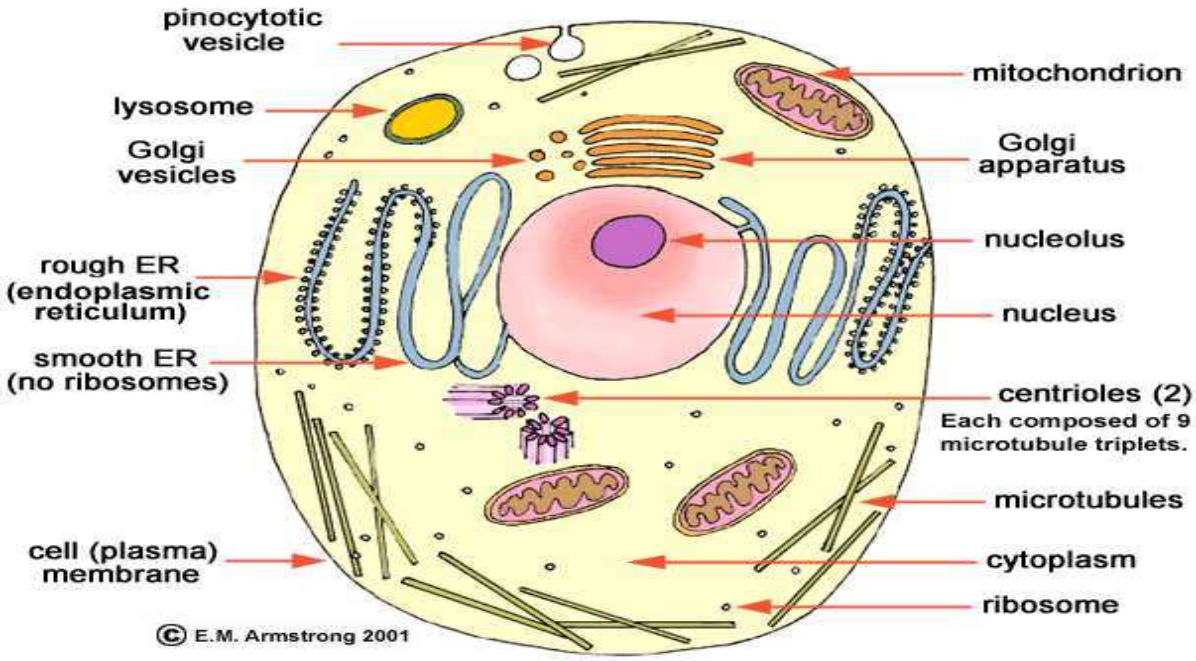
FORENSIC DNA FINGERPRINTING

INTRODUCTION:

DNA (Deoxyribo Nucleic Acid) is the hereditary material in humans and almost all other organisms. It is the polymer of nucleotides present in the nucleus of the cell. The basic unit of life is the cell. Every species has a characteristic set of chromosomes in each cell. The human cell consists of 46 chromosomes in 23 similar pairs, except in reproductive cells i.e., egg & sperm, where they differ with one pair of chromosomes as xx & xy respectively (22 pairs of autosomes and a pair of sex chromosomes). Genes are the basic units of heredity located on chromosomes. Each gene is actually composed of DNA.

Genome is the total genetic makeup of an organism. DNA is the building block of life. It directs all cellular functions. More than 99% of human DNA is identical in all people. Less than 1% makes them different. Parts of this DNA are polymorphic: they exist in more than one form. The observable variation in human DNA is called polymorphism. A human embryo is formed when an egg is fertilized with a single sperm cell. Half of the DNA is inherited from a person's mother and the other half from the father. Siblings inherit different combinations of DNA from the same parents and are therefore different from each other. Each generation of people is a new and different combination of genetic material from the previous generation. DNA Fingerprinting is the technique to use the highly variable polymorphic region of DNA to identify individuals by preparing the DNA profiles.

