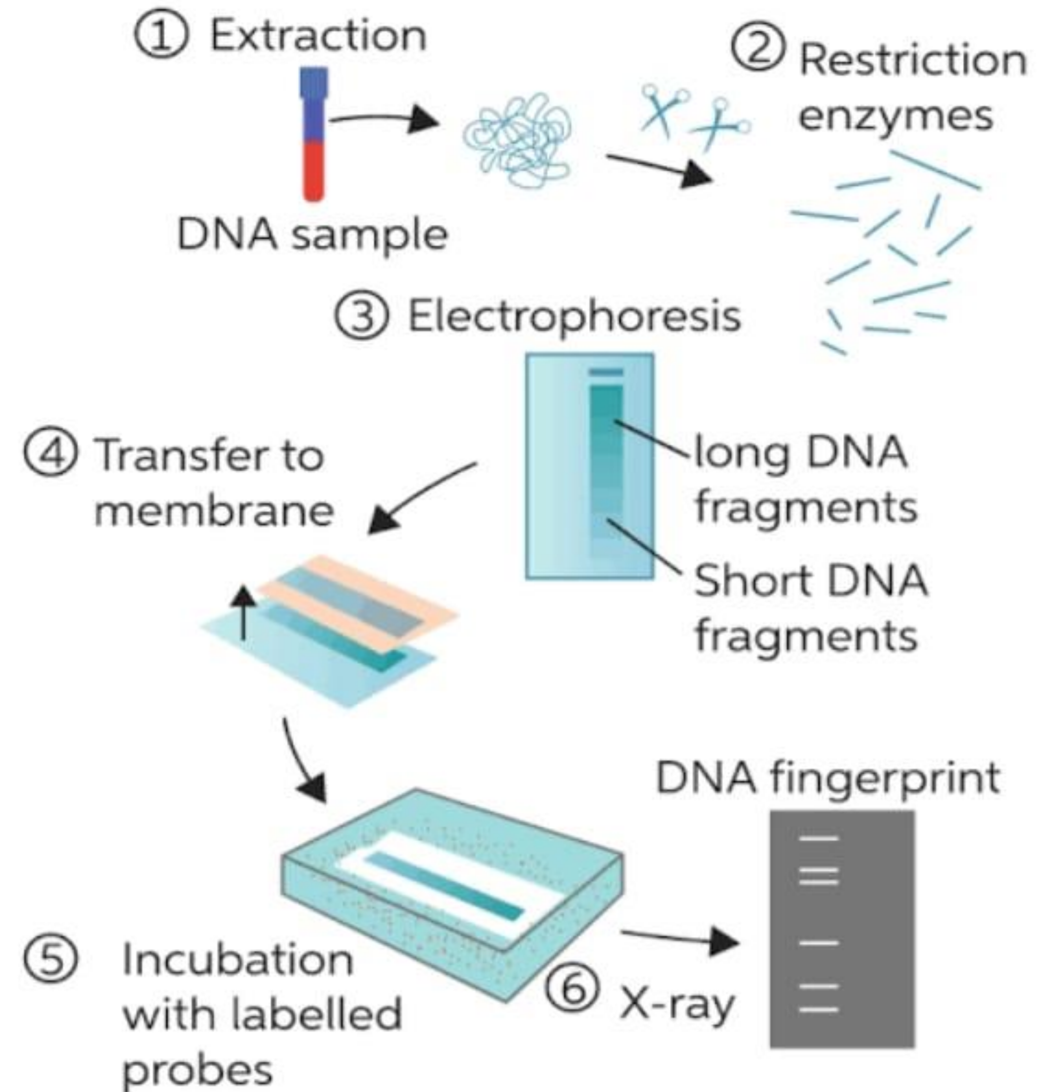
2. PCR technique - called as Polymerase Chain Reaction

3. STR method - Short Tandem Repeats

4. Mitochondrial DNA analysis

RFLP DNA Typing

Restriction Fragment Length Polymorphism



RFLP DNA Typing

- Now this method is not used
- The DNA is extracted from sample
- DNA is subjected to restriction enzymes (called as endonucleases). The restriction enzymes cut the DNA into pieces.
- The restriction enzymes are of different types, for example, Eco-R-1, PST-1, Hin-F-I etc. These enzymes recognized a particular sequence.
- When DNA is subjected to the enzymes, the enzyme recognizes a particular sequence and cut these sequences in one or two or more fragments.

