Applications of Biotechnology in Medicine

By

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Biotechnology: Overview

- **Recombinant DNA technology**
- **Genetic engineering**
- **Gene cloning**

What is Biotechnology?

Biotechnology = Biology + Technology

Understanding & Manipulation of Genes (The code of life) and Their Products for Useful Purposes

The Goals of Biotechnology in Medicine:

- To identify and manage the root cause of diseases
- To find ways to improve lives through better health

To fight and cure diseases

Medical Biotechnology

- Production of Drugs and Therapeutics
- Genetically modified organisms
- Analysis of the Genes in genetic disease
- Correction of genetic defects

Molecular Medicine in the Era of Biotechnology

- Improved diagnosis of disease
- Earlier detection of genetic predispositions to disease
- Rational drug design
- Gene therapy and control systems for drugs
- Pharmacogenomics "custom drugs"

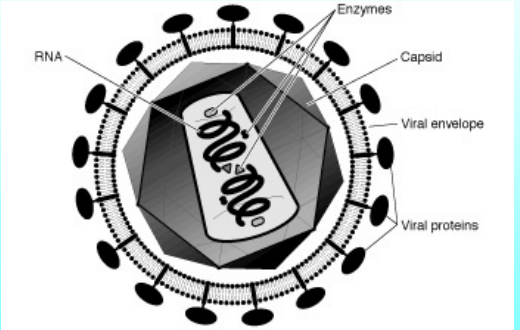
Biotechnology Employs Natural, Organic Tools:

- Cells,
- Genes,
- Proteins,
- Enzymes,
- Antibodies

Viruses

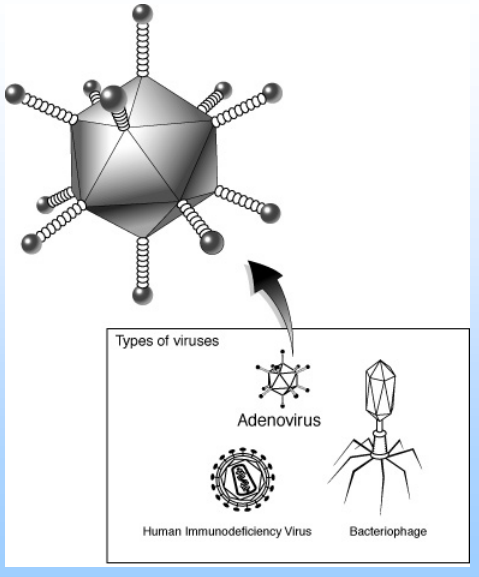
The Experimental Material for Biotechnologists

HIV - (Human Immunodeficiency Virus)



Structure of Human Immunodeficiency Virus (HIV)

Adenovirus



Retrovirus

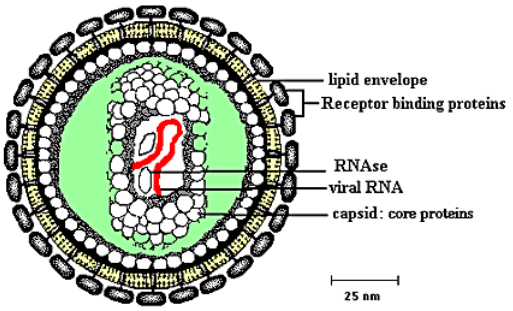
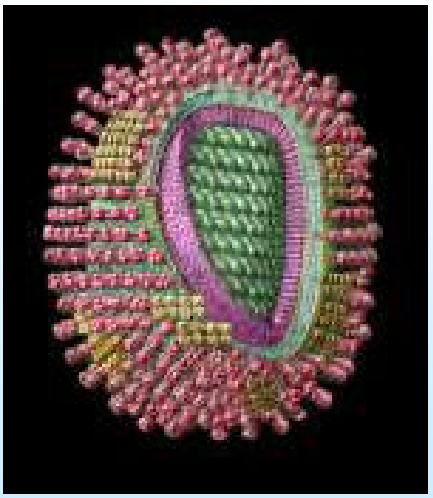