Anatomy of the animal cell



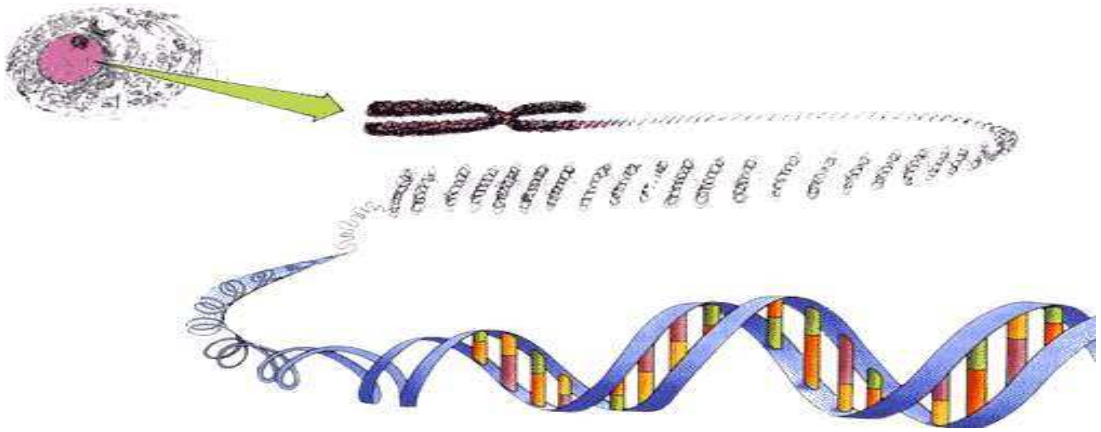
CELL – THE BASIS OF LIFE:

The basic unit of life is the cell which is a miniature factory producing the raw materials, energy and waste removal capabilities necessary to sustain life. Inside each cell in the human body, with in the nucleus, there are strands of genetic material called chromosomes. Along the chromosomes, like beads on a thread, genes are arranged.

The gene is the fundamental unit of heredity. It instructs the body cells to make proteins that determine every character and function of the body. Each gene is actually composed of DNA. DNA is the structural and functional chemical unit of chromosomes.

