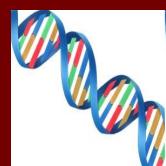
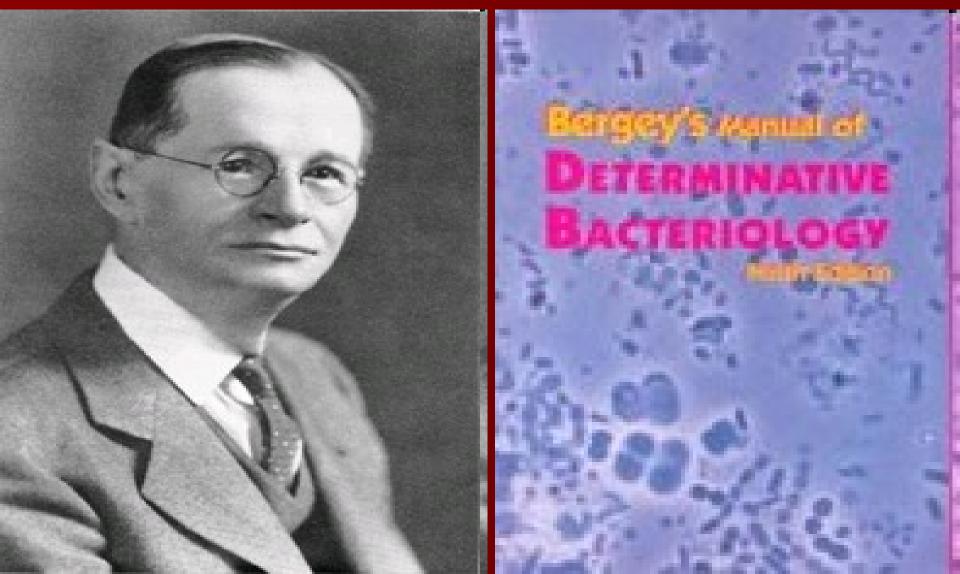
# **Molecular Biology** *Principles and Basic Tools*

Dr.T.V.Rao MD



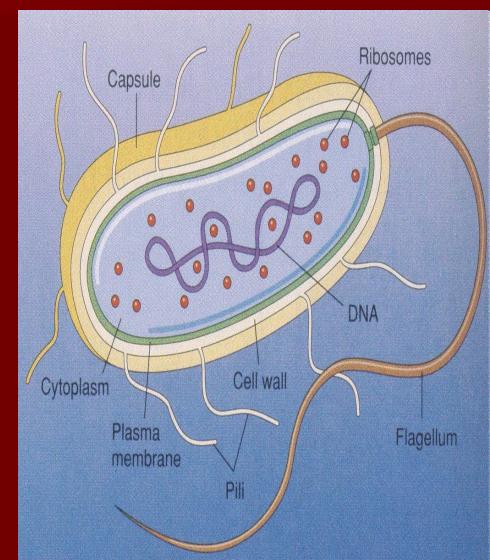
### David H. Bergey, Bergey's Manual identifies the Bacteria to Minute details



# **Traditional Microbial Diagnostic Methods**

- Morphology
- Staining properties
- Grow on different laboratory conditions and media.
- Phenotyping protocols use purely biological phenomena and usually refer to study of Proteins and enzymes.

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# Disadvantages of Phenotyping Methods

- Lack of Reproducibility
- Poor Discriminatory power
- Difficulties in Typing
- Genomic changes with Antibiotic resitance patterns
- Technical manpower costs. (Developed World)

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#### **Molecular Biology - Definition**

Molecular biology is the study of biology at a molecular level. The field overlaps with other areas of biology and chemistry, particularly genetics and biochemistry.

**Molecular Biology Interdisciplinary Science** Molecular biology chiefly concerns itself with understanding the interactions between the various systems of a cell, including the interactions between DNA, RNA and protein biosynthesis as well as learning how these interactions are regulated

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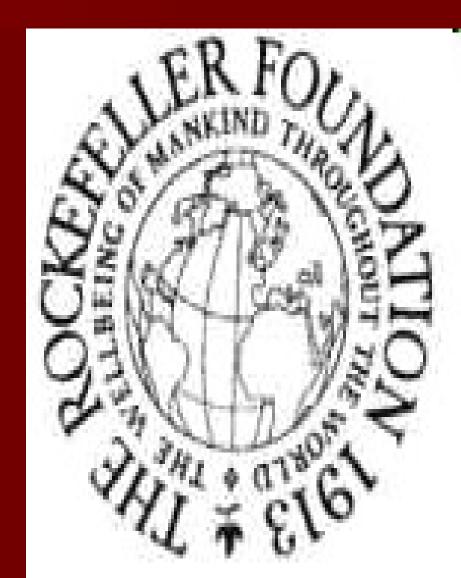
# **Beginning of Molecular Biology**

The Modern of molecular biology begins in the 1930s with the convergence of various, previously distinct biological disciplines: biochemistry, genetics, microbiology, and virology. With the hope of understanding life at its most fundamental level, numerous physicists and chemists also took an interest in what would become molecular biology.

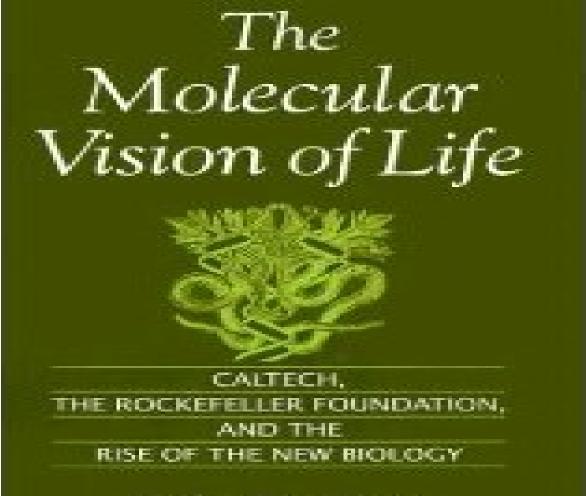
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# Warren Weaver

While molecular biology was established in the 1930s, the term was first coined by Warren Weaver in 1938. Warren was the director of Natural Sciences for the Rockefeller Foundation...



# Early Vision on Molecular Biology



Lily E. Kay

# **Watson and Crick**

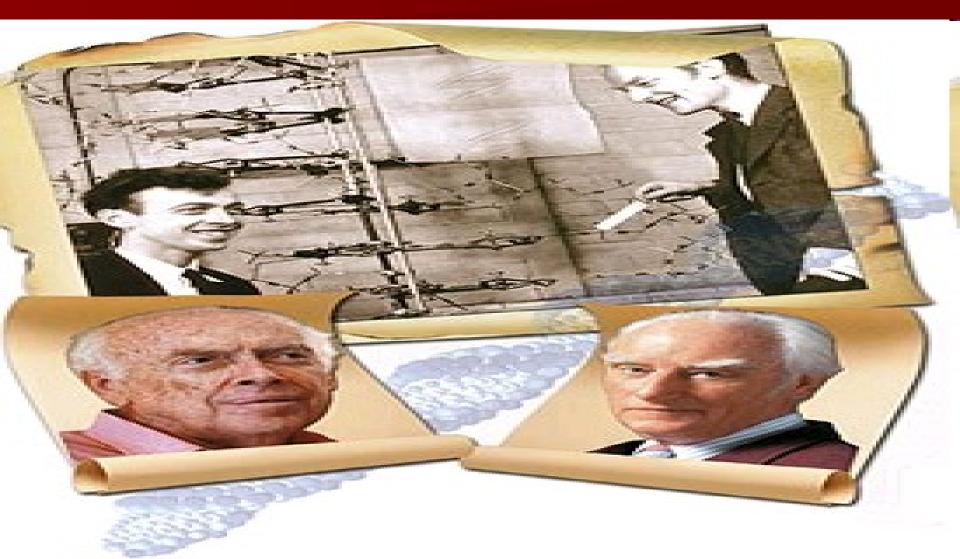
The structure of DNA was described by British Scientists Watson and **<u>Crick</u>** as long double helix shaped with its sugar phosphate backbone on the outside and its bases on inside; the two strand of helix run in opposite direction and are antiparallel to each other. The DNA double helix is stabilized by hydrogen bonds between the bases

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# Watson and Crick discovers DNA / Feb 28<sup>th</sup> 1953



# Watson and Crick Builds a Model DNA 7<sup>th</sup> March 1953



#### First Document on DNA published in Nature 25<sup>th</sup> April 1953

(Reprinted from Nature, Vol. 171, p. 737, April 25, 1953)

#### MOLECULAR STRUCTURE OF NUCLEIC ACIDS

#### A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey<sup>1</sup>. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

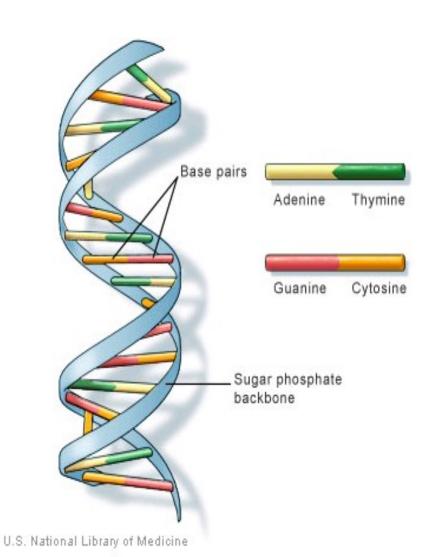
We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining  $\beta$ -D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's<sup>2</sup> model No. 1 ; that is, the bases are on the inside of the helix and the phosphates on the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same

# Watson and Crick - Awarded Nobel Prize in 1962

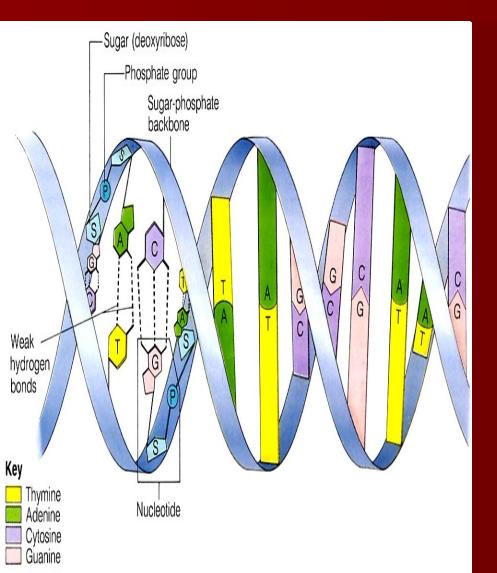


# **DNA - Structure**

The nucleotide, however, remains as the fundamental unit (monomer) of the nucleic acid polymer. There are four nucleotides: those with cytosine (C), those with guanine (G), those with adenine (A), and those with thymine (T).