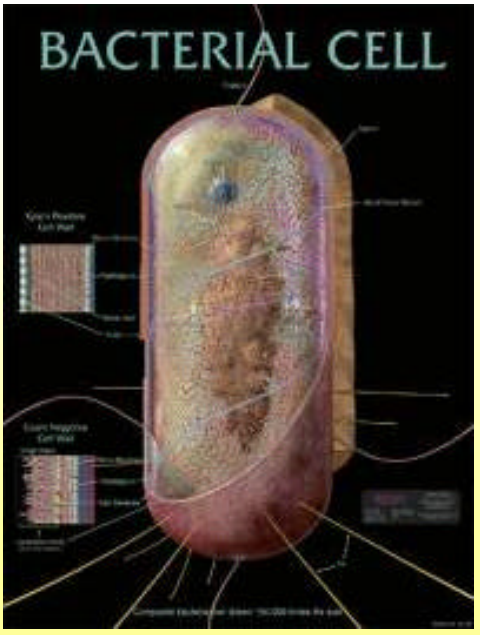
Diagram of a Retrovirus

Influenza Virus

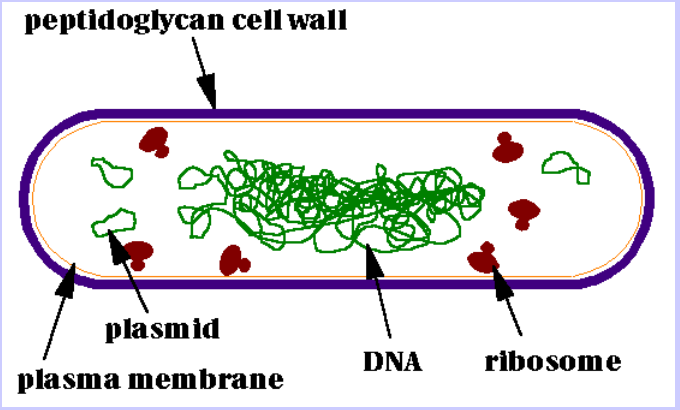


Bacteria

The most favorite of Biotechnologists



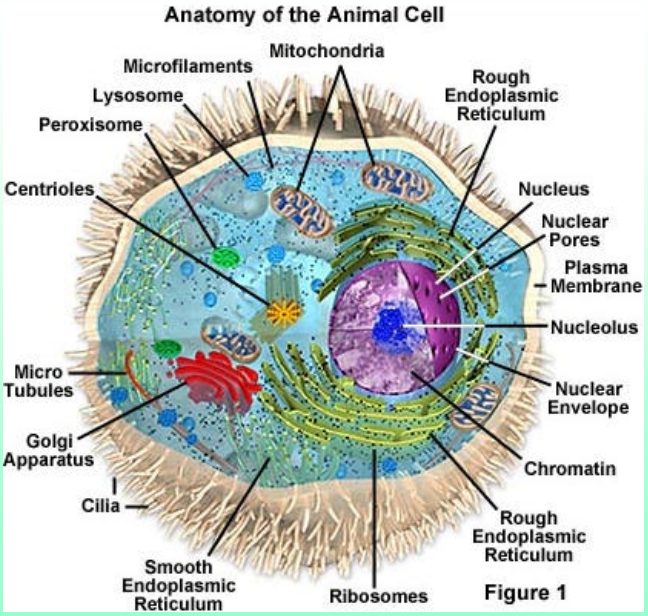
Bacteria: More on Morphology

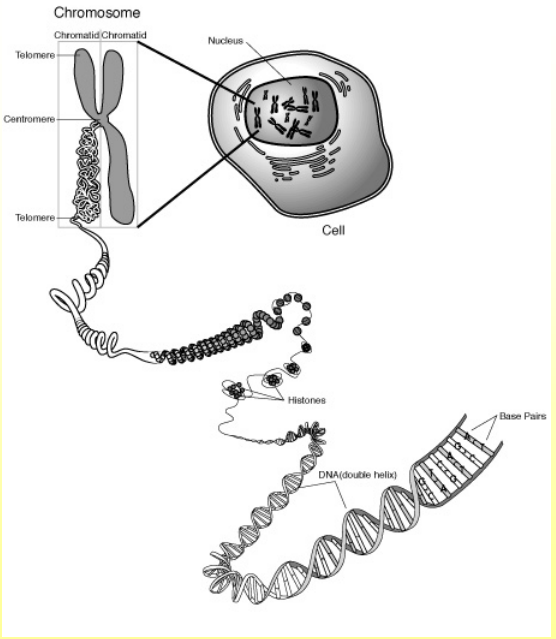


Animal or Human Cell

The most challenging for Biotechnologist to Engineer it

Human/Animal Cell Structure

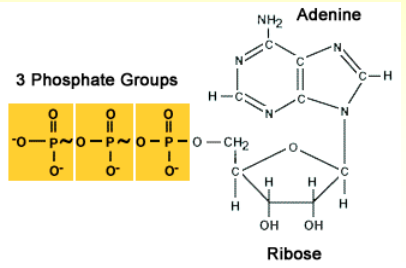


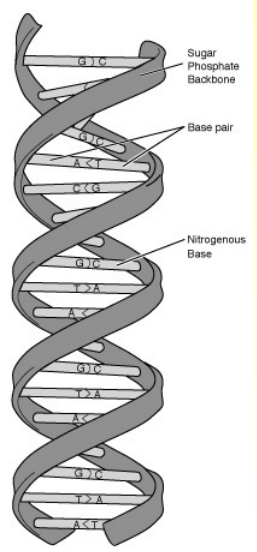


- **Chromosome**
- **Genes**
- **DNA**

Basic Genomics

- DNA
- Genes and other sequence information
- Base pairs A-T, C-G comprised of nucleotides
  - A-adenine
  - T-thymine
  - G-guanine
  - C-cytosine

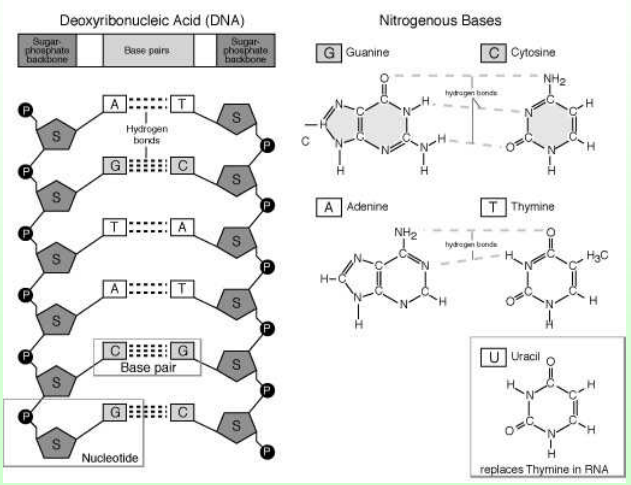




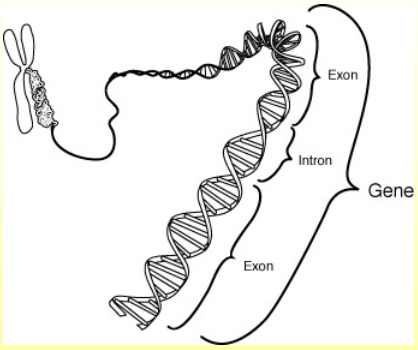
DNA:

The Secret of Life

Base Pairs - A More Detailed Picture



Where Genes Are Located?



Basic Proteomics

Proteins

↑

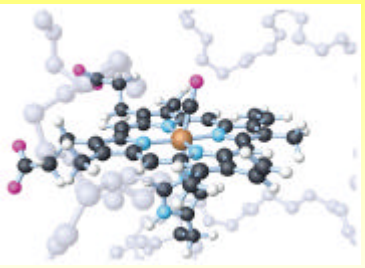
Polypeptides

↑

Peptides

↑

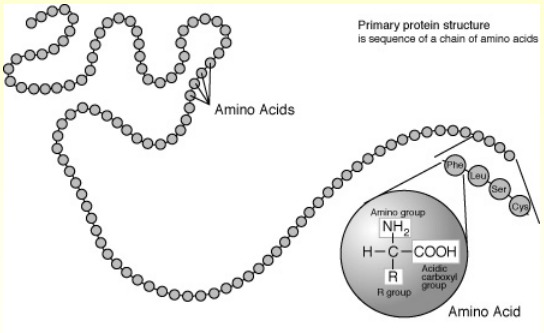
Amino Acids



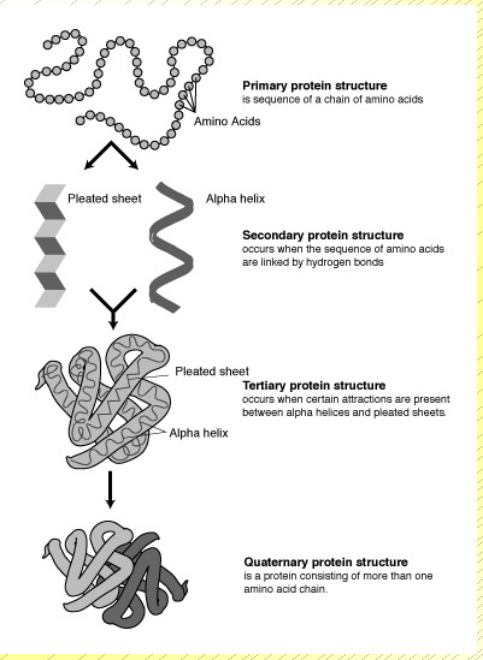
Hemoglobin

Amino Acids:

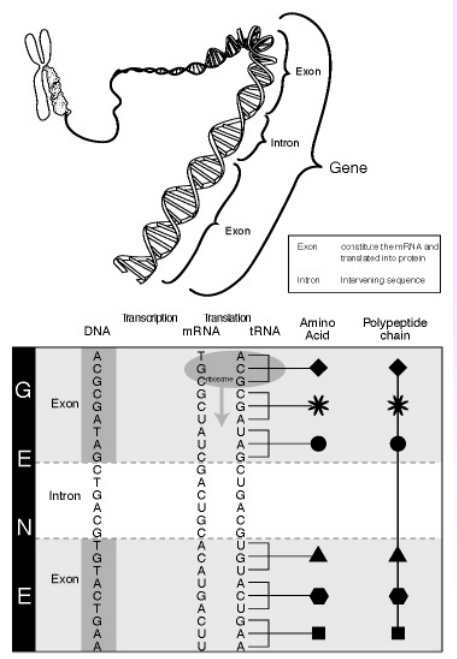
The basic units of the protein

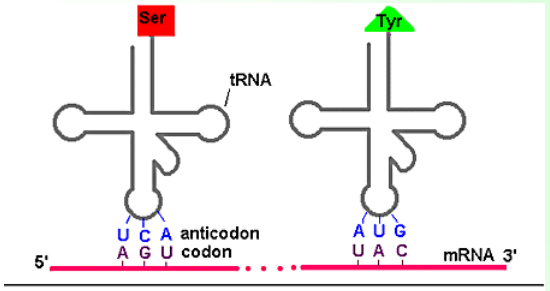


Proteins



Gene - A More Detailed Description



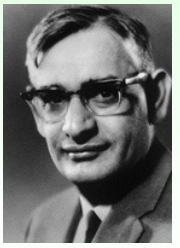


2nd base in codon

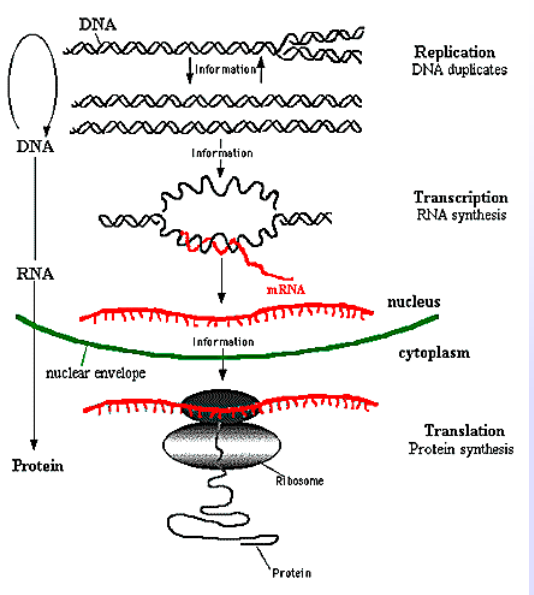
1st base in codon	U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The Genetic Code

The Genetic Code

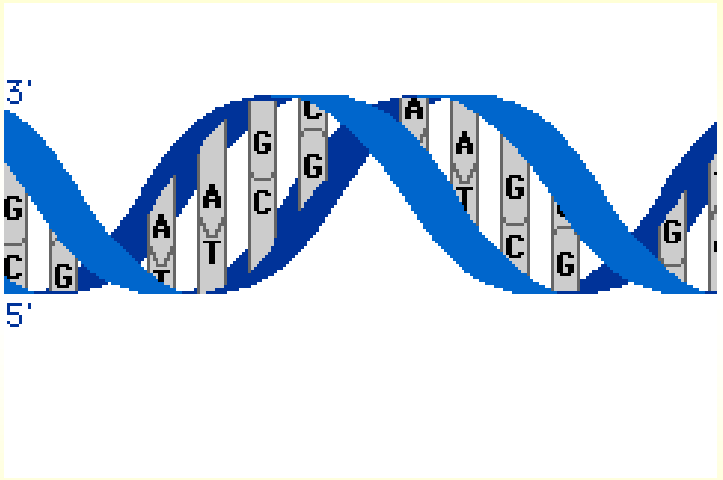


Har Gobind Khorana

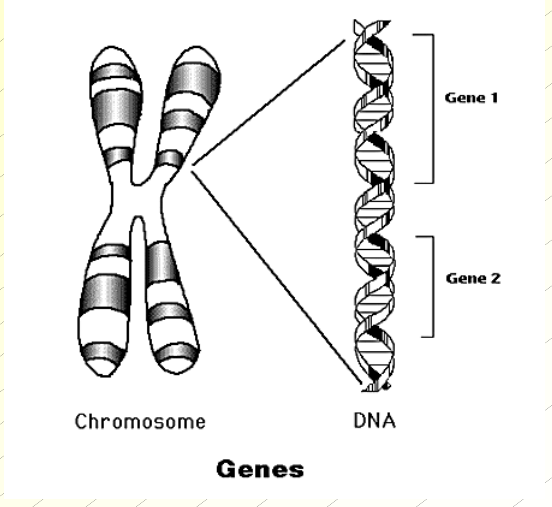


The Central Dogma of Molecular Biology

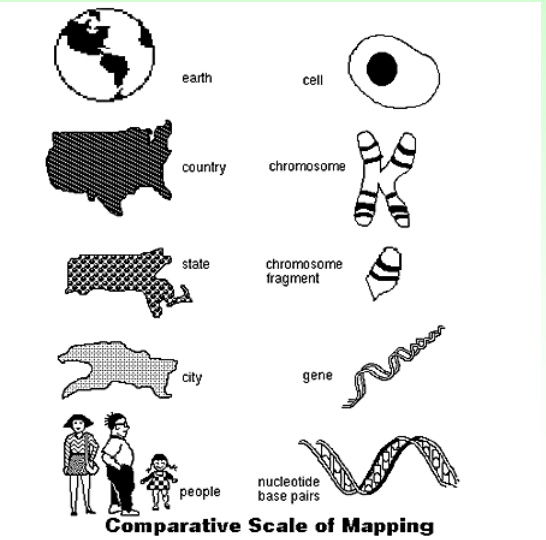
The Central Dogma of Molecular Biology



Genes & Genome



Comparative Scale of Mapping



Comparative Scale of Mapping

Human Genome Project

A globe-wide effort to to find out the sequence of DNA on all human chromosomes

Gene Facts

- size of human genome: 3.4 billion base pairs (bp)
- number of human genes: ~100,000 (was estimated but is 30,000 – 35, 000 now)
- genes vary in length and can cover thousands of bases
  - avg. size: ~3,000 bp
- only about 5% of the human genome contains genes
- function of much of the genome is unknown

Tools of Biotechnologist

- DNA/RNA isolation,
- Gel electrophoresis,
- Restriction Enzymes,
- PCR (Polymerase chain reaction),
- Blotting methods (Southern, Dot/Slot, Northern, Western),
- DNA Sequencing,
- Gene Expression
- Gene Cloning etc.