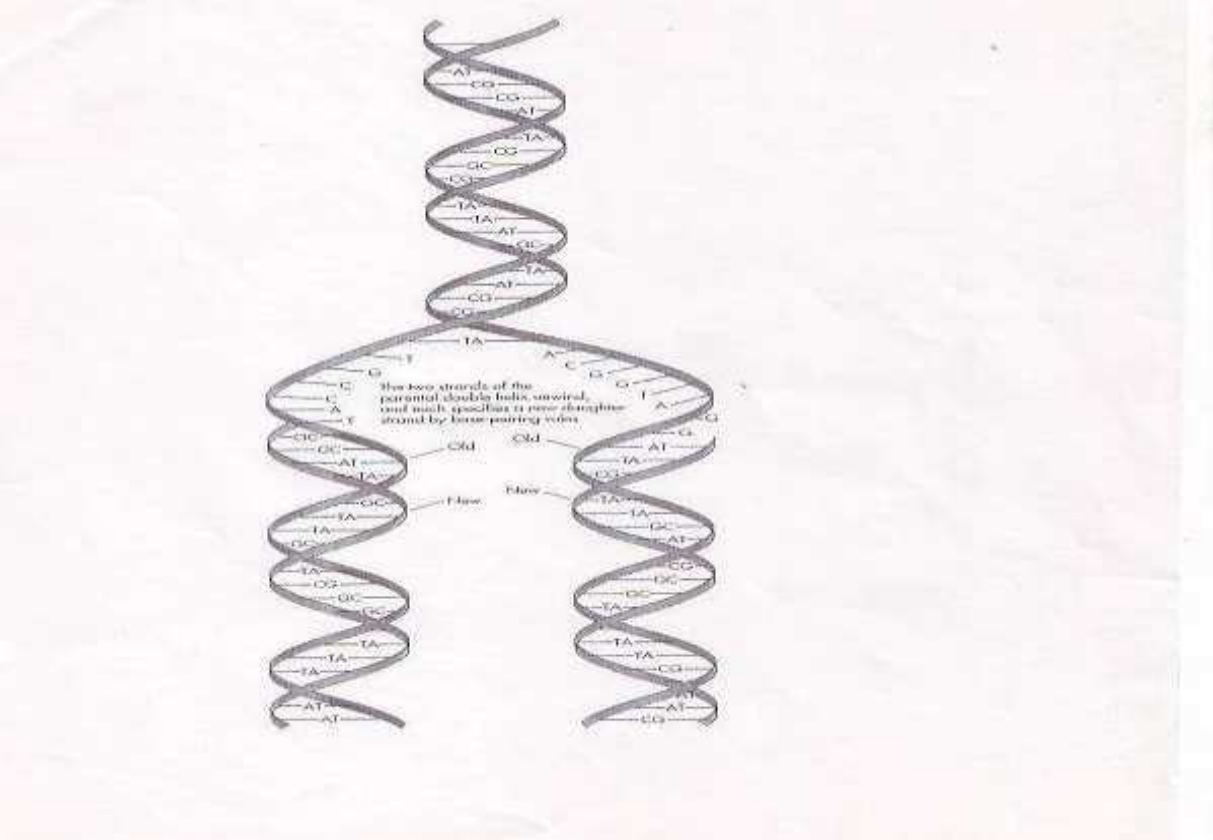
Genes that govern a given character are similarly positioned on chromosomes inherited from the mother & the father (Alleles). Genes instruct the body cells to make proteins – determining characteristics of a person – hair colour to susceptibility to diseases. The inheritable traits that are controlled by DNA which directs the production of complex molecules called proteins. Proteins are actually made by linking together a combination of up to 20 known amino acids. The sequence of amino acids-determines the shape & function of a protein. The observable variation in human DNA is called polymorphism. These polymorphic segments in the DNA molecule serve as a means to identify individuals. On the basis of these findings, it was revealed that, except for monozygotic twins, each individual can be differentiated from another.

DNA in the Cell



Nuclear DNA is a unique type of molecule. Its shape is called a double helix. Consider a very long ladder. This ladder has two poles connected by many rungs. Each rung consists of two complementary pieces that are joined together. Now, if the ladder is twisted many times throughout its length, it resembles a spiral staircase. That is the shape of the DNA molecule.

