Recombinant DNA technology is one of the important tool for the diagnosis of several diseases. The diagnostic technique involves the construction of **probes consisting of short segments of single stranded DNA** attached to a radioactive or fluorescent marker. Such probes are used to identify infections agents such as *Salmonella* (cause food poisoning), Staphy/ococcus (pus forming bacterium), hepatitis virus, HIV; muscular dystrophy, cystic fibrosis and so on. Recombinant DNA technology can also be employed to predict the inheritance of genetic disorders from carrier parents. The chances of birth of an affected child can be predicted by testing DNA of repetitive prospective genetic disorder carrier parents.

GENE THERAPY

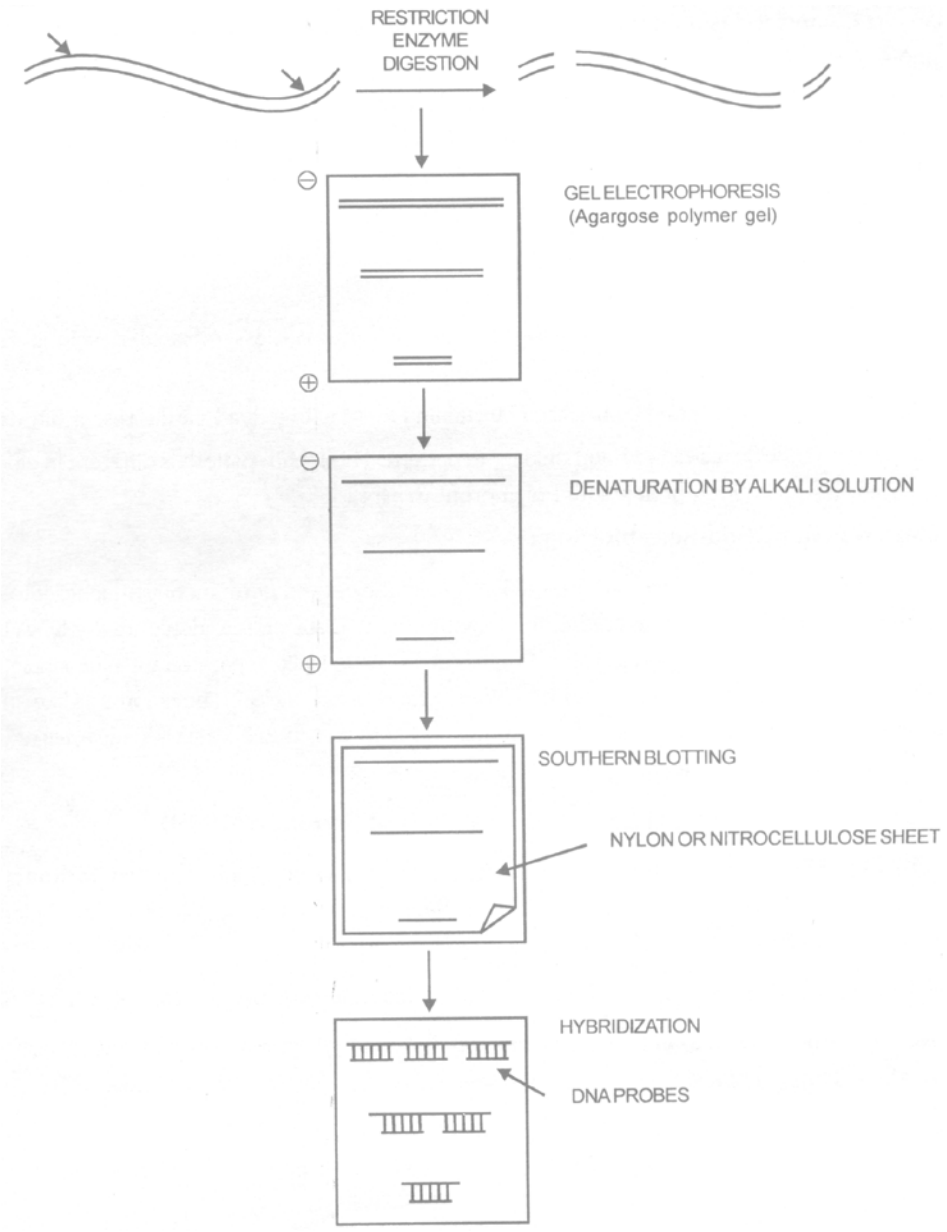
- Technique for curing gentic disease through replacing a **"Faulty Gene"** by a normal healthy functional gene.
- The first gene therapy used in **severe combined immuno deficiency** (SCID) pateint.
  About the 25% of infant with SCID disorder lack the *enzyme* adenosine deaminase (ADA)
- ADA enzyme involved in purine metabolism.
- These patients have no functioning T & B lymphocytes.
- The affected child of SCID develops recurrent infection early in life because they have no immune response against invading pathogen.
- The ideal approach would be to give the patient a functioning ADA by gene therapy.