Nucleic Acid (DNA/RNA) Isolation/Purification

Sources:

Viruses, Bacteria and Virtually every Eukaryotic cell,

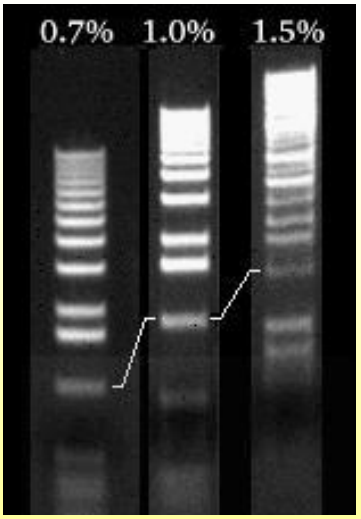
Human: blood

Gel Electrophoresis

Agarose Gel Electrophoresis

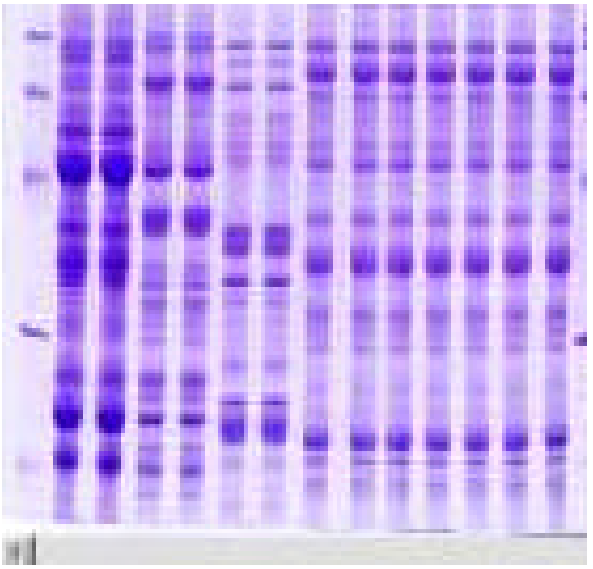
Pulse Field Gel Electrophoresis

Polyacrylamide Gel Electrophoresis



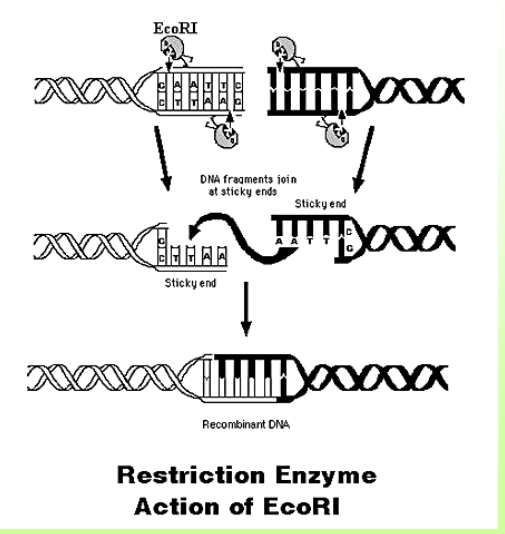
Agarose Gel Electrophoresis of DNA

Polyacrylamide Gel Electrophoresis



Restriction Enzymes

Selective Scissors for Cutting at specific sites in DNA

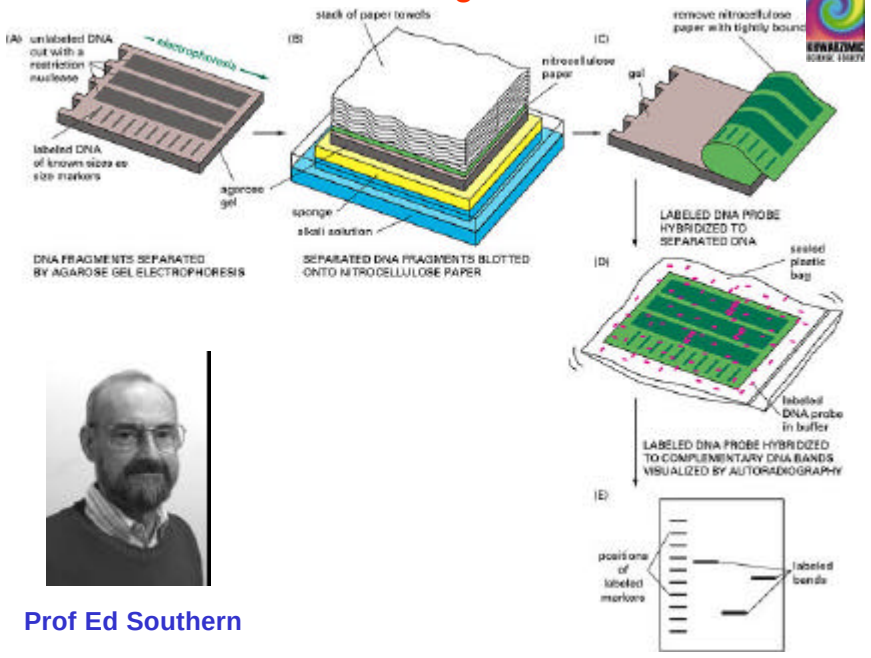


Restriction Enzyme Action of EcoRI

Blotting Methods

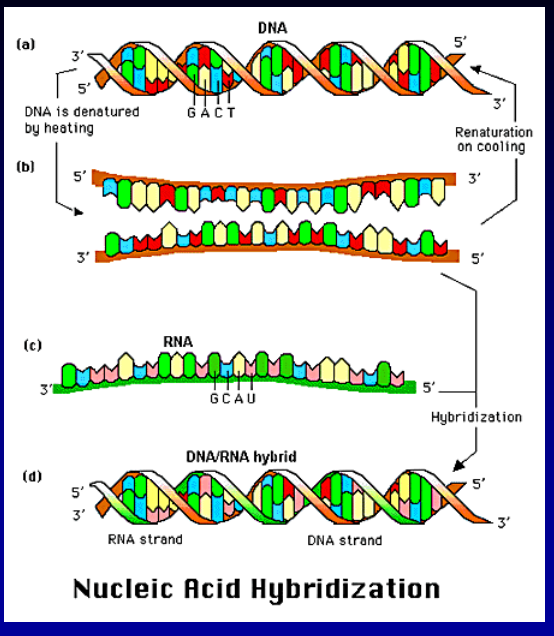
- Southern Blotting
  - Dot/Slot Blotting
- Northern Blotting

Southern Blotting: Gel Transfer

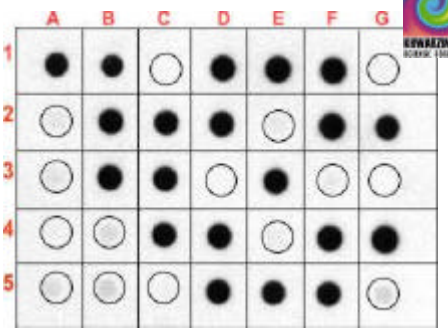


Prof Ed Southern

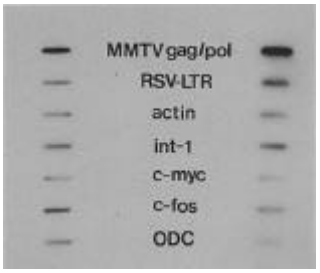
Nucleic Acid Hybridization



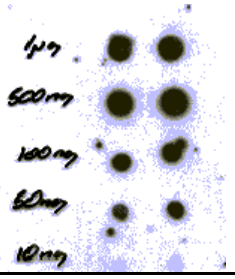
Dot-Blot



Slot-Blot



Reverse Dot Blot



Polymerase Chain Reaction

Amplification of fragments of DNA or Genes

Requirements:

DNA sample,

Primers,

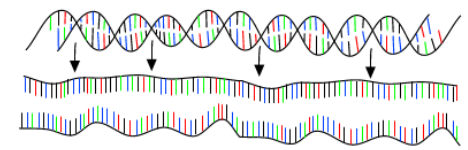
dNTPs,

Taq Polymerase enzyme,

Thermal Cycler

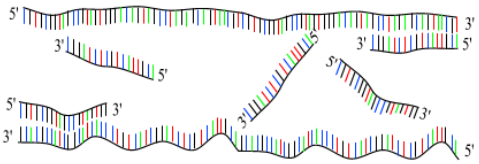
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