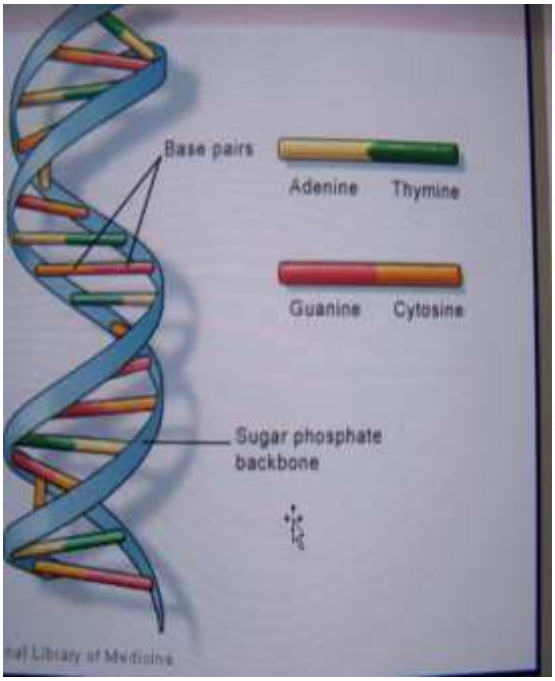
DOUBLE HELICAL STRUCTURE OF DNA

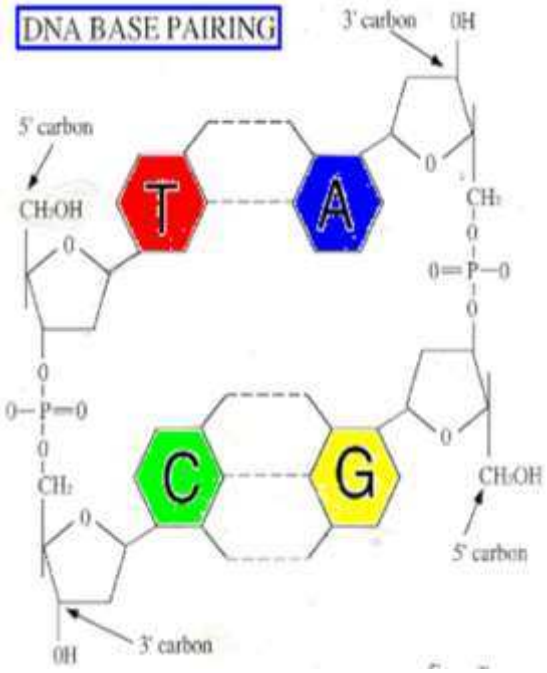


Watson and Crick have described the structure of DNA molecule.

DNA molecule is a composition of two DNA strands coiled into a double helix.

DNA is a polymer. A polymer is a very large molecule made by linkage of a series of repeating units known as nucleotides. A nucleotide is composed of a sugar molecule, a phosphorous containing group and a nitrogen containing molecule called a base, which is either Adenine (A), Guanine (G), Cytosine (C) or Thymine (T). The only arrangement possible in the double-helix configuration was the pairing of bases - A to T and G to C, a concept that is known as “base pairing”. Although A-T and G-C pairs are always required, there are no restrictions on how the bases are to be sequenced on a DNA strand.





T-C-G-A can be paired with the base sequence.

A-G-C-T in a double-helix configuration.

Any base can follow another on a DNA strand. The average human chromosome has DNA containing 100 million base pairs. All the human chromosomes taken together contain about 3 billion base pairs. That is the diversity of DNA and hence the diversity of living organisms.

DNA Finger printing is the technique to use the highly variable polymorphic regions of DNA to identify individuals by preparing their DNA profiles. There are so many millions of base pairs in each person's DNA and every person has a different sequence.

Using these sequences every person could be identified solely by the sequence of their base pairs.

No two human beings have identical sequences with an exception of identical twins.

SCOPE AND APPLICATIONS OF DNA FP IN FORENSIC INVESTIGATIONS

DNA is present in every nucleated cell and is therefore present in biological materials left at crime scenes. DNA can be successfully isolated and analyzed from a variety of biological materials.

DNA finger printing is a genetic technique i.e. decoding of genetic information. Each individual has an entirely unique genetic 'signature'/the'DNA fingerprint'. DNA finger printing is also called DNA typing/DNA profiling. It was first described in 1985 by an English geneticist- Sir Alec J. Jeffreys—the Father of DNA Fingerprinting. Dr.Lalji Singh is the scientist who developed & applied DNA FP in criminal justice system.

DNA typing is the most important advancement in the field of Forensic Science. It serves as a powerful forensic tool, not only because of its high level of discrimination among individuals, but also because it can be done with many sources of biological materials. The DNA of any in dividual is identical whether it is extracted from blood, semen, tissue, bone or any other biological specimen.

When DNA profiling is done with appropriate care, the results are highly reproducible and conclusive enough to prove the guilt or innocence of the suspect beyond reasonable doubt.

Some facts about DNA…

DNA is very stable.

DNA can be isolated from the biological remains of 1000s of year old and even from fossil, which is 2.5-million-year-old.

DNA pattern of an individual from every tissue of the body is the same. DNA does not change with the age.

DNA Fingerprinting is useful in the following Cases:

- Murder
- Rape
- Identity of Missing and Dead
- Identity of persons in mass disasters
- Disputed Paternity
- Wild Life poaching
- Identification and comparison of Plant Materials

General types of physical evidence:

- Blood
- Blood stains
- Seminal stains, vaginal swabs etc.
- Saliva on cigarette butts, bottles, glasses etc.
- Hairs with roots
- Teeth with pulp
- Bones with marrow
- Tissues & organs
- Skin
- Seeds etc. plant material.

