Diagnosis of Genetic Diseases
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- Family History*
- Estimation of Haematological parameters
- Clinical Presentation*
- Estimation of Biochemical Parameters
- Chromosomal Analysis
- Determination of Enzyme Activity or Specific Protein
- Recombinant DNA Technology

* Important for all genetic diseases
1. Family History

- Consanguinity of parents.
- Presence of other siblings with the same disorder.
- Occurrence of the disorder in other members of the family.
- Repeated abortions or still births,
- Mother and father's ages.
- Drawing punnett square helps to determine the mode of inheritance of the genetic disorders.
  - Autosomal or X-linked
  - Dominant or recessive
2. Clinical Presentation

Certain clinical features are specific for a disease:

- **Chronic anaemia:**
  - Haemoglobinopathies
  - Thalassaemia
  - Other genetic anaemias
- **Acute anaemia, under certain stressful conditions.**
  - G-6-PD deficiency
- **Hypoxia – sickle cell disease.**
- **Dependence on blood transfusion - β-thalassaemia (major)**
- **Severe immune deficiency – ADA deficiency.**
- **Emphysema - α1 anti-trypsin deficiency.**
- **Hypercholesterolaemia – familial hypercholesterolaemia.**
- **Delayed blood coagulation – Haemophilia (decrease in factor VIII or IX).**
- **Mental retardation – Fragile syndrome (in X chromosome) or phenylketonuria (PKU).**
- **Muscular weakness and degeneration – Duchenne muscular dystrophy.**
Recombinant DNA Technology
( Genetic Engineering)
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Techniques for cutting and joining DNA
Recombinant DNA

- The DNA created by joining DNA from different origins e.g. human DNA sequence of interest and bacterial or other DNA molecule.
- It is capable of duplication in the laboratory.
Requirements for DNA technology

- Restriction endonucleases
- Primers
- Vectors
- NTPs
- Probes
- DNA
- Other enzymes e.g. ligases, Taq polymerases
- Special chemicals and equipment
Restriction Endonuclease

- Endonucleases.
- Synthesized by procaryotes. Do not restrict host DNA.
- Recognize and cut specific base sequence of 4-6 bases in double helical DNA.
- The sequence of base pairs is palindromic i.e. it has two fold symmetry and the sequence, if read, from 5’ or 3’ end is the same.

5’-GAATTC-3’
3’-CTTAAG-5’
Produce either Blunt Ends or Staggered ends:

**Restriction Endonuclease**

5'-GAATTC-3'  5'-GAA TTC-3'
3'-CTTAAG-5'  3'-CTT AAG-5'

or

5'-GAATTC-3'  5'-G AATTTC-3'
3'-CTTAAG-5'  3'-CTTAA G-5'

or

5'-GAATTC-3'  5'-GAA TTC-3'
3'-CTTAAG-5'  3'-CTT AAG-5'
Uses of Restriction Endonuclease

- Obtaining DNA fragments of interest.
- Gene mapping.
- Sequencing of DNA fragments.
- DNA finger printing
- Recombinant DNA technology
- Study of gene polymorphism.
- Diagnosis of disease.
- Prenatal diagnosis
Sources of DNA

Genomic DNA
DNA extracted from cells

Synthesis of DNA
Using DNA synthesiser

cDNA
Synthesised from mRNA using reverse transcriptase
cDNA Synthesis

mRNA → Poly A tail

Viral reverse transcriptase

Hair pin loop

NaOH (Hydrolysis of RNA)

dNTP → DNA polymerase

DNA nuclease (single-strand specific)

Double strand cDNA
Vectors

- DNA molecules.
- Can replicate in a host e.g. bacterial cells or yeast.
- Can be isolated and re-injected in cells.
- Presence can be detected.
- Can be introduced into bacterial cells e.g. E. coli.
- May carry antibiotic resistance genes.

Cloning vesicles
<table>
<thead>
<tr>
<th>Type</th>
<th>Insert size</th>
</tr>
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<tbody>
<tr>
<td>I. Plasmid: circular, double stranded</td>
<td>• &lt;5-10 kb.</td>
</tr>
<tr>
<td>cytoplasmic DNA in procaryotic e.g.</td>
<td></td>
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<tr>
<td>PBR 3 of Ecoli.</td>
<td></td>
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<tr>
<td>II. Bacteriophage lambda: a bacterial</td>
<td>• Upto 20kb.</td>
</tr>
<tr>
<td>virus infects bacteria.</td>
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<tr>
<td>III. Cosmids: a large circular cytoplasm</td>
<td>• Upto 50kb.</td>
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<tr>
<td>ic double stranded DNA similar to</td>
<td></td>
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<tr>
<td>plasmid.</td>
<td></td>
</tr>
<tr>
<td>IV. Yeast Artificial Chromosomes (YAC)</td>
<td>• ~100-1000kb.</td>
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</tbody>
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Inserting a DNA Sample into a Plasmid
Plasmid Insertion