- These restriction fragments are then separated by gel electrophoresis.
- The gel electrophoresis separate pieces of DNA based on their size. These fragments migrate toward the positive electrode and in this, the smaller fragments move faster than larger fragments thus separating the DNA samples into bands.
- These double stranded fragments are then denatured by high alkali treatment into single strands.
- These single stranded separated fragments are then transferred (blotted) to nitrocellulose filter or nylon membrane. This transfer technique (blotting) is called as Southern Blot (the name is given after EM Southern who first described the technique).

- Next step is to make these single stranded fragments into double stranded fragments. This process of making single stranded into double stranded is known as hybridization. This is done by addition of probe to the nylon membrane (or nitrocellulose filter).
- A probe is single stranded DNA segment (or synthetic DNA) and is tagged with radioactive marker such as P When the probe DNA mixes with single stranded fragments on nylon membrane, the probe will attach to particular segments (I.e. complementary sequence). Thus, the attached fragment will be radioactive.
- The nylon membrane is then washed with 0.05% SDS and in this process, the loose probe are removed.

- Then the nylon membrane is put in contact with X-ray film and the X-ray film is exposed. The X-ray film is then developed. The X-ray film shows gray to black bands. These bands represent hybridized radioactive probe with complementary sequences.

Advantages of RFLP Method

1. It can differentiate two samples originating from different sources, using fewer loci than other systems.

2. Determine more readily whether single sample contains DNA from more than one person.

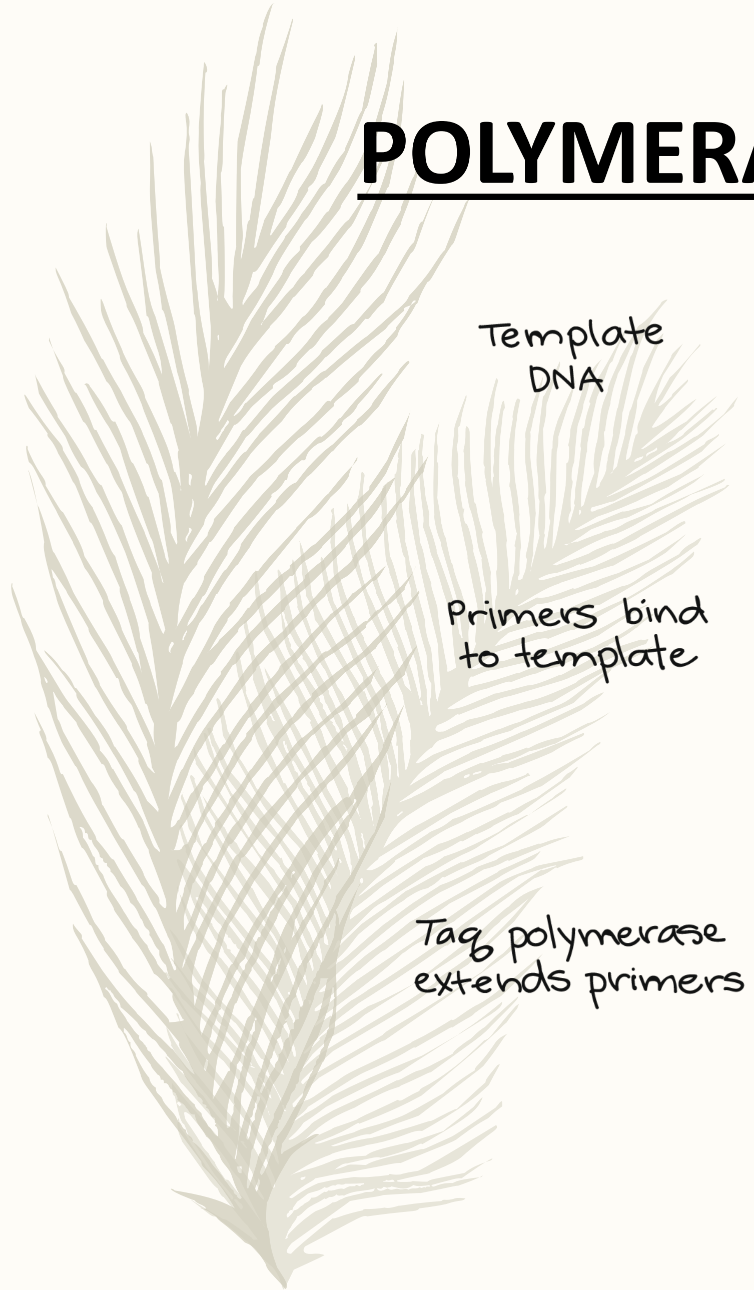
Disadvantages of RFLP Method

1. Requires high-molecular weight, high-quality DNA

2. Require large sample

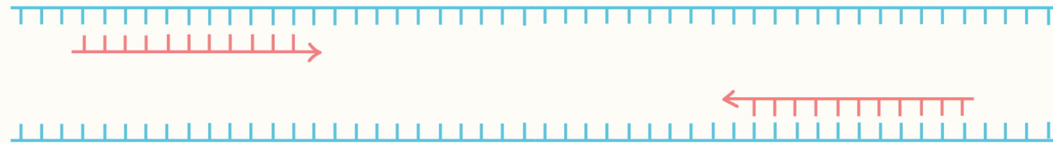
3. Requires more time

POLYMERASE CHAIN REACTION (PCR)

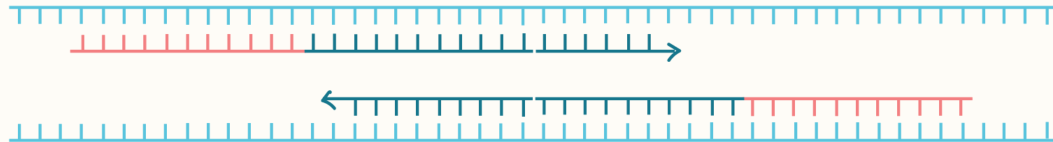


Template DNA

Primers bind to template



Taq polymerase extends primers



PCR Method

When very small sample is available, this method is convenient and used. In this method, DNA is extracted from sample and the extracted DNA is mixed with short fragments of known DNA called primers. It is three step procedure:

1. First step-extract DNA, denatured the DNA to form single strand DNA
.2. Second step-mix this single strand DNA with single strand primer DNA
3. Third step-DNA is synthesized by primer. At the end of third cycle, identical double strands DNA appear.

Advantages of PCR Technique

1. Require only trace amount of DNA.

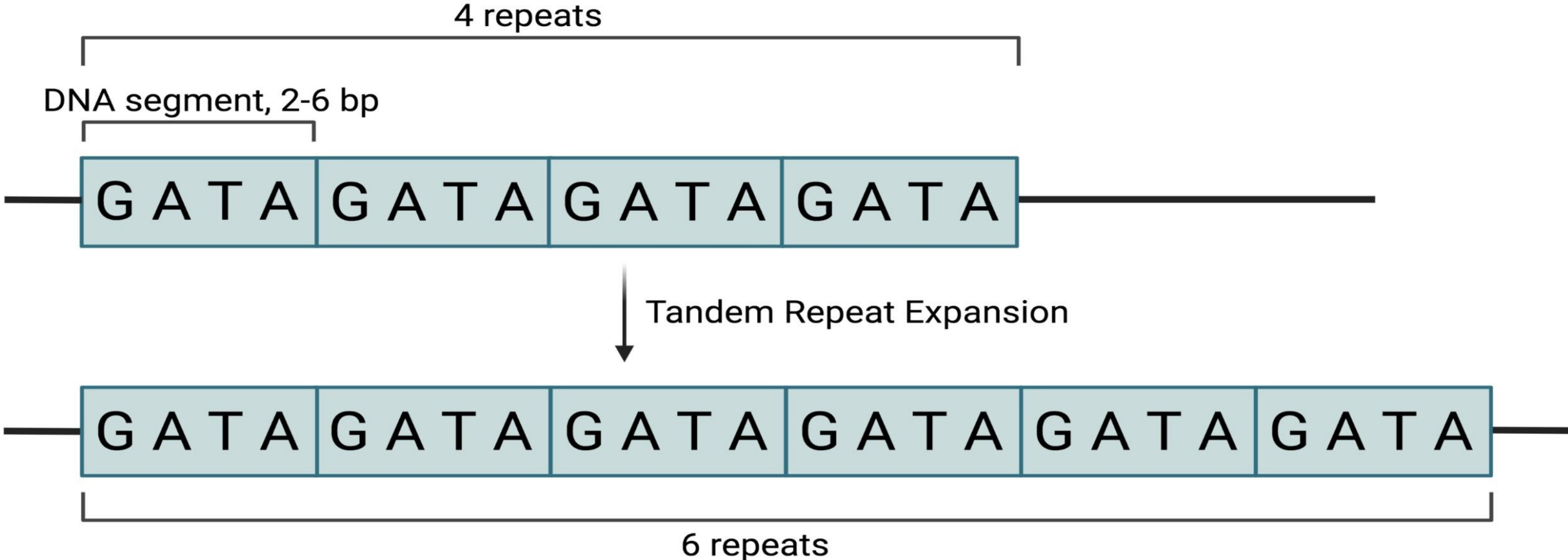
2. Procedure is fast and requires less time.