Crime Scene with Blood

BLOOD STAINS ON KNIFE, ON FLOOR, ON CLOTHES

Use of DNA technique in crime investigation: -

a) To solve paternity problems

b) To establish family relationship for immigration

c) To identify the offender in rape & murder cases

d) To identify mutilated bodies from tissue remnants

e) To maintain social security record

f) To identify unknown dead body

g) To identify the true parents of the trafficking minors

h) To identify the exchanged babies in hospitals

i) To identify the people died in mass disaster

Samples obtained from a crime scene or a paternity investigation are subjected to standard & defined technical procedures to get the DNA profiles of the samples. Identification and comparison of crime and control DNA patterns are done and conclusions are drawn.

GENERAL APPLICATIONS OF DNA PROFILING:

DNA profiling has application in a broad cross section of disciplines, including human forensic science, diagnostic medicine, family relationship analysis, animal & plant sciences and wildlife forensic science.

The applications of DNA profiling can be listed as below:

DNA profiling has application in a broad cross section of disciplines, including human forensic science, diagnostic medicine, family Relationship analysis, animal and plant sciences and wildlife forensic science.

- To solve paternity problems
- To establish family relationship for immigration
- To identify the offender in rape & murder cases
- To exonerate persons wrongly accused of crimes
- To identify mutilated bodies from tissue remnants
- To maintain social security record
- To identify unknown dead body
- To identify the true parents of the trafficking minors
- To identify the exchanged babies in hospitals
- To identify the people died in mass disaster

- To match organ donors with recipients in human organ transplant programs
- To identify endangered and protected species in wildlife poaching cases
- To determine pedigree for seed or livestock breeds.

Briefly the uses of DNA Technique in Forensic Investigations:

- In social security record identification.
- DNA profiling is applicable in a number of areas in medicine, including in pedigree analysis, pathogen identification etc.
- For defence records – DNA FP of service personnel can be of immense help in establishing their identity during mass disasters like air crash or during war from their physical remains and records.
- DNA profile pattern is a more stable, meaningful identity record than any other known method.

The principles of individual uniqueness and identical DNA structure within all tissues of the same body provide the basis for DNA profiling in Forensic Science.

The cases can be broadly categorised under two heads: -

1. Civil Cases: For cases such as paternity/maternity disputes, immigration cases, swapping of new-born children in hospitals etc., fresh blood samples are received for examination.

2. Criminal Cases: For cases such as rape, murder, missing identity, child swapping, abduction, mass disasters etc., body fluids, hair with root, bones, teeth, tissues, animal tissues and skin, plants and other botanical evidence also are received for analysis.

The different types of the biological evidence can be used to associate or to exclude an individual from involvement with the crime. In particular, the direct transfer of DNA from one individual to

another individual or to an object can be used to link a suspect to a crime scene.

EVIDENCE COLLECTION FOR DNA TESTS:

Before a DNA test can be performed on a sample, it must be collected and the DNA isolated and put in the proper format for further characterization. If the samples are not handled properly in the initial stages of an investigation, then no amount of hard work in the final analytical or data interpretation steps can compensate. The process begins with the receipt of a forensic sample (above biological sample) which is later compared to the blood-relative of an individual's DNA (typically called "reference sample").

Identification of DNA sample sources and their collection:

DNA is present in every nucleated cell and is therefore present in biological materials left at crime scenes. DNA Examinations are required in forensic samples collected from the crime scene, suspect and victim in the form of evidentiary material recovered. Types of cases recovered include:

1. Disputed Paternity/Maternity
2. Murder
3. Sexual Assault
4. Cases of identity in the missing and the dead
5. Identification of mutilated bodies and exhumed bodies and mass disasters
6. Wild life poaching
7. Identification and comparison of plant materials

DNA has been successfully isolated and analysed from a variety of biological materials. Introduction of the Polymerase Chain Reaction (PCR) has extended the range of possible DNA samples that

can be successfully analysed because many copies are made of the DNA markers to be examined.