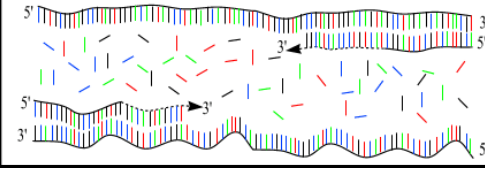
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



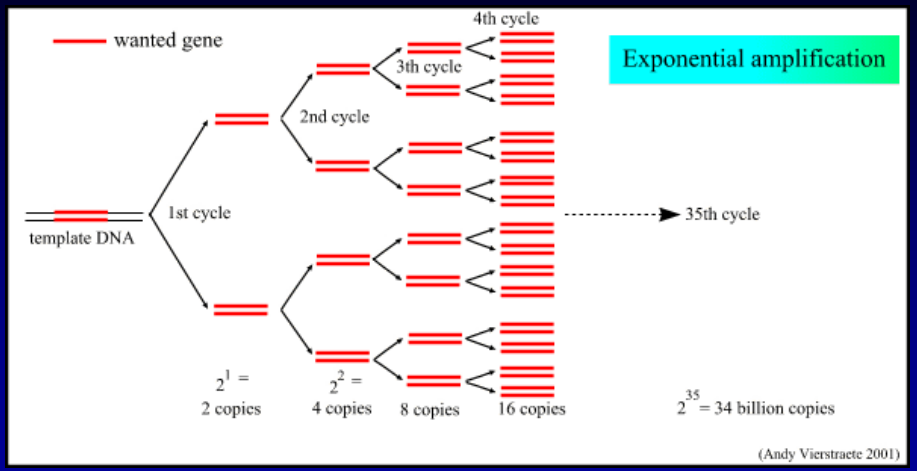
Step 3 : extension

2 minutes 72 °C

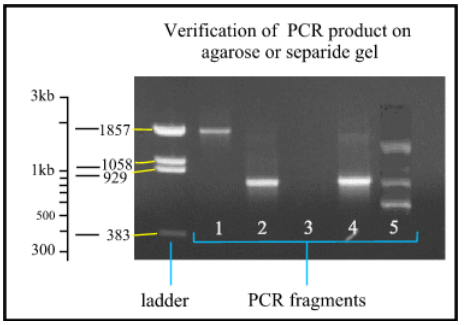
only dNTP's

(Andy Vierstraete 1999)

PCR Product



PCR Product Analysis



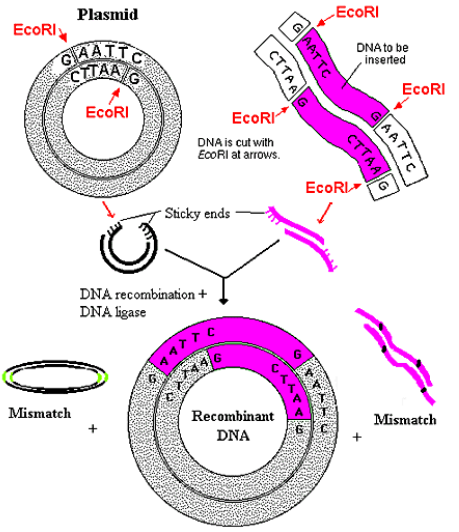
DNA Sequencing

Gene Cloning

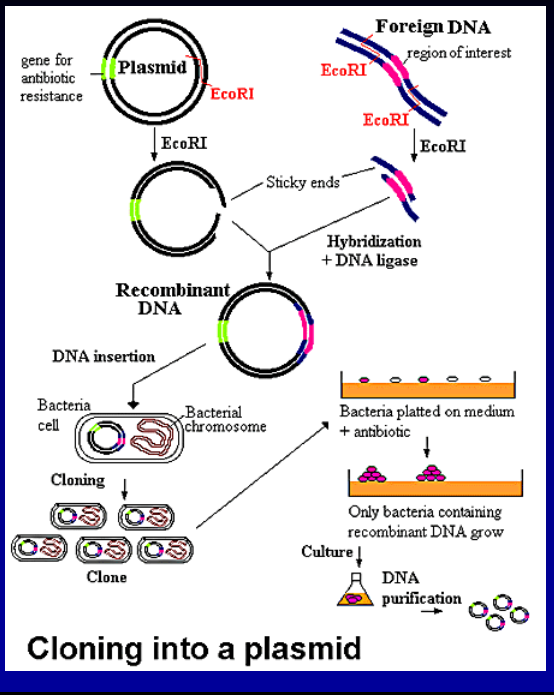
Gene Cloning: Vectors

- Plasmid
- Bacteriophage lambda
- Cosmid
- Yeast artificial chromosome or (YAC)

Inserting a DNA Fragment into a Plasmid

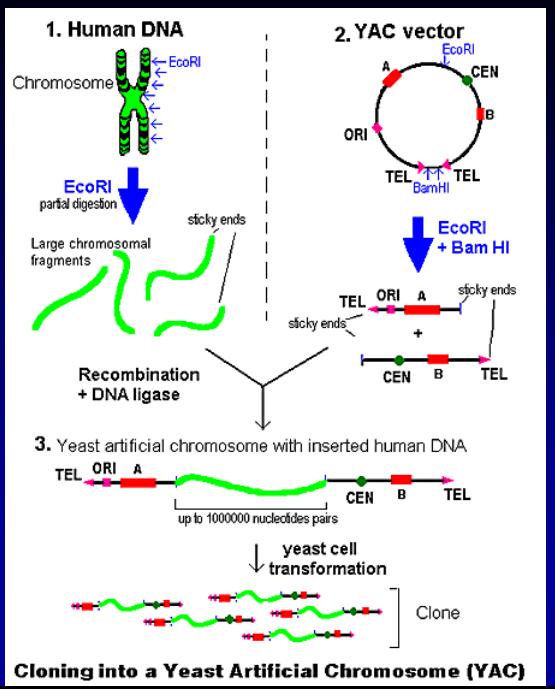


Inserting a DNA Sample into a Plasmid

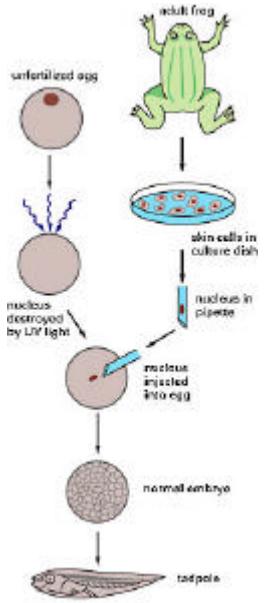


Cloning into a plasmid

Cloning into a Plasmid

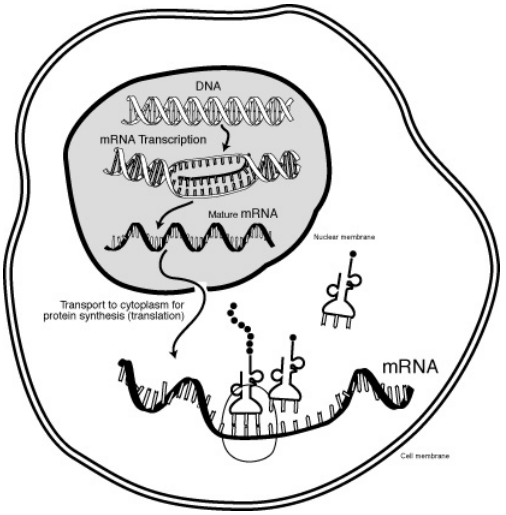


Cloning into a Yeast Artificial Chromosome (YAC)