DNA FINGER PRINTING / DNA TYPING / DNA PROFILING/ DNA TEST

- It is technique to identify a person on the basis of his/her DNA specificity. This technique was invented by sir Alec. Jeffery (1984).
- In India DNA Finger printing has been started by **Dr. V.K. Kashyap & Dr. Lal Ji Singh.**
- DNA of human is almost the same for all individuals but very small amount that differs from person to person that forensic scientists analyze to identify people.
  These differences are called Polymorphism (many forms) and are the key of DNA typing. Polymorphisms are most useful to forensic scientist. It is consist of variation in the length of DNA at specific loci is called Restricted fragment. It is most important segment for DNA test made up of short repetitive nucleotide sequences, these are called VNTRs (variable number of tandem repeat).
  VNTR's also called minisatellites were discovered by Alec Jeffery. Restricted fragment consist of hypervariable repeat region of DNA having a basic repeat sequence of 11-60 bp and flanked on both sites by restriction site.
- The number and position of minisatellites or VNTR in restriction fragmnt is different for each DNA and length of restricted fragment is depend on number of VNTR.
- Therefore, when the genome of two people are cut using the same restriction enzyme the length of fragments obtained is different for both the people.
- These variations in length of restricted fragment is called RFLP or Restriction fragment length polymorphism.
- Restriction Fragment Length Polymorphism distributed throughout human genomes are useful for DNA Finger printing.
- DNA Fingerprint can be prepared from extremely minute amount of blood, semen, hair bulb or any other cellj of the body.
  DNA content of 1 - Microgram is sufficient.

Technique of DNA Finger printing involves the following major stpes.

1. **Extraction** - DNA extracted from the cell by cell lysis. If the content of DNA is limited then DNA can be amplified by Polymerase chain reaction (PCR). This process is amplification.



Steps of DNA Fingerprinting

2. **Restriction Enzyme Digestion** : Restriction enzyme cuts DNA at specific 4 or 6 base pair sequences called restriction site.
   Hae III (Haemophilus aegyptius) is most commonly used enzyme. It cuts the DNA, every where the bases are arranged in the sequence GGCC. These restricated fragment transferred to Agarose Polymer gel.
3. **Gel Electrophoresis :**

- Gel electrophoresis is a method that separates macromolecules-either nucleic acid or proteins-on the basis of size, electric charge.
- A gel is a colloid in a solid form. The term electrophoresis describes the migration of charged particles under the influence of an electric field. Electro refers to the energy of electricity. Phoresis, from the Greek verb phoros, means "to carry across." Thus, gel electrophoresis refers to the technique in which molecules are forced across a span of gel, motivated by an electrical current. Activated electrodes at either end of the gel provides the

driving force. A molecule's properties determine, how rapidly an electric field can move the molecule through a gelatinous medium.

- Many important biological molecules such as amino acids, peptides, proteins, nucleotides, and nucleic acids posses ionisable groups and, therefore, at any given pH, exist in solution as electrically charged species either as cation (+) or anions (-). Depending on the nature of the net charge, the charged particles will migrate either to the cathode or to the anode.
- By the gel electrophoresis these restricted fragments move towards the positive electrode (anode) because DNA has -ve electric charge (PO_4^{-3}).
- Smaller Fragment more move towards the positive pole due to less molecular weight. So after the gel electrophoresis DNA fragment arranged according to molecular weight.
  These separated fragments can be visualized by staining them with a dye that fluoresces ultraviolet radiation.
- This appears the specific restricted fragment length pattern. This length pattern is different in different individual. This is called Restricted Fragment length Polymorphism (RFLP).

4. Southern transfer / Southern blotting :

The gel is fragile. It is necessary to remove the DNA from the gel and permanently attaches it to a solid support. This is accomplished by the process of Southern blotting. The first step is to denature the DNA in the gel which means that the double-stranded restriction fragments are chemically separated into the single stranded form. The DNA then is transferred by the process of blotting to a sheet of nylon. The nylon acts like an ink blotter and "blots" up the separated DNA fragments, the restriction fragments, invisible at this stage are irreversibly attached to the nylon membrane the "blot".
This process is called Southern blot by the name of **Edward Southern (1970).**

5. Hybridization : To detect VNTR locus on restricted fragment, we use single stranded **Radioactive (P^{32}) DNA** probe which have the base pair sequences complimentary to the DNA sequences at the VNTR locus. Commonly we use a combination of at least 4 to 6 separate DNA probes 6.Labelled Probes are attached with the VNTR loci of restricted DNA Fragments, this process is called Hybridization. **Autoradiography** : Nylon membrane containing radio active probe exposed to X-ray. Specific bands appear on X-ray film. These bands are the areas where the radioactive probe bind with the VNTR. These allow analyzer to identify a particular person DNA, the occurance and frequency of a particular genetic pattern contained in this X-ray film. These x-ray film called DNA signature of a person which is specific for each individual. The probability of two unrelated individual having same pattern of location and *repeat* number of minisatellite (VNTR) is one in ten billion (world population 6.1 billion) In India the centre for DNA finger printing and diagnosis (CDFD - center for DNA finger printing & diagnosis) located at Hyderabad.

Application of DNA Finger printing

1. Paternity tests. The major application of DNA finger printing is in determining family relationships. For identifying the true (biological) father, DNA samples of Child, mother and possible fathers are taken and their DNA finger prints are obtained. The prints of child DNA match to the prints of biological parents.

2. Identification of the criminal. DNA finger printing has now become useful technique in forensic (crime detecting) science, specially when serious crimes such as murders and rapes are involved. For identifying a criminal, the DNA fingerprints of the suspects from blood or hair or semen picked up from the scene of crime are prepared and compared. The DNA fingerprint of the person matching the one obtained from sample collected from scene of crime can give a clue to the actual criminal.