The samples are classified into:

- Whole Blood
- Blood Stains
- Semen, seminal stains, vaginal swabs etc.
- Saliva on cigarette butts, bottles, glasses etc.
- Bone with marrow
- Tissues & Organs
- Teeth with pulp
- Hair with root
- Amniotic fluid
- Skin
- Seeds etc. plant material

Note: - Other biological evidences such as tears, perspiration, serum and other body fluids are not useful for DNA analysis.

The conditions in which biological molecules exist in the body are carefully controlled and very specific. From the moment biological material is out of the body, it is in a foreign environment and changes begin to take place. DNA is packed very tightly in the chromosomes of a cell. Outside of its natural, protected environment, DNA is subject to degradation. Factors leading to the degradation of DNA include time, temperature, humidity, light and chemical or biological contamination. However, DNA is much more stable than the conventional genetic markers used in forensic

examinations. Under normal environmental conditions, DNA can remain stable and typable for years.

BIOLOGICAL EVIDENCE AT CRIME SCENES:

The different types of biological evidence discussed can be used to associate or to exclude an individual from involvement with a crime. In particular, the direct transfer of DNA from one individual to another individual or to an object can be used to link a suspect to a crime scene. As noted by Dr. Henry Lee (Lee et al. 1991), this direct transfer could involve:

1. The suspect's DNA deposited on the victim's body or clothing;
2. The suspect's DNA deposited on an object;
3. The suspect's DNA deposited at a location;
4. The victim's DNA deposited on the suspect's body or clothing;
5. The victim's DNA deposited on an object;
6. The victim's DNA deposited at a location;
7. The witness' DNA deposited on victim or suspect; or
8. The witness' DNA deposited on an object or at a location

DNA evidence collection from a crime scene must be performed carefully and a chain of custody established in order to produce DNA profiles that are meaningful and legally accepted in courts. DNA testing techniques have become so sensitive that biological evidence too small to be easily seen with the naked eye can be used to link suspects to crime scenes. The evidence must be

carefully collected, preserved, stored and transported prior to any analysis conducted in a Forensic DNA Laboratory.

EVIDENCE COLLECTION AND PRESERVATION:

The following suggestions may be helpful during evidence collection to preserve it properly.

- Avoid contaminating the area where DNA might be present by not touching it with your bare hands or sneezing and coughing over the evidence.
- Use clean latex gloves for collecting each item of evidence. Gloves should be changed between handling of different items of evidence.
- Each item of evidence must be packaged separately.
- Bloodstains, semen stains and other types of stains must be thoroughly air-dried prior to sealing the package.
- Samples should be packaged in paper envelopes or paper bags after drying. Plastic bags should be avoided because water condenses in them, especially in areas of high humidity and water can speed the degradation of DNA molecules. Packages should be clearly marked with case number, item number, collection date and initiated across the package seal in order to maintain a proper chain of custody.
- Stains on unmovable surfaces (such as table or floor) may be transferred with sterile cotton swabs and distilled water. Rub the stained area with the moist swab until the stain is transferred to the swab. Allow the swab to air-dry without touching any others. Store each swab in a separate paper envelope.

Collection, Preservation, Packing and Transportation of DNA samples:

Blood: Should be collected in sterile vial with EDTA (Ethylene Diamine Tetra Acetic acid) as preservative.

2ml of blood in case of adults and 1ml of blood in case of children. To be stored and transported in thermos flask containing ice to the lab within 24-48 hrs.

Note: 1. Invariably the persons should be directed to FSL for blood collection.

2. Attested passport size photographs of concerned persons should accompany the samples.

Blood-stained clothes:

Blood-stained garments should be thoroughly dried in shade and packed in separate paper packets or in dry moisture resistant polythene bags and should be sealed.

Blood-stained articles/weapons:

In case the stains are dry, scrape the stained area, keep in paper envelope and seal; or swab the stains with sterile gauge cloth soaked in saline; dry and pack separately.