# DNA

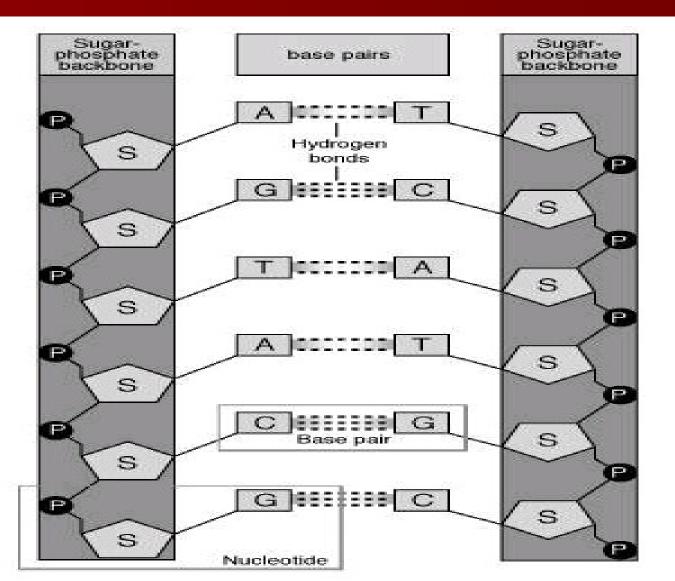


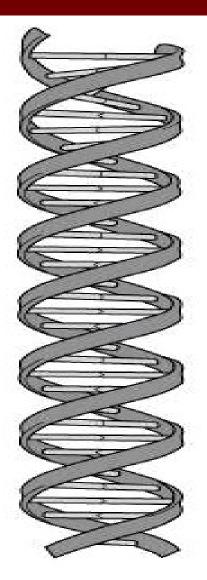
A purine always links with a pyrimidine base to maintain the structure of DNA.

Adenine ( A ) binds to Thymine ( T ), with two hydrogen bonds between them.

Guanine (G) binds to Cytosine (C), with **three hydrogen bonds** between them.

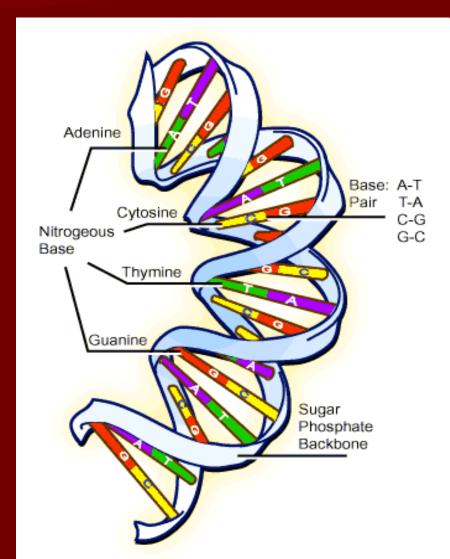
# **Chemical structure of DNA**





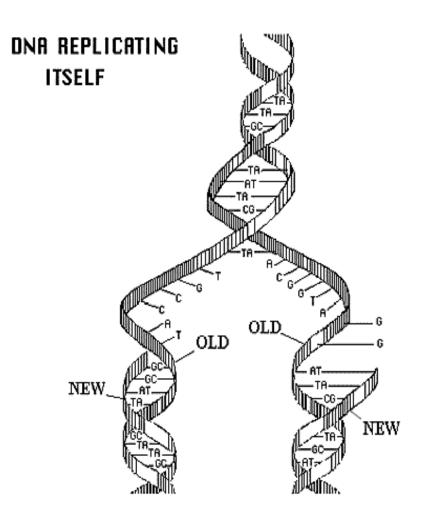
# **DNA is Endless structure**

- The rungs of the ladder can occur in any order (as long as the base-pair rule is followed)
- Those 4 bases have endless combinations just like the letters of the alphabet can combine to make different words.



# **DNA makes a Copy of Self**

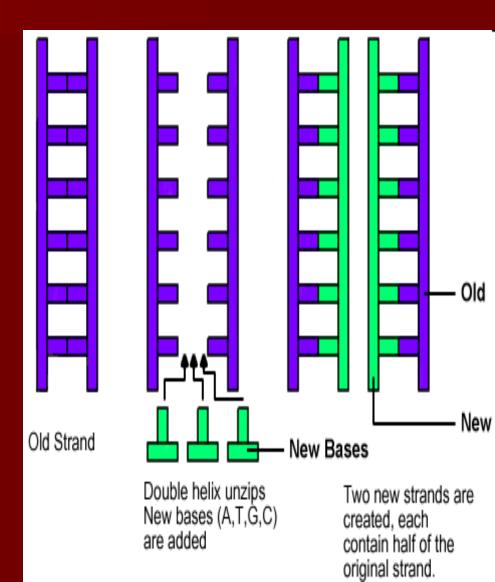
Replication is the process where DNA makes a copy of itself. Why does DNA need to copy? Simple: Cells divide for an organism to grow or reproduce, every new cell needs a copy of the DNA or instructions to know how to be a cell. DNA replicates right before a cell divides.



# **DNA Replication**

DNA replication is semi-conservative. That means that when it makes a copy, one half of the old strand is always kept in the new strand. This helps reduce the number of copy errors.

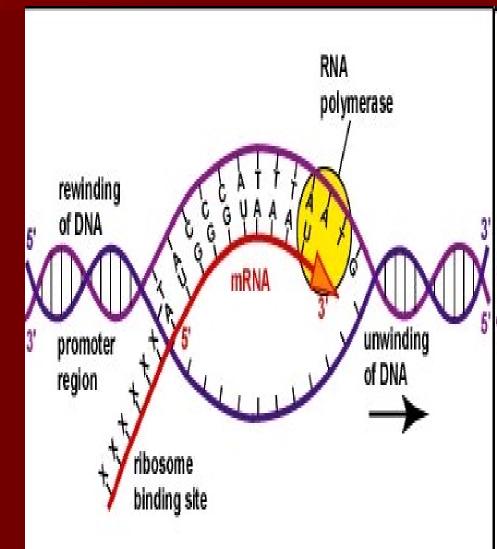
So we remained what we were ?



# DNA to RNA creates functional translations

DNA remains in the nucleus, but in order for it to get its instructions translated into proteins, it must send its message to the ribosome's, where proteins are made. The chemical used to carry this message is **Messenger RNA** 

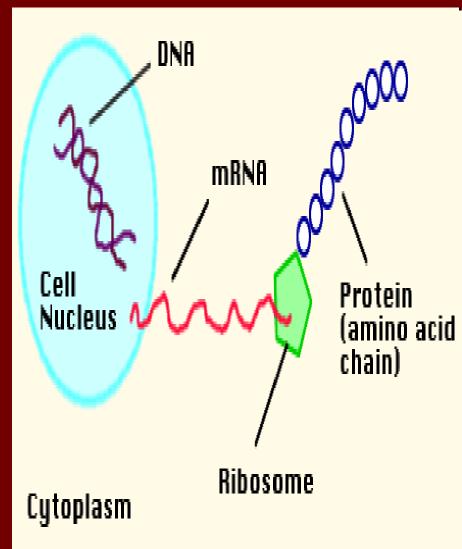
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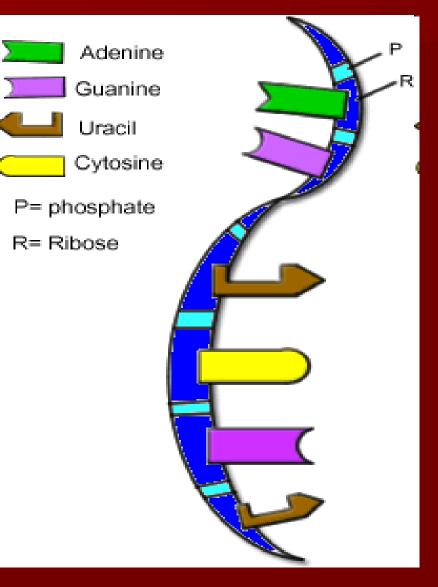
# DNA – RNA – DNA a never ending cycle

- RNA has the job of taking the message from the DNA to the nucleus to the ribosome's.
- Transcription RNA is made from DNA
- Translation Proteins are made from the message on the RNA

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# **RNA = Ribonucleic acid.**