3. Highly sensitive method.

Disadvantage of PCR Technique

Susceptible to contamination.

STR METHOD



STR Method

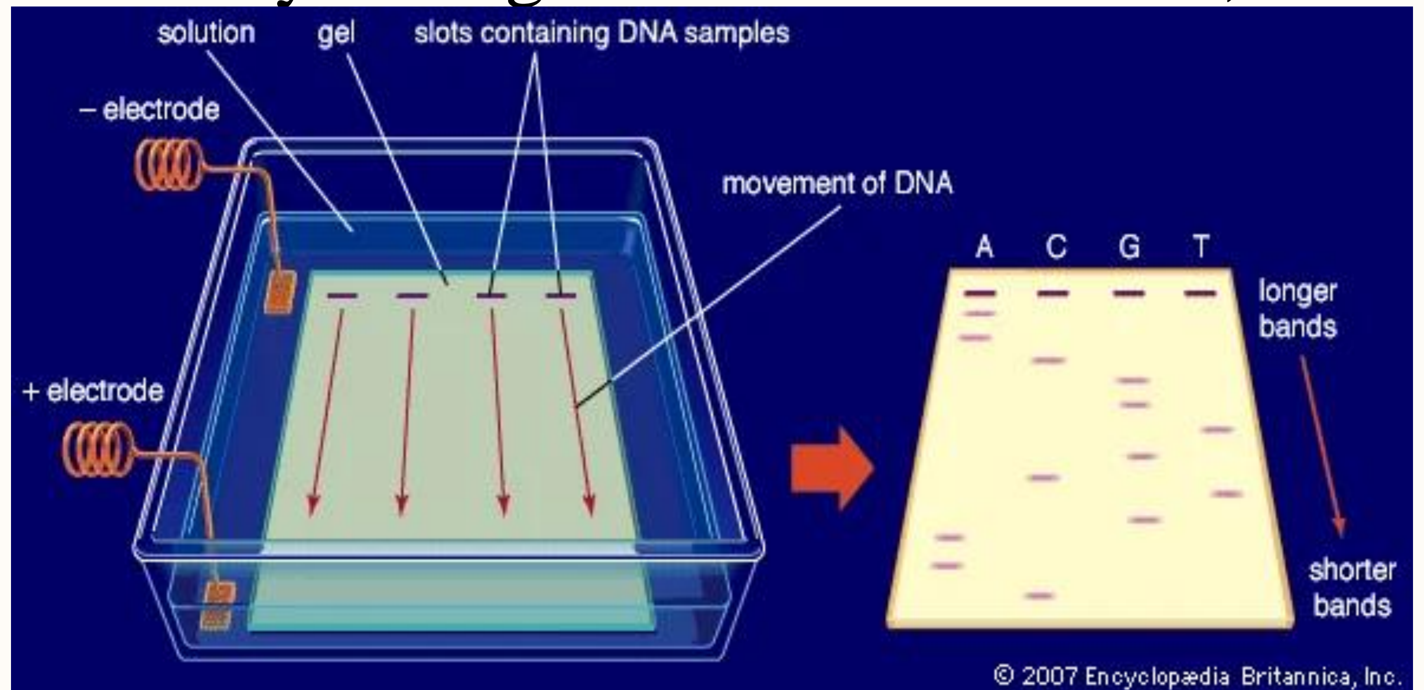
- Tandem repeated DNA sequences are present in human genome and they show variability in different individuals.

- These tandemly repeated regions of DNA are classified into several groups depending on the size of the repeat region such as:

1. Minisatellites-variable number of tandem repeats - VNTRS
2. Microsatellites-short tandem repeats - STR - have repeats with 2-5 bp

- STR and PCR technique has replaced RFLP. this technique is rapid and can be performed on small amount of DNA

1. Isolating the DNA
2. Replicating the STR fragments by PCR
3. Performing gel electrophoresis
4. With the help of software analysis of generated data is done, i.e. profiles are compared.