Semen-Stained clothing/vaginal swabs etc.:

To be air dried and packed in paper covers / cloth parcels and sealed. Avoid folding, crushing the stained area in the process of packing.

From immovable articles - Collect using a sterile gauze cloth wetted in normal saline.

Air dry and pack them separately in paper covers/cloth parcels and seal.

Vaginal swabs – to be collected by M.O. Air dry and pack in dry glass bottle/paper pack and seal.

Saliva stains:

Stained cigarette butts, beedi butts, drinking vessels etc.-

Air dry and pack separately in paper covers and seal.

Tissues - Muscle tissues found at Crime scene –

Collect in a clean glass bottle, add 20% Dimethyl Sulphoxide saturated with Sodium Chloride/or in normal saline/or in salt solution. Decomposed dead bodies - Muscle tissues close to the femur or humerus bone should be dissected. 100gms to be sent in clean glass bottle.

In case tissues are highly degraded, whole bones are to be sent.

Bones: Whole bones like femur, humerus etc. to be collected and packed in separate paper packets or should be kept in aluminium foil and frozen.

Teeth: Teeth should be packed in dry paper packets and sealed (molar teeth are preferred).

Visceral matter: Collect intestine, spleen and heart (avoid liver).Add 20% dimethyl sulphoxide saturated with sodium chloride as preservative.

STORAGE AND TRANSPORT OF DNA EVIDENCE:

Carelessness or ignorance of proper handling procedures during storage and transport of DNA from the crime scene to the laboratory can result in a specimen unfit for analysis.

Most biological evidence is best preserved when stored dry and cold. These conditions reduce the rate of bacterial growth and degradation of DNA. In general, biological processes are slowed by removing moisture and lowering the temperature. Thus, the goal of the crime scene investigator is to dry a sample and freeze it as soon as it is practical. Inside the laboratory, DNA samples are either stored in a refrigerator at 4°C or in a freezer at -20°C. For long periods of time, extracted DNA samples may even be stored at -70° C.

Once the sample is dried, refrigerated and in the laboratory, the potential for contamination is mostly from other samples undergoing processing at the same time. This is where the training, qualifications of the analyst and quality control of the laboratory come into play.

Blood samples are to be collected as reference samples from known persons. When blood samples are taken, they should be put in tubes that already contain a preservative such as ethylenediamine tetraacetic acid (EDTA).

Evidence from animals:

a) Skin

b) Tissues

c) Blood etc.,

To ascertain, whether the skin/tissue/blood belong to the particular animal or not.

Evidence from plant material:

- Roots
- Twigs
- Leaves
- Flowers
- Seeds etc.,

To know whether the plant material belongs to the particular origin/source or not.

Importance of collection and preservation of biological evidence:

The ability to perform successful DNA analysis on biological evidence recovered from a crime scene depends very much on what

kinds of specimens were collected and how they were preserved. Unless the evidence is properly documented, collected, packaged and preserved, it will not meet the legal and scientific requirements for admissibility into a court of law.

CONCLUSION:

Physical evidence that is not properly recognised, documented, collected and preserved is of no value in a criminal investigation. DNA analysis of biological evidence is an extremely powerful tool for the identification of an offender but if the above parameters are not followed, the forensic laboratory may not be able to use this tool in the interest of justice.

PROBATIVE VALUE -

The main objective is to isolate DNA and prepare a profile of the individual and match with DNA profiles prepared from the test samples, so as to exclude innocent and connect criminal to the crime.

In disputed parentage cases, the DNA profiles of the child, suspected father/mother are compared to ascertain whether the child is born to the father/mother in dispute so as to fix the biological parentage.

In cases of murder and rape, DNA profiles obtained from blood samples of suspects/victims will be matched with DNA profiles prepared from the stains collected from crime scene, victims & suspects; finally, to connect the criminal to the crime.

In case of unidentified dead bodies, the DNA profile obtained from the source (bone, tissue, teeth etc.) of the deceased is matched with DNA profile prepared from the blood samples of nearest blood relations of suspected victim persons - to establish the biological relationship and thereby to identify the dead bodies.