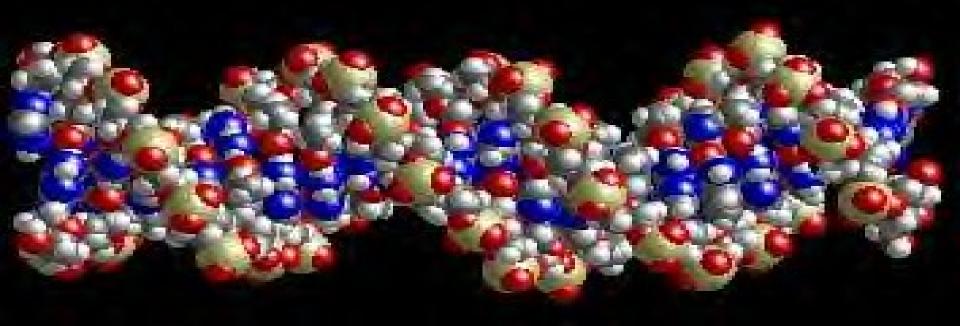
#### RNA is similar to DNA except:

It has one strand instead of two strands. Has uracil instead of thymine 3.Has Ribose instead of Deoxyribose

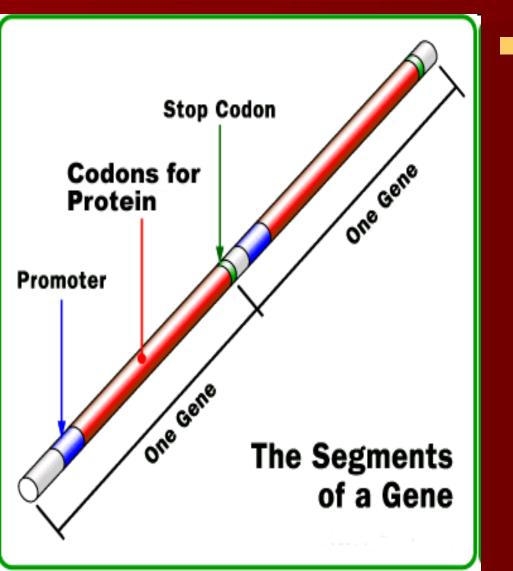


- Example
- First strand
- Second strand

# GGGTTTAAACCC CCCAAATTTGGGG

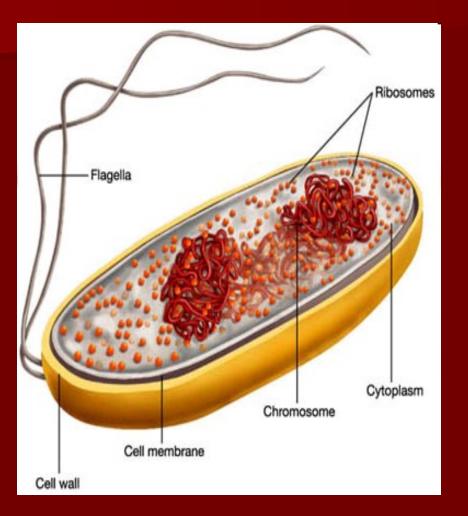


# What is Gene



The gene, the basic units of inheritance; it is a segment within a very long strand of **DNA\_with specific** instruction for the production of one specific protein. **<u>Genes</u>** located on chromosome on it's place or locus.

# **Bacterial Chromosome**



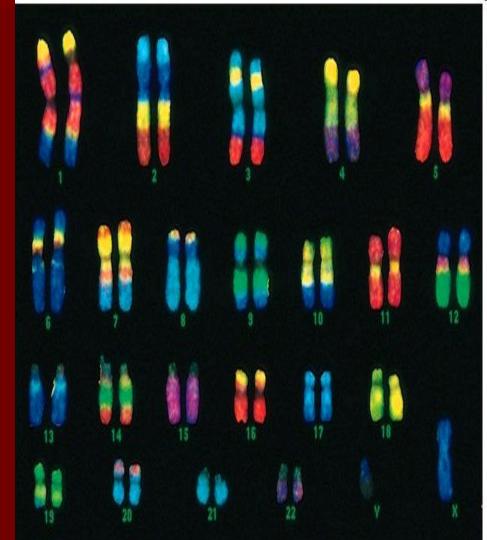
 The bacterial DNA contains ds DNA arranged in circular form 1,000 microns in length

The bacterial DNA is about 4,000 kb

The Human genome is about 3 million kb

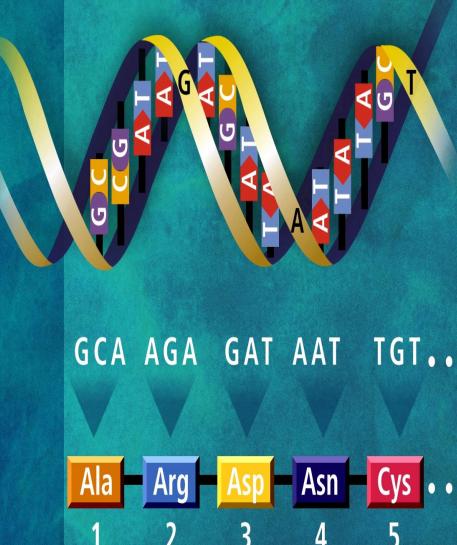
# Chromosomal variation defines the Species

- The Chromosome, the storage place for all genetic information, the number of chromosomes varies from one species to another.
- The human genome is complex contains 3 million kb.
- Non functional codons called as introns
- Functional are exons
- All sequences multiply

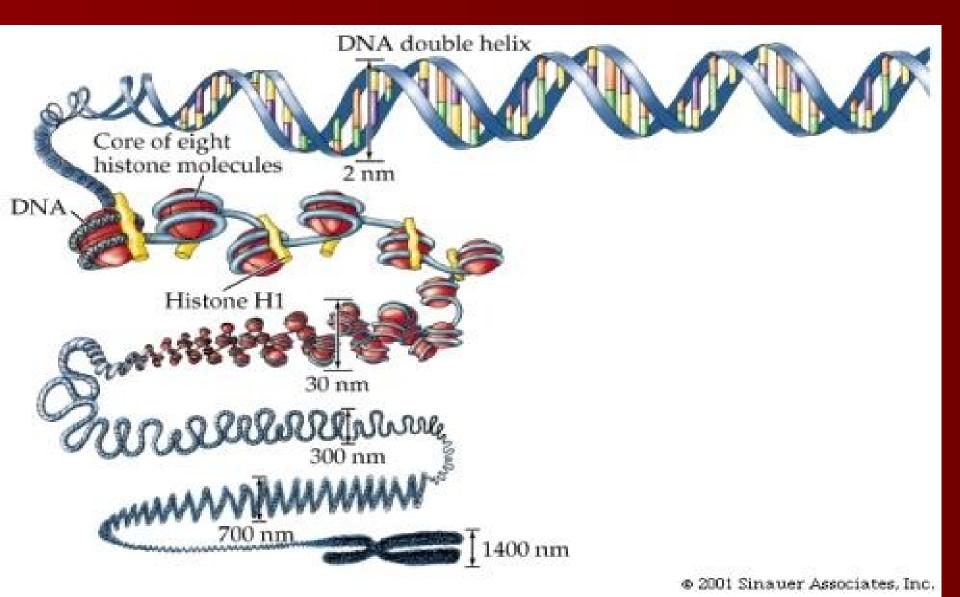


# Genome defines total Information

The genome of an organism is the totality of genetic information and is encoded in the **DNA** (or, for some viruses, RNA).



#### **Genomic DNA Organization**



# **Biology to Molecular Biology**

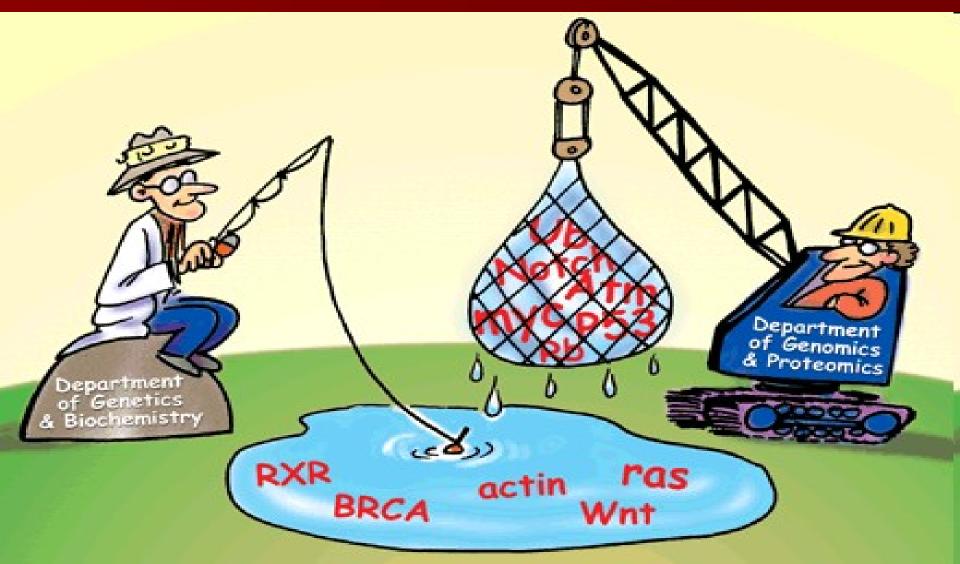
- Molecular biology; the study of gene structure and functions at the molecular level to understand the molecular basis of hereditary, genetic variation, and the expression patterns of genes.
- The Molecular biology field overlaps with other areas, particularly genetics and biochemistry
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# Data base Libraries can be created

- The genome database is organized in six major organism groups: eukaryotes, bacteria, archaea, viruses, viroids and plasmids.
- All living things are grouped into three domain eukaryotes; prokaryotes and archaea.