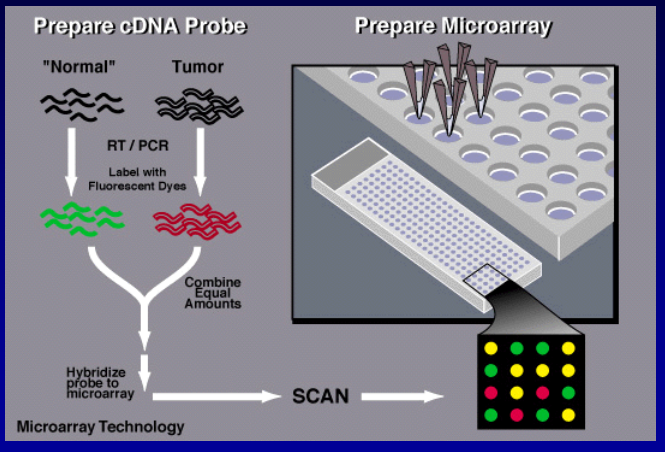


Cloning from Adult Vertebrate Cells

Gene Expression



Microarrary Technology



Drugs and Therapeutics

Antibiotics, Hormones, Proteins
Anitbodies, and Genetic vaccines.

Biotechnologists now "program" bacteria/cells
to make many other types of drugs

Safer, Cheaper Medicines From Biotechnology

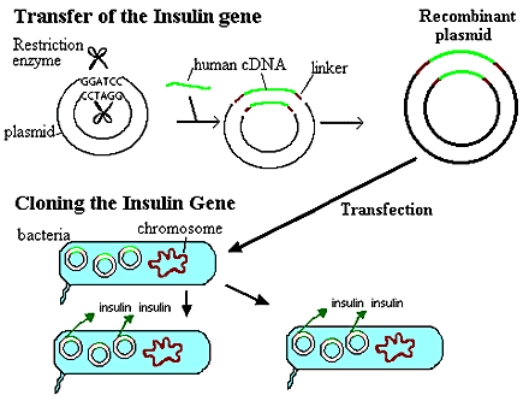
Medicines from ‘cultured’ cells and Microorganisms

Antibiotic Production

A bacterium – *Streptomyces* – is a source of antibiotics, immunosuppressants, anti-cancer and anti-parasitic agents.

- Penicillin
- Streptomycin for the treatment of tuberculosis
- Hybrid antibiotics

Transfer and Cloning of the Insulin Gene

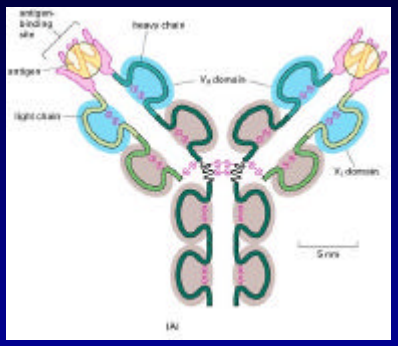


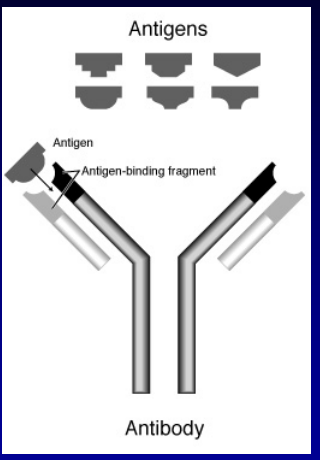
Transfer and cloning of the Insulin gene

Therapeutics

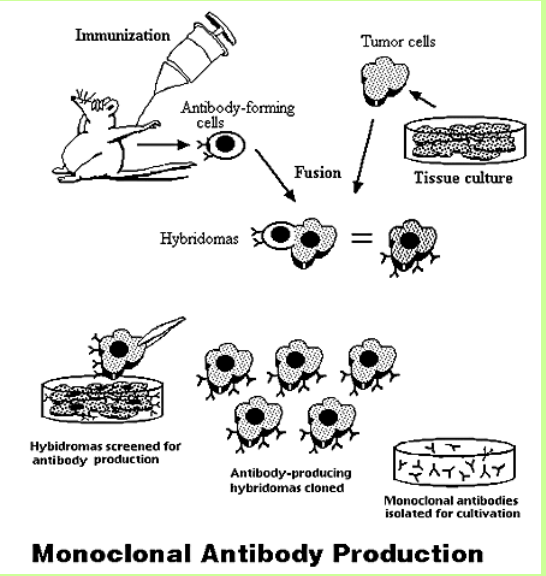
- Erythropoietin: for anemia
- Glucocerebrosidase (Cerozyme): Gauchers disease
- Growth hormone
- Factor VIII: Haemophilia factor replacement
- Facto IX:……
- Interferon alpha-2b: for HBV and HCV infections

Antibodies





Monoclonal Antibody Production



Genetic Vaccines

DNA Vaccines

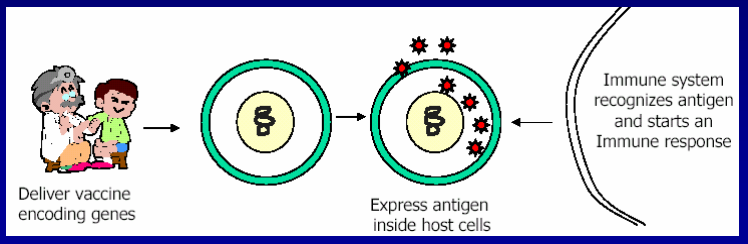
DNA vaccines are possibly the most hopeful and powerful alternative to traditional vaccines.

A genetically engineered vaccine is already widely used against the liver infection hepatitis B

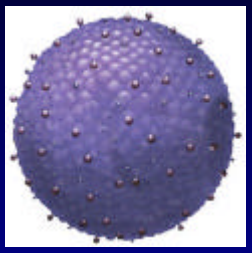
DNA Vaccines: What are they?

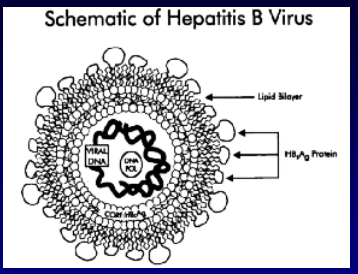
Recombinant antigen proteins etc.