Advantages

1. Rapid
2. Small sample required
3. Degraded DNA may be typed using STRS.

Disadvantage of STR Method

1. .Susceptible to bacterial contamination.

Advantages of DNA fingerprinting

1. Conclusive method of identification of an individual
2. Method can be applied to old stains or biological material.
3. Small quantity of sample is required.
4. Degraded DNA may be typed using STRS.

Disadvantages of DNA Fingerprinting

1. DNA profiling cannot differentiate between monozygotic twins
2. Expensive
3. Interpretation requires trained manpower
4. Subjected to contamination

Applications of DNA profiling

1. To establish Identity of the person

sexual crimes:- rape
Violent crimes :- Murder
Missing person
Mistaken identity
war fighters
accidents/ mass disasters

2. To acquit a falsely implicated person from such similar crime.
3. Disputed paternity
4. Disputed Maternity

5. <u>To resolve disputes of</u>
Child born out of rape
Custody of child born
false implication on person being father of child

<u>6. Determination of twin zygosity</u>
<u>7. To identify sex.</u>

Collection, preservation and forwarding of sample for analysis

DNA analysis are equally important. Unless the samples are properly collected and preserved, it will not be useful for investigation. The consequences of improper collection and preservation of samples are:

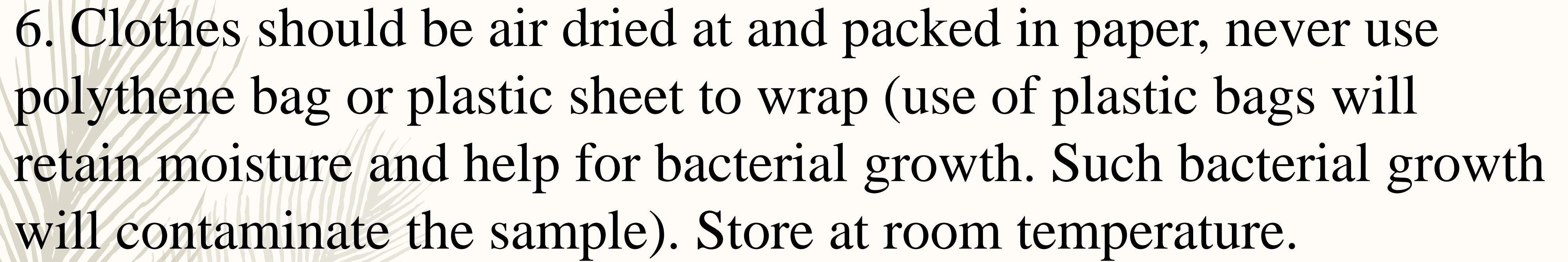
1. If not properly collected, the biological activity of sample may be lost.

2. If improperly packed, cross contamination may occur.

3. If improperly preserved, decomposition and degradation may occur. DNA extraction from degraded sample is difficult and challenging.

COLLECTION AND PRESERVATION OF SAMPLE

1. Practically one can collect any samples that contains cells, i.e. nucleus. Nucleus is the main source for DNA. extraction. Non-nucleated cells like mature RBC are not important
2. Dried blood stains/samples can be lifted from nonporous surface with conventional adhesive tape
3. Liquid blood: Collect 2-5 mL intravenous blood; place it in clean and sterile test tube. Add 4% EDTA as preservatives.
4. Semen/vaginal swabs should be preserved in clean and sterile container. In gang rape case, more than two vaginal samples/swabs should be collected and send in separate tubes.
5. swab of saliva taken should be air dried



6. Clothes should be air dried at and packed in paper, never use polythene bag or plastic sheet to wrap (use of plastic bags will retain moisture and help for bacterial growth. Such bacterial growth will contaminate the sample). Store at room temperature.

7. In exhumation cases-dry tissues are placed in a sterile container without adding any preservative and sent to laboratory at room temperature.

Mitochondrial DNA

- In nucleus DNA 2 copies per cell is present one from father one from mother
- In mitochondrial DNA multiple copies of mitochondrial DNA (up to 100,000 copies) are present in human cell
- Inherited from maternal side
- This is method of choice in samples that are degraded because of environment like mass disaster, exhumation etc.
- For forensic identification purpose selected loci are used.

Cell

Mitochondria

Mitochondrial DNA