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# **Every body fishing for New Ideas**



#### **Central Dogma of Molecular Biology**

Replica	tion DNA	mRNA		ranslation synthesis)
xoodoox =	± x000x → 200E	40000-		P
	Transcripti (RNA synth	on F nesis)	≀ibosome	$\sim \sim$
			· · · · · · · · · · · · · ·	Protein
	DNA		RNA	Protein

# **Common Tools of Molecular**

**Biology** 

- Nucleic acid fractionation
- Polymerase chain reaction
- Probes, Hybridization
- Vector, Molecular cloning
- Nucleic acid enzymes
- Microarray
- DNA sequencing
- Electrophoretic separation of nucleic acid
- Detection of genes:

**DNA:** Southern blotting; inSitu hybridization; FISH Technique

**RNA:** Northern blotting

Protein: Western blotting,

immunohistochemistry

# Molecular Biology Interdisciplinary approach

Molecular biology is facilitating research in many field including biochemistry, microbiology, immunology and genetics,... ATGC CGAT saga continues to strengthens the hands of science.....

Various methods in molecular biology diagnose the different human diseases; diagnosis of an infectious agent, in malignancy, the presence of the genetic disease and in transplantation, paternity and forensic analysis.

# Current Uses of molecular Biology

The most recent applied technologies, genetic engineering, DNA finger-printing in the social and forensic science, pre and postnatal diagnosis of inherited disease, gene therapy and drug Design.

Molecular biology allows the laboratory to be predictive in nature, it gives information that the patients may be at risk for disease (future). Major tool in Diagnosis of Infectious

**Restriction Endonuleases** changes the Biology If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another.

## What are *Restriction Endonuleases*

- Restriction endonucleases are enzymes that cleave DNA molecules at specific nucleotide sequences depending on the particular enzyme used. Enzyme recognition sites are usually 4 to 6 base pairs in length.
- The recognition sequences are randomly distributed through the DNA and recognizes different nucleotide sequences, and snips through DNA molecule.

## **Types of Endonuleases**

- Type I split the target randomly
- Type II split the molecule only at recognition site
- Type III split the DNA at a fixed distance from the recognition site
- Type II and III played major role in identification of sequence of bases in DNA
- Today's genetic engineering and genethreapy dependent on Endonuleases.
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## Arber, Nathans and Smith – Nobel Prize 1978

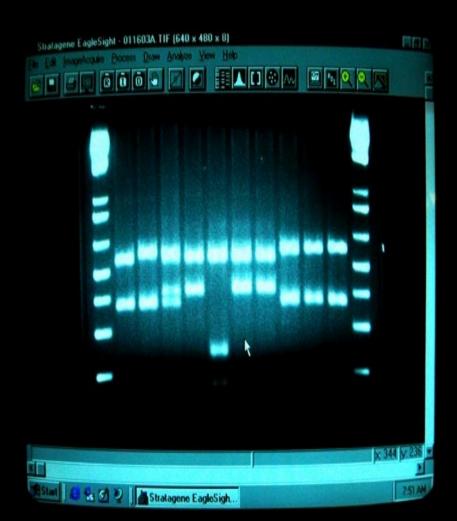
- The restriction endonulease is the basis of science of molecular genetics , genetic engineering or recombinant DNA technology
- Futher advances with Frederick Sanger, Walter Gilbert contribute to Nucleotide sequncesing DNA ligase.

#### **Restriction endonuleases**

- Restriction endonulease is an enzyme which cleave double sequence.
- The enzyme Eco RI HIND III Taq 1
- Causes of entry of foreign substances in cell.
- Restriction enzymes split DNA strands into fragments varying lengths
- Separated by gel electrophoresis and stained with ethydium bromide and photographed.

## Development of Gel electrophoresis

Berg, Sanger and Gilbert awarded Nobel prize in 1980 Gel electrophoresis reads nucleotide sequencing



## **Cutting DNA -Sticky ends**

The first type of enzyme are **restriction** endonulease. Their job is to cut a strand of DNA at a specific area of the code called a recognition site. Genetic engineers have a large number of restriction endonulease for different recognition sites so that cuts can be made in specific places.



## Sticky ends an Advantage to propagation of Life

			Re			Restriction endonuclease		
G	C	T	T	A	G	C	C	G
C	G	A	A	T	С	G	G	C
								с Істтіс

 $\begin{array}{c|c} G & |C & T & T & A & G & C & C \\ C & G & A & T & C & G & G \end{array}$ 

After the DNA has been removed using this enzyme you get sticky ends which is at the end of the fragment and is 'sticky' because it wants to become part of a DNA strand again.

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Sticky ends

#### **Signal Amplification Methods**

 Combines some type of nucleic acid usually probe with generation of signal.
The signal is amplified through enzymatic reaction eg as Signal from fluorescence in situ hybridization (FISH) may be directly observed

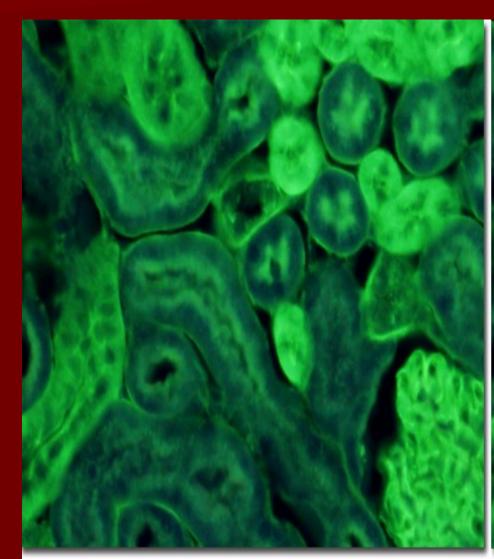
#### **Important Methods**

Nucleic acid probes
Hybrid capture
Branched chain DNA
Situ hybridization



### **Nucleic-acid probes**

Nucleic-acid fragment that is complementary to another nucleic-acid sequence and thus, when labelled in some manner, as with a radioisotope, can be used to identify complementary segments present in the nucleicacid sequences of various microorganisms



## Nucleic acid probe

Nucleic acid fragment, labelled by a radioisotope, biotin, etc., that is complementary to a sequence in another nucleic acid (fragment) and that will, by hydrogen binding to the latter, locate or identify it and be detected; a diagnostic technique based on the fact that every species of microbe possesses some unique nucleic acid sequences which differentiate it from all others, and can be used as identifying markers or "fingerprints."

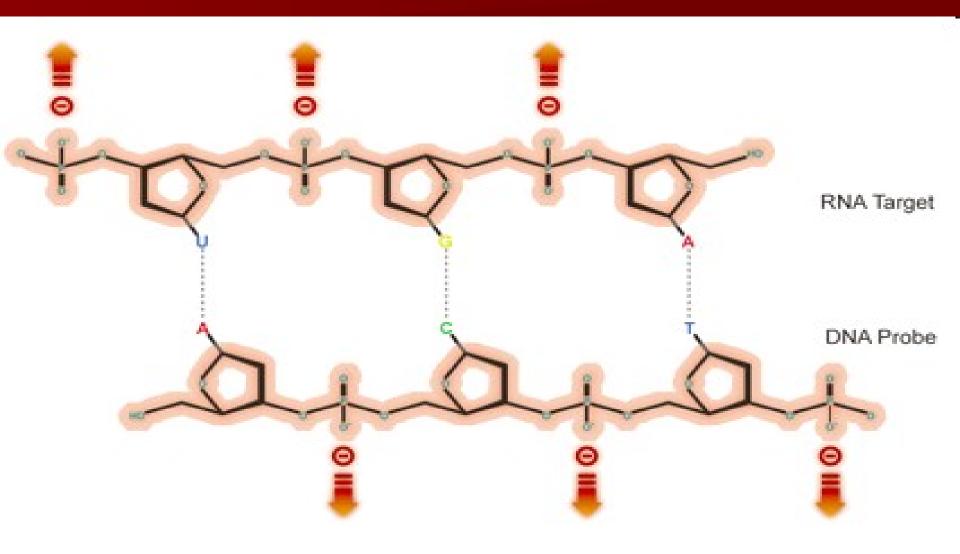
## **Hybridization probes**

Hybridization probe is a fragment of DNA or RNA of variable length (usually 100-1000 bases long), which is used to detect in DNA or RNA samples the presence of nucleotide sequences (the DNA target) that are complementary to the sequence in the probe. The probe thereby hybridizes to single-stranded nucleic acid (DNA or RNA) whose base sequence allows probetarget base pairing due to complementarily between the probe and target.