DNA vaccines: Genes encodes for antigenic proteins of interest



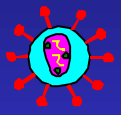
Hepatitis B Virus (HBV):





A recombinant HBsAg vaccine produced in yeast

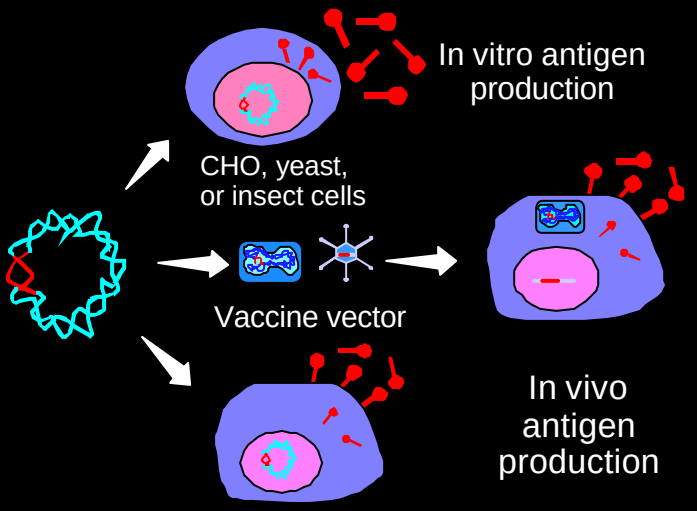
Traditional Approaches to Vaccine Development



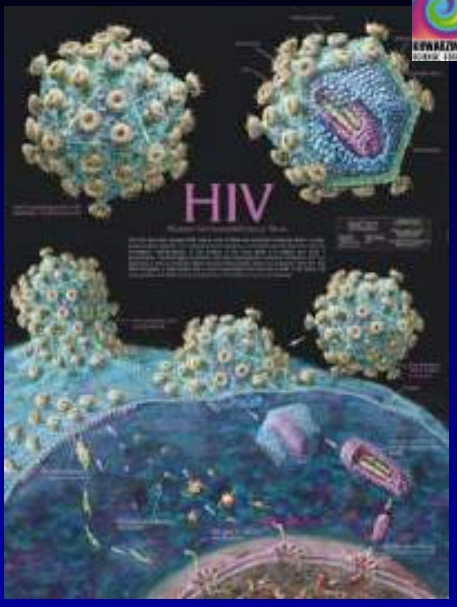
Live attenuated virus

Whole killed virus

Recombinant DNA Technology and Vaccine Development



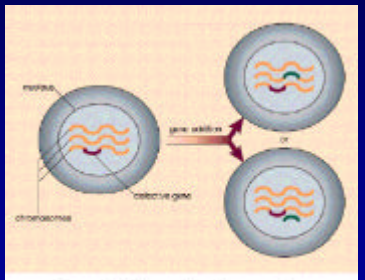
A waiting candidate for vaccine



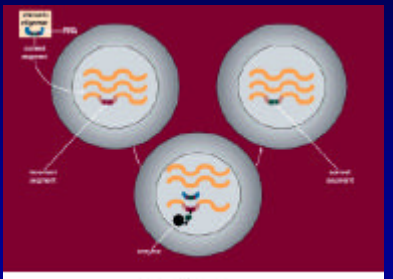
Gene Therapy

What is Gene Therapy?

Correction or Replacement of
a Disease causing Gene



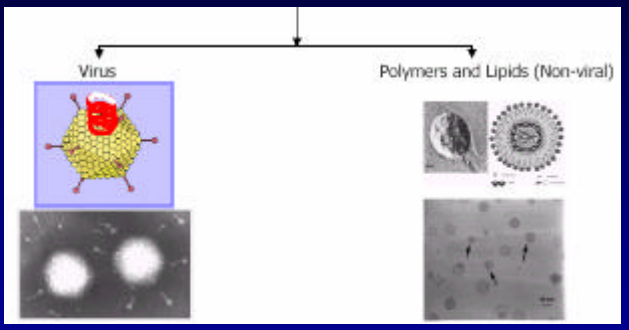
Gene Addition or Replacement

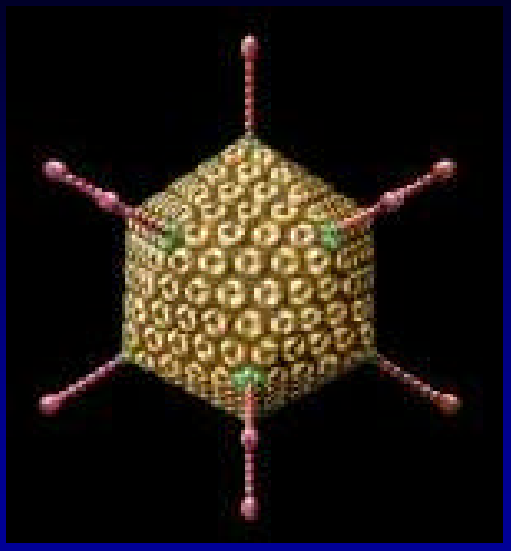


Gene Repair

How can we put genes inside cells?

We need a carrier





Adenovirus

Gene therapy is being used for ;

- **ADA (adenosine deaminase),**
- **Haematopoetic diseases,**
- **PKU,**
- **Cystic fibrosis**

Molecular Diagnosis

Infectious Diseases

e.g. HCV, Tuberculosis

Genetic Diseases

e.g. Chromosomal abnormalities, Thalassemias

Molecular Techniques

• Dot-blot, Southern blot, in-situ hydridization,

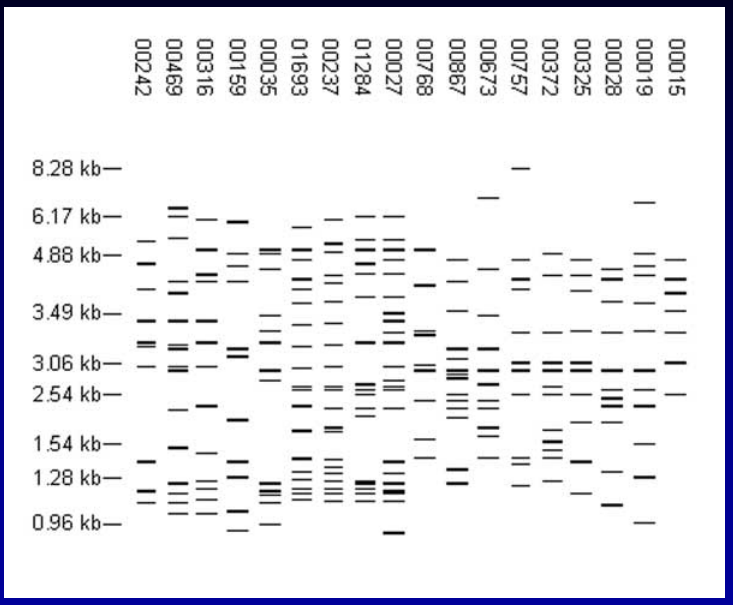
•PCR and related techniques

TUBERCULOSIS

Identification and Molecular Characterization of Changes Associated with Multi-drug Resistance among Isolates of *M.tuberculosis*

PCR-based methods are now being developed for identification of drug resistant strains

DNA Fingerprinting of *M. tuberculosis*



Diagnosis of Hepatitis C

PCR – Positive in >95% of infected individuals.