Gene for antibiotic resistance
Plasmid

Foreign DNA with gene of interest
Cutting sites for endonuclease

Sticky ends
Mismatch
Desired recombinant plasmid

Mismatch
Bacterial chromosome

Recombinant plasmid
Cloned or synthetic nucleic acids used for DNA:DNA or DNA:RNA hybridization reactions to hybridize to DNA of interest.

- DNA or RNA.
- cDNA.

Labeling of probes:
- $^3\text{H}$ (Radioactive)
- $^{32}\text{P}$
Hybridization

- target
- separate and immobilize
- nitrocellulose
- add probe
- remove unbound probe
- detect probe
Recombinant DNA Technology

Amplification of DNA
- DNA cloning
- Polymerase chain reaction
  - DNA sequencing
  - RT PCR

Study of DNA structure and functions
- DGGE
- RT PCR
- ARMS
- DGGE
- Others
- Dot blot analysis

Others
**Principles of Molecular Cloning**

**Involves:**

- Isolation of DNA sequence of interest.
- Insertion of this DNA in the DNA of an organism that grows rapidly and over extended period e.g. bacteria.
- Growing of the bacteria under appropriate condition.
- Obtaining the pure form of DNA in large quantities for molecular analysis.

![Cloning into a plasmid diagram](image)
1. Human DNA

Chromosome

EcoRI partial digestion

Large chromosomal fragments

sticky ends

Recombination + DNA ligase

2. YAC vector

EcoRI + BamHI

3. Yeast artificial chromosome with inserted human DNA

up to 1000000 nucleotides pairs

yeast cell transformation

Clone

Cloning into a Yeast Artificial Chromosome (YAC)
Polymerase Chain Reaction (PCR)

- Method to amplify a target sequence of DNA or RNA several million folds.
- Based on Enzymatic amplification of DNA fragment flanked by primers i.e. short oligonucleotides fragments complimentary to DNA. Synthesis of DNA initiates at the primers.

```
5' ATCAGGAATT CATGCCAAGGTTGATCGATGATCGATCGATCGATCGATTGAT 3'
3' AGCTAGCTAGCT 5'
```
POLYMERASE CHAIN REACTION

1. DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

2. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

5. Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.
Application of PCR

- Diagnosis of genetic disease by amplification of the gene of interest, followed by detection of mutation.
- Detection of infectious agent e.g. bacteria and viruses.
- DNA sequencing.
- In forensic medicine.
1. Clinical Chemistry:
   - Diagnosis of disease e.g. sickle cell anaemia by Mst II.
   - Prenatal diagnosis
Southern Blotting

1. **DNA**
2. **Enzyme Cutting Sites**
3. **Restriction Enzyme**
4. **Agarose Gel Electrophoresis**
5. **Blotting**
6. **Nitrocellulose Paper**
7. **Add Probe of Interest**
8. **Hybridization**
9. **Autoradiography**
10. **Detect DNA Sequence of Interest**
Pathogenesis of α-Thalassaemia

1. **Withdraw blood**
2. **Extract DNA**
3. **Treat with BglII**
4. **Electrophoresis**
5. **Southern Blotting**
6. **Visualize**

- **BglII**
- **BamHI**
- **14.5Kb**
- **12.5Kb**
- **7.0Kb**

**Steps:**
- Withdraw blood
- Extract DNA
- Treat with BglII
- Electrophoresis
- Southern Blotting
- Visualize
2. Human Genetics
   • Mutations in genes causing hereditary disease e.g. diagnosis of fibrosis Channes disease.

3. Forensic Medicine
   • Analysis of stains of blood, semin.

4. Virology
   • Detection of viral diseases e.g. hepatitis

5. Microbiology
   • Using specific gene probes for detection of E.coli

6. Cytology, Histology and Pathology
   • Used in detection of tumor.

7. Synthesis of protein in bacterial
   • Insulin
   • GH
   • Somatostatin
   • Interferon
Transfer of the Insulin gene

Restriction enzyme

plasmid

human cDNA

linker

Recombinant plasmid

Cloning the Insulin Gene

bacteria

chromosome

Transfection

Transfer and cloning of the Insulin gene
Transgenic Mice