- Accu probes from Gene probe, In which it contain Chemiluminescent label and target the rRNA of the Microorganisms of interest.
- It reads events in vivo or during the multiplication of organims.
- The presence of light considered positive reaction
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#### Probes identifies related Molecules

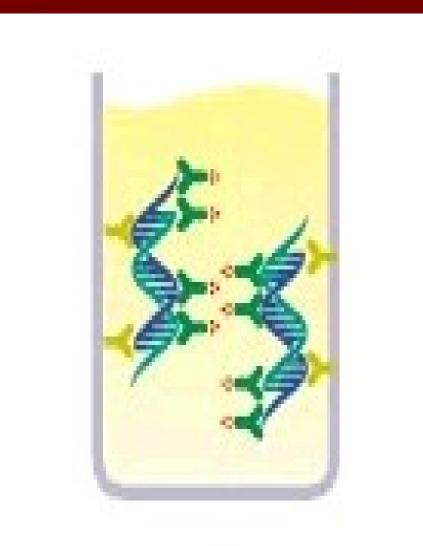


#### **Nucleic Acid probe applications**

- Helps the direct detection of bacteria in clinical specimens or culture
- Helps in N gonorrhea and C.trachomatis,
- Group A streptococcus
- Rapid identification of Mycobacterium complex.Listeria
- Dimorphic fungi
- Why popular in developed countrels ?
- Reached a specificity from 90 100%
- Time saving and Labour costs, but expensive
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## **Hybrid Capture**

Hybrid Capture (HC) technology detects nucleic acid targets directly and uses signal amplification to provide sensitivity that is comparable to target amplification methods



## **Hybrid Capture**

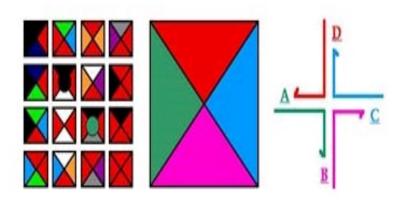
- It involves a signal amplification technology that consists of retention of DNA – RNA molecule complex in a tube or micro titer plate
- Useful in detection of Human papilloma virus (Repeat pap smear positives), comparable to PCR technology. CMV, HBV C.trachomatis. N.gonnorhea
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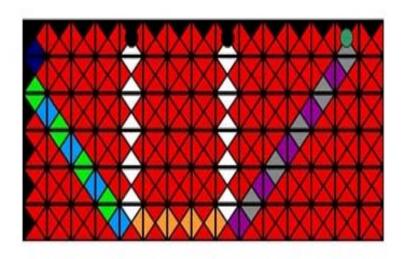
# Hybrid Capture

Tests are available to detect Human Papillomavirus (HPV), Chlamydia trachomatis (CT), Neisseria gonorrhoea (GC), and blood viruses such as Hepatitis B virus (HBV) and Cytomegalovirus (CMV). An assay for Herpes Simplex Virus (HSV) is in development.

### Branched DNA makes the complicated matters simple

- The technology uses variety of branched DNA ( bDNA ) probes and signal amplification reporter molecules
- And generate Chemiluminescent signal.
- The signal correlates with target nucleic acid
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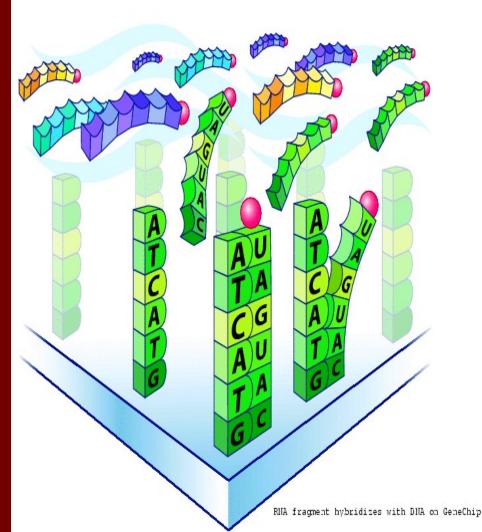




## **Branched DNA applications**

RNA fragments with fluorescent tags from sample to be tested

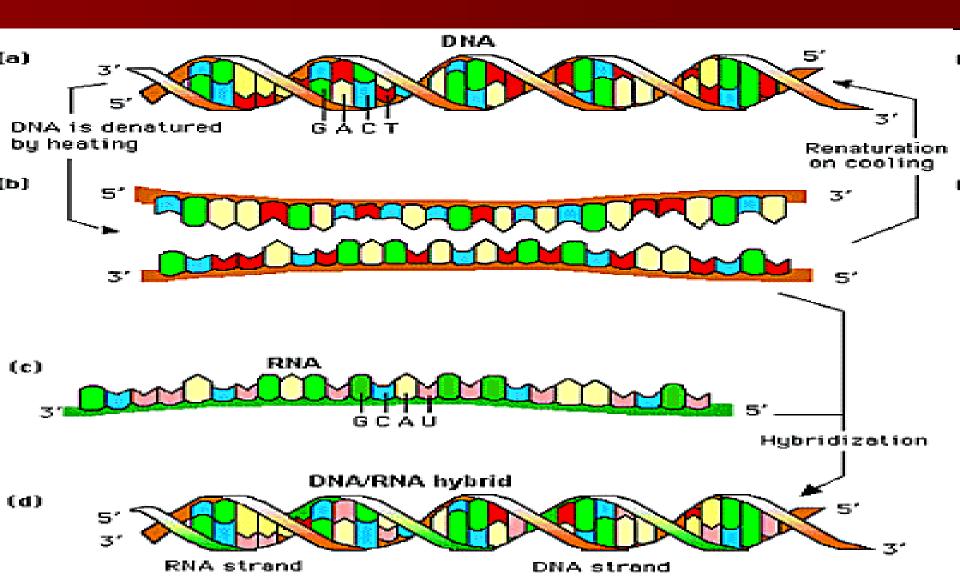
- Detection HIV, HBV, and HCV
- Measures viral loads
- Less sensitive than PCR



## In Situ Hybridization

- More in use Chromosomal translocations
- Useful in CMV, HSV, VZV
- Sub types of papilloma virus
- Useful in Mycobacteria, fungi and parasites
- Helicobacter pylori from gastric biopsies
- Legionella pneumophila
- Pneumocystis jiroveci
- Tests done on paraffin embedded specimen
- Need applications in Infectious diseases
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#### **In Situ Hybridization**



#### Protocols - In Situ Hybridization

The labelled probe is first denatured (by heating or under alkaline conditions) into single DNA strands and then hybridized to the target DNA (Southern blotting) or RNA (northern blotting) immobilized on a membrane or in situ.

## Nucleic acid probe

- Nucleic acid fragment, labelled by a radioisotope, biotin, etc., that is complementary to a sequence in another nucleic acid (fragment) and that will, by hydrogen binding to the latter, locate or identify it and be detected; a diagnostic technique based on the fact that every species of microbe possesses some unique nucleic acid sequences which differentiate it from all others, and can be used as identifying markers or "fingerprints."
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## **Blotting Methods**



## What is Blotting

Blots are techniques for transferring DNA, RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis. The Southern blot is used for transferring DNA, the Northern blot for RNA and the western blot for PROTEIN

#### TYPES OF BLOTTING TECHNIQUES

#### Blotting technique

Southern Blot

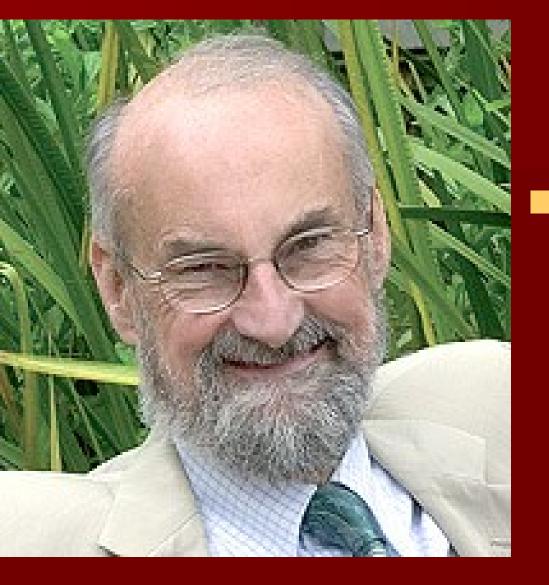
It is used to detect DNA.

#### Northern Blot

It is used to detect RNA.

#### Western blot

It is used to detect protein.



#### Professor Sir Edwin Southern

Professor Sir Edwin Southern, Professor of Biochemistry and Fellow of Trinity developed this method in 1975.

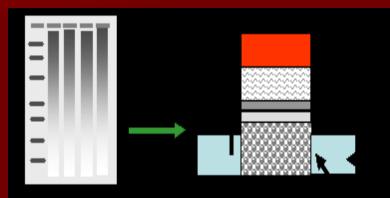
### **Nucleic Acid Hybridizations**

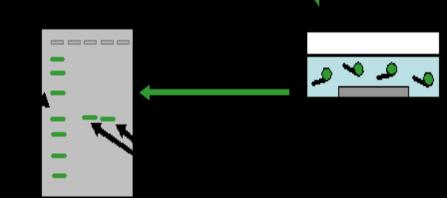
- The hybridization of a radioactive probe to filter bound DNA or RNA is one of the most informative experiments that is performed in molecular genetics. Two basic types of hybridizations are possible.
- Southern hybridization hybridization of a probe to filter bound DNA; the DNA is typically transferred to the filter from a gel
- Northern hybridization hybridization of a probe to filter bound RNA; the RNA is typically transferred to the filter from a gel

## **Southern hybridization**

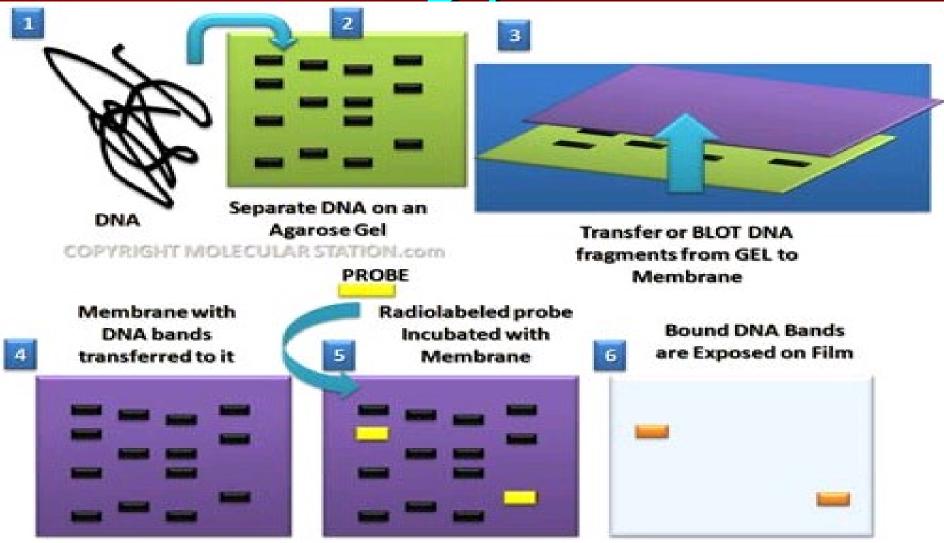
Southern hybridization

- hybridization of a probe to filter bound DNA; the DNA is typically transferred to the filter from a gel
- Under optimal conditions, Southern blotting detects
  ~ 0.1 pg of the DNA of interest





## Southern Blot working protocol



## **Uses of Southern Blotting**

Southern blots are used in gene discovery and mapping, evolution and development studies, diagnostics and forensics.

In regards to genetically modified organisms, Southern blotting is used as a definitive test to ensure that a particular section of DNA of known genetic sequence has been successfully incorporated into the genome of the host organism.

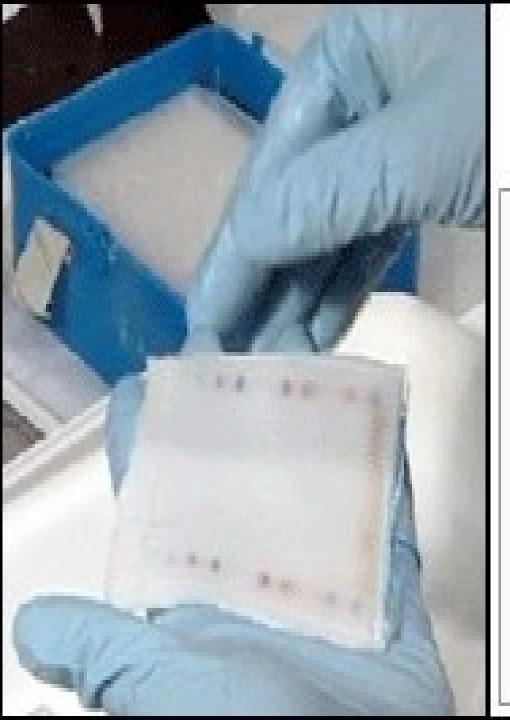
Used in prognosis of cancer and in prenatal diagnosis of genetic diseases

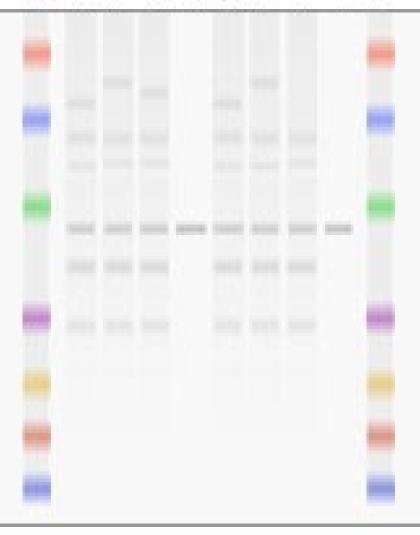
## Western blot

- The western blot (alternatively, immunoblot) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein
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## Western blotting

- Western blotting is an Immunoblotting technique which rely on the specificity of binding between a molecule of interest and a probe to allow detection of the molecule of interest in a mixture of many other similar molecules.
- In Western blotting, the molecule of interest is a protein and the probe is typically an antibody raised against that particular protein.





markens sample 1 sample 2 sample 3 sample 1 sample 2 sample 3 pure sample markens

# **Gel electrophoresis**

The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel.

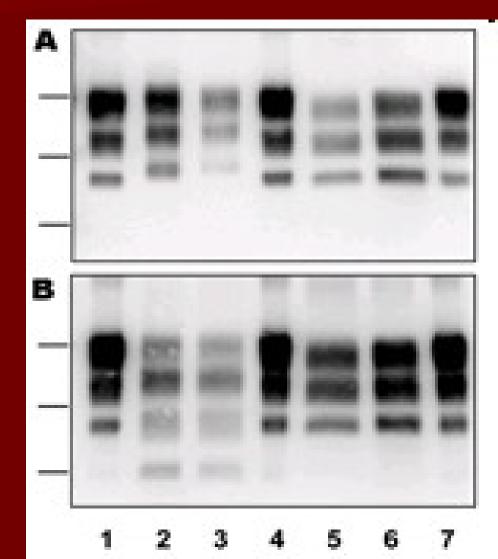
# Western Blot

The confirmatory HIV test employs a Western blot to detect anti-HIV antibody in a human serum sample. Proteins from known HIVinfected cells are separated and blotted on a membrane as above. Then, the serum to be tested is applied in the primary antibody incubation step; free antibody is washed away, and a secondary anti-human antibody linked to an enzyme signal is added. The stained bands then indicate the proteins to which the patient's serum contains antibody.

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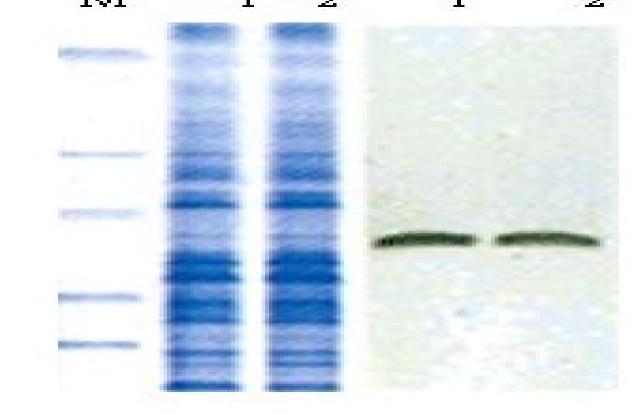
# **Other uses of Western Blot**

- A Western blot is also used as the definitive test for Bovine spongiform encephalopathy (BSE, commonly referred to as 'mad cow disease').
- Some forms of Lyme disease testing employ Western blotting



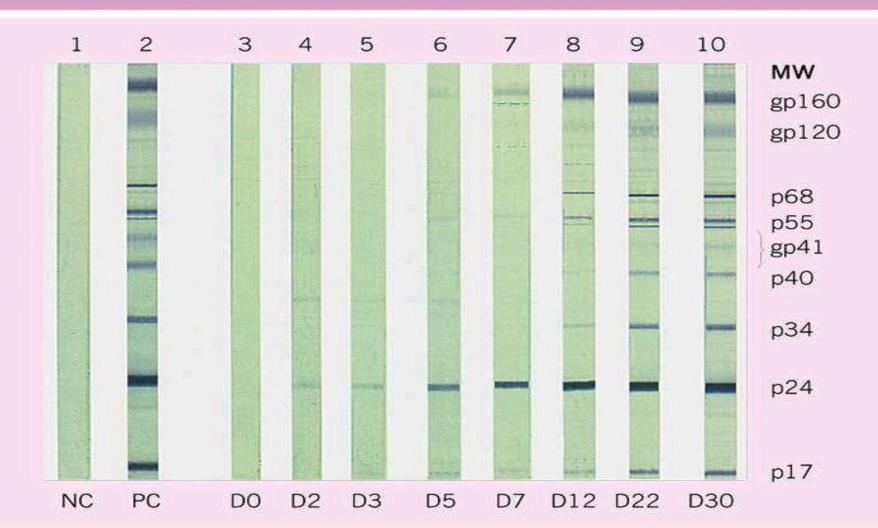
# **Results documented and userfriendly approaches**

#### <SDS-PAGE> <Western Blot> M 1 2 1 2



# **Results Documented**

#### WESTERN BLOT REACTIVITY IN ONE HIV-1 SEROCONVERTER



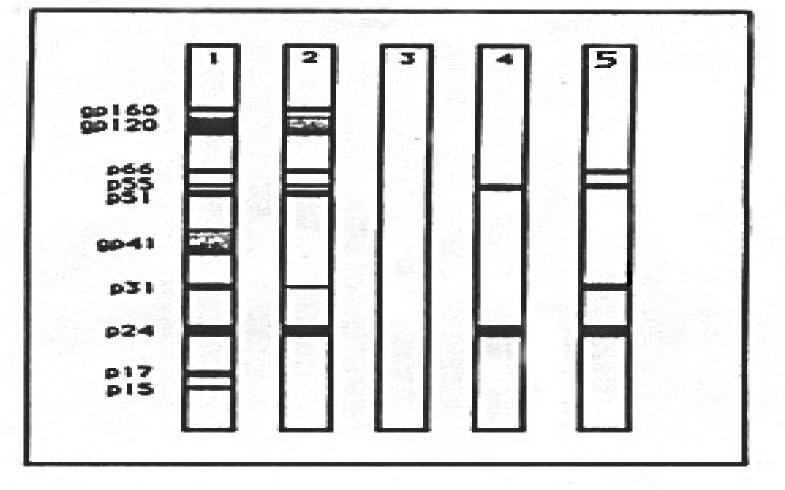


Figure:

Examples of reactions by an HIV-1 Western blot:

```
1. Positive control (strong)
```

```
2. Positive control (weak)
```

```
Negative control
```

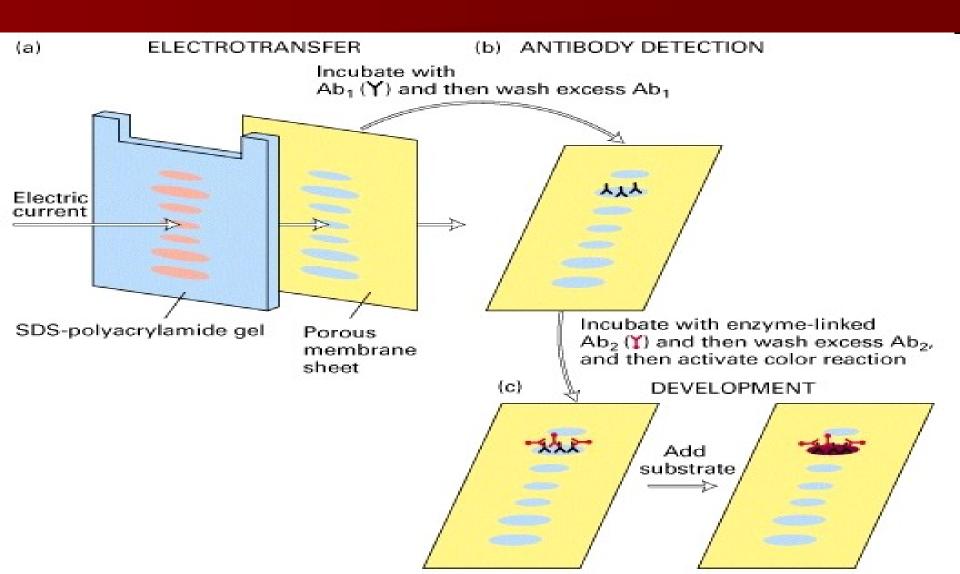
```
4. Indeterminate profile
```

```
5. Indeterminate profile (highly suggestive)
```

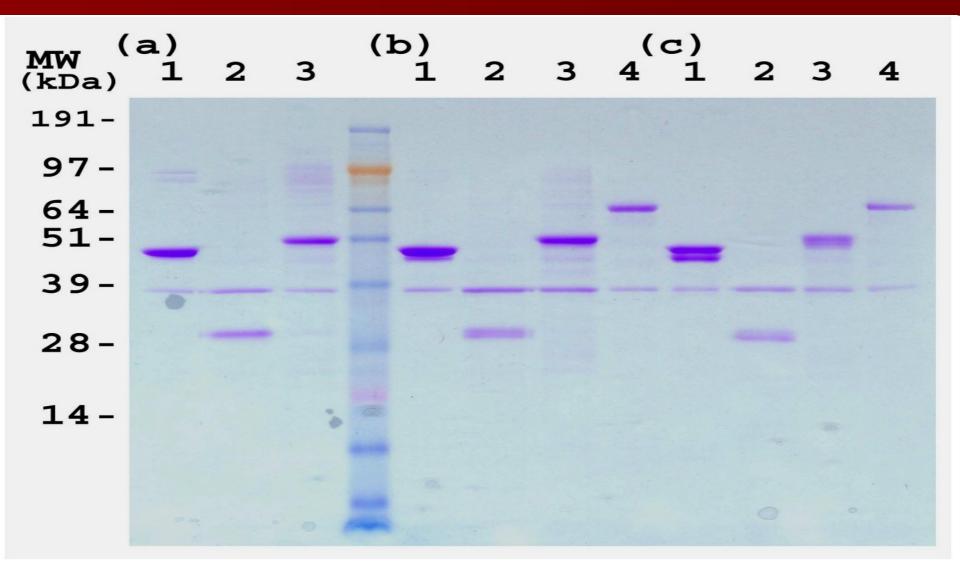
# SDS PAGE

- SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight as well as higher order protein folding
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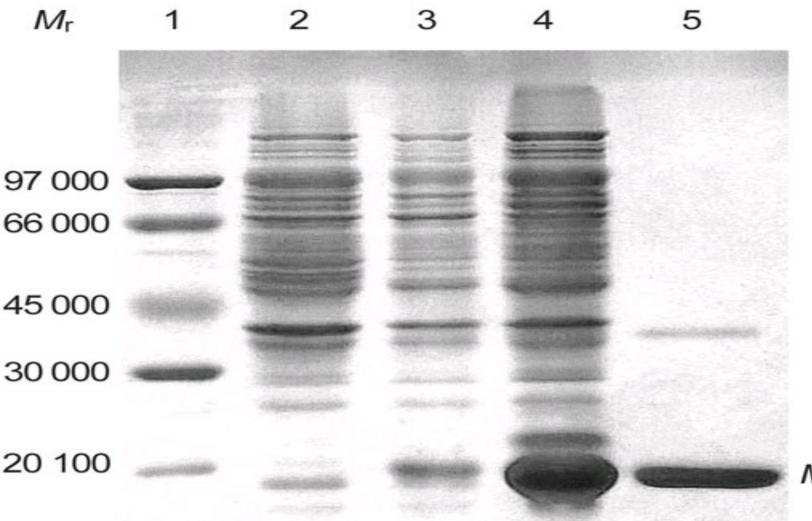
# **Documentation of SDS PAGE**



## **Becomes a Legal record**



# **SDS Page** *Reading frames*

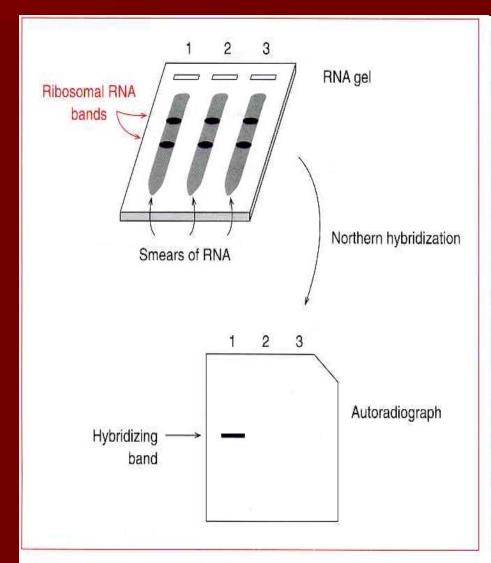


M<sub>r</sub> 17200

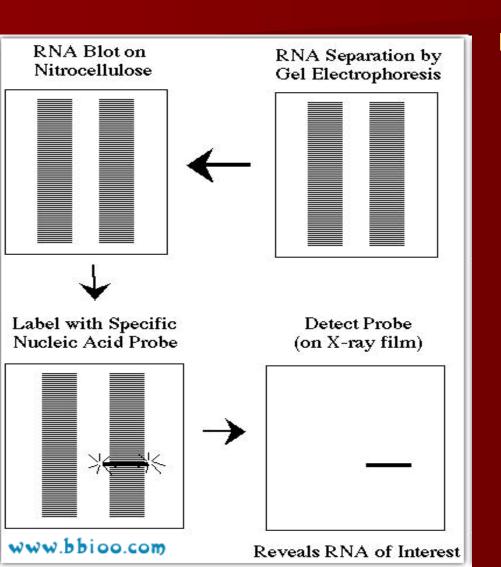
# Northern Blot

- The northern blot is a technique used in molecular biology research to study gene expression by detection of RNA in a sample.
- Northern blotting involves the use of electrophoresis to separate RNA samples by size, and detection with a hybridization probe complementary to part of or the entire target sequence.





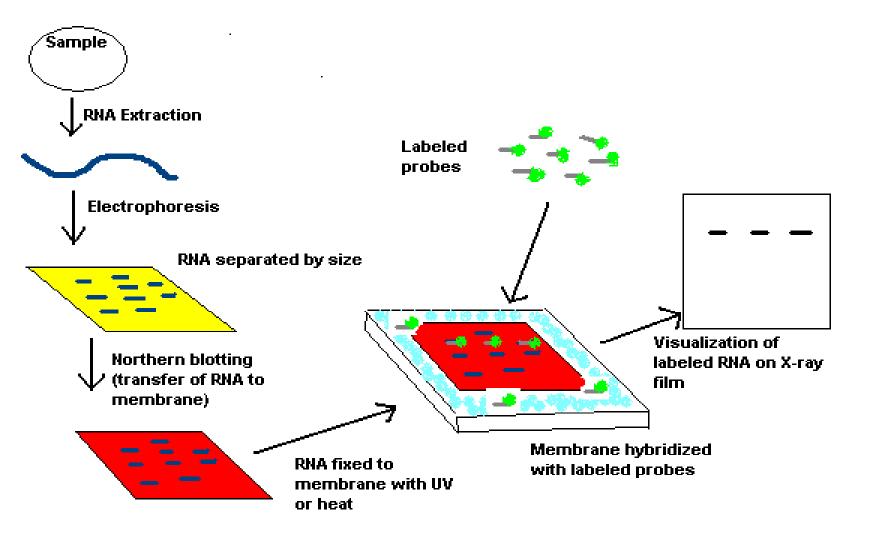
# Northern hybridization



Northern hybridization hybridization of a probe to filter bound RNA; the RNA is typically transferred to the filter from a gel.

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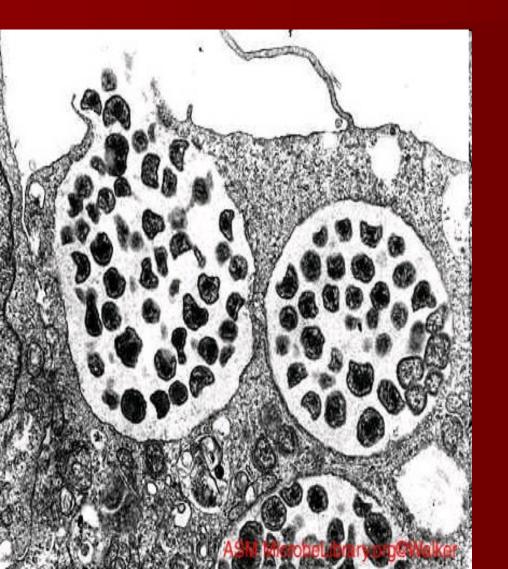
# **Northern Blot**



# **Eastern Blot**



# **Eastern Blot in Ehrlichia**



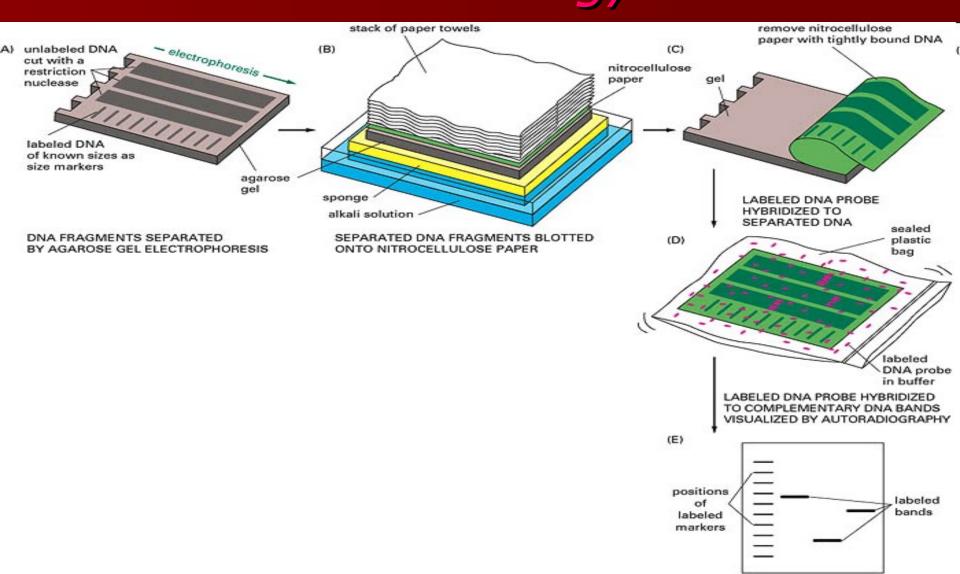
Developed at the Dept. of Pathology, University of Texas Medical Branch, Galveston, Texas, while working on the intracellular bacteria, *Ehrlichia* 

# **Eastern Blotting**

- The technique was developed to detect protein modifications in two species of *Ehrlichia-E. muris* and Cholera toxin B subunit (which detects lipids)
- Expression of post translated proteins is important in several diseases.



### Eastern Blotting Methodology



# Far Eastern Blotting

Far-Eastern blotting is a technique developed in the 1990s by T. Taki and colleagues at the Cellular Technology Institute of Otsuka Pharmaceutical Co., Japan for the analysis of lipids separated by high-performance thin layer chromatography (HPTLC). The lipids are transferred from the HPTLC plate to a PVDF membrane for further analysis, for example by enzymatic or ligand binding assays or mass spectrometry.

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# Uses of Far Eastern Blotting

- Purification of glycosphingolipids and phospholipids.
- Structural analysis of lipids in conjunction with direct mass spectrometry.
- Binding study using various ligands such as antibodies, lectins, bacterium, viruses, and toxins, and
- Enzyme reaction on membranes.
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# Restriction fragment length polymorphism

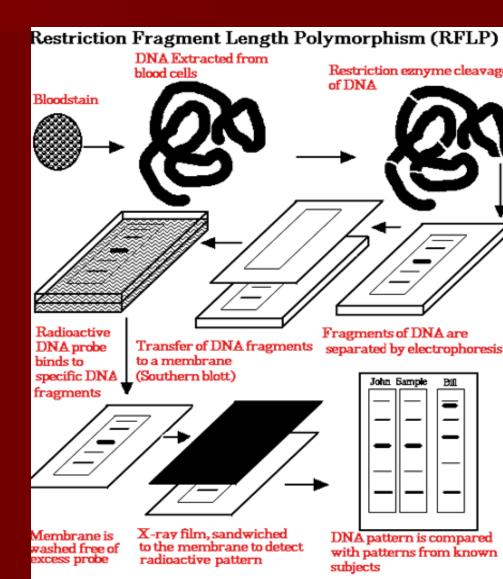
Sir Alec Jeffreys developed restriction fragment length polymorphism (RFLP), which quickly became the standard technique for DNA testing throughout the 1980s. RFLP provided the world with the first form of genetic testing based on DNA, the body's genetic material

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## Restriction Fragment Length Polymorphism (RFLP)

- Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA.
- Defines how we differ from others

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# RFLP

The basic technique for detecting RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest. The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure.

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#### **How the RFLP Process Works**

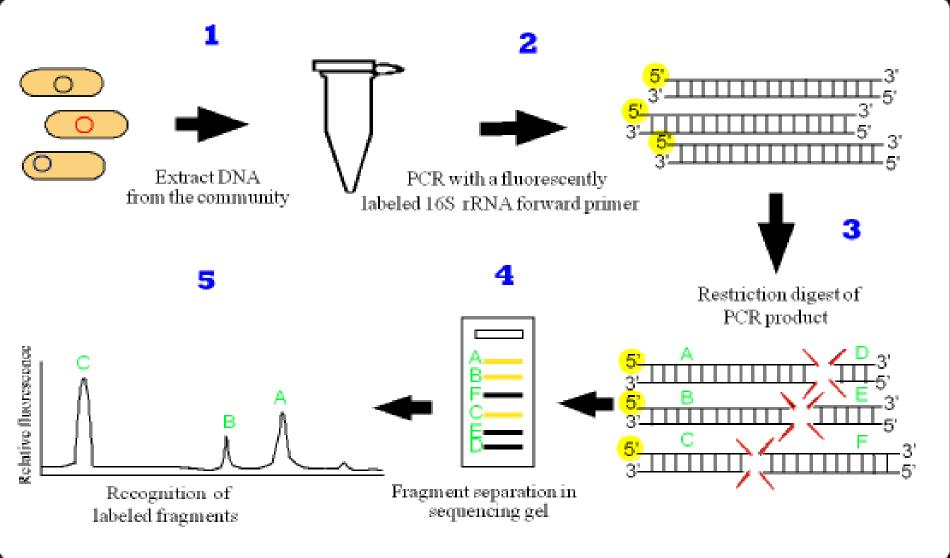
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Sponge A B C Gel Nitrocellulose Paper paper towels Alkaline solution 3 Alkaline solution is DNA Samples pulled upward through the 2 Electrophoresis gel to a sheet of nitrowith added separates the restriction cellulose laid on the top restriction enzymes fragments. Each sample of it, transferring the DNA produce restriction forms a characteristic to the paper. fragments. pattern of bands. Paper blot Plastic bag 6 DNA probe 2 A photographic film laid on top solution

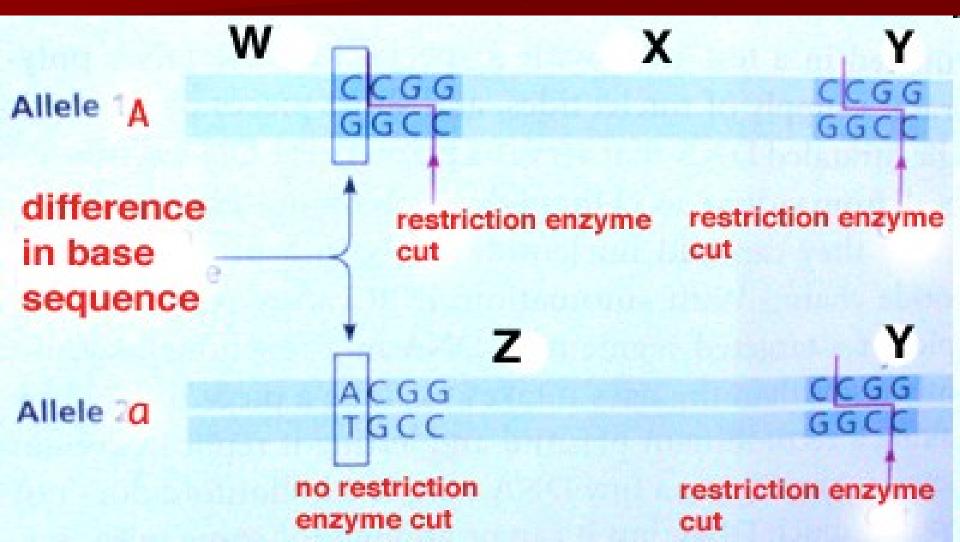
The paper is exposed to a solution containing radioactively-labeled probe.

A photographic film laid on top of the paper is exposed by the radioactivity in the bond probe to form an image corresponding to the DNA bands.

## Creation of fragments and Documentation



### Cutting the Desired sequences is basis of success in Molecular Biology



# Spoligotyping

Spoligotyping, a new method for simultaneous detection and typing of M. tuberculosis complex bacteria, has been recently developed. This method is based on polymerase chain reaction (PCR) amplification of a highly polymorphic