Currently the most sensitive tests available.

the 5'-untranslated region (UTR)
is used for genotyping of HCV

HCV Diagnosis

Blood/Serum

↓

RNA Isolation

↓

cDNA

↓

RT-PCR

↓

Gel Electrophoresis

↓

Results Analysis

Diagnosis of Genetic Diseases

(e.g. Thalassemia and Chromosomal abnormalities)

Leukaemia and lymphoma

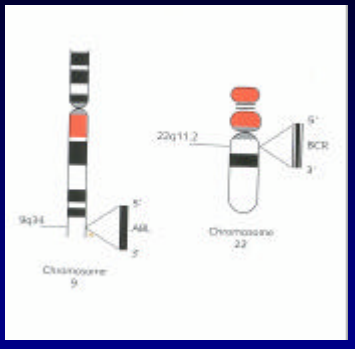
Chromosomal translocations

Dysregulation of a proto-oncogene or alternatively the formation of a chimeric fusion gene

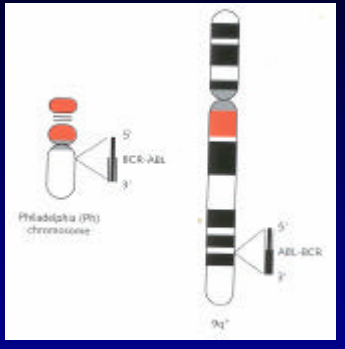
These translocations are demonstrable by PCR

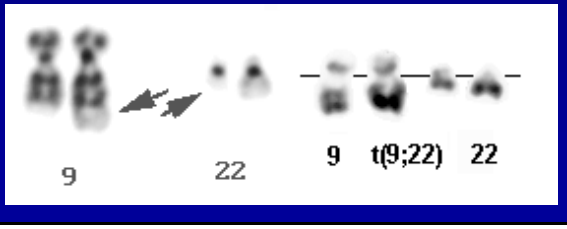
Leukemias: CML, AML & ALL common translocations by RT - PCR

Normal configuration of chromosomes 9 and 22

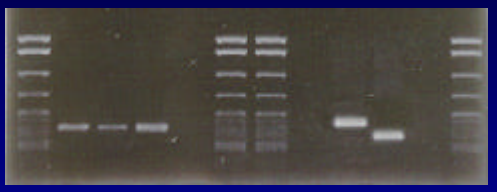


Rearranged chromosome 9 ($9q^+$) & 22 (Ph)





ABL-ABL BCR-ABL



M 1 2 3 4 M M 1 2 3 4 M

Diagnosis by Molecular Methods

Thalassemias (alpha & beta),

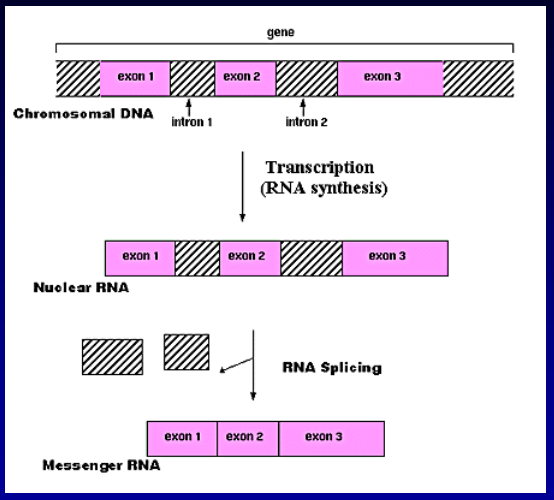
Sickle Cell Disease,

Duchenne Muscular Dystrophy,

Hemophilia

HLA Typing

Beta-Globin Gene



Beta-Thalassemia Diagnosis

Blood

↓

DNA Isolation

↓

PCR (i.e. ARMS-PCR)

↓

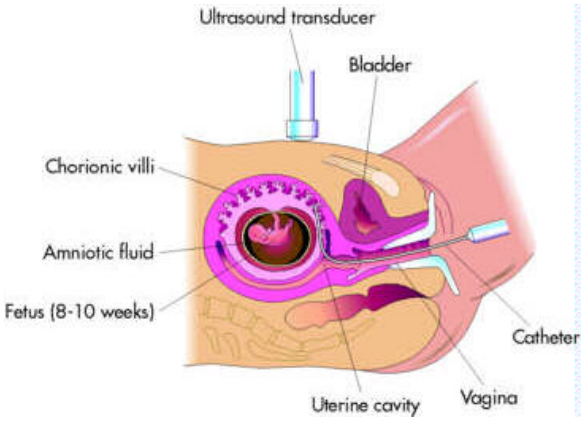
Gel Electrophoresis

↓

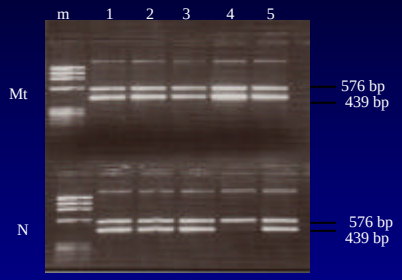
Mutation Analysis

Prenatal Diagnosis

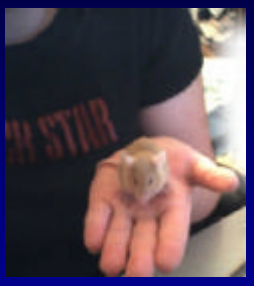
Chorionic villus sampling



Prenatal diagnosis of genetic diseases e.g. thalassemias.



Transgenic Animals



Transgenic animals:

The models of diseases for better understanding of disease course and pathology.

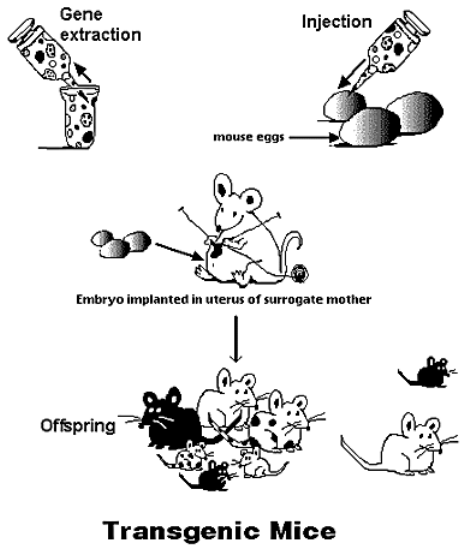
Tay–Sachs and Sandhoff Mice Models

b-hexosaminidase isoenzymes

Hexa -/- (a null)

Hexb -/- (b null)

Transgenic Mice



Conclusion

The future findings of DNA technology can open new methods and procedures in different parts of medicine and answer so many questions which have not been detected until now.

Family of Biotechnology is expanding

- **Transcriptomics**
- **Proteomics**
- **Structural genomics**
- **Glycomics**
- Metabonomics

+ ??….omics

Public awareness and understanding of these scientific advancements.

- **Privacy and confidentiality of genetic information.**
- **Fairness in the use of genetic information**
- **Psychological impact, stigmatization, and discrimination**
- **Uncertainties associated with gene tests for susceptibilities and complex conditions**
- **Health and environmental issues**
- **Commercialization of products**

Are We Aware of These Issues

???

??

?

More to Explore

Scientific American

New Scientist

Nature

Science

+ INTERNET

Thanks to,

All the websites for literature,

KSS Team,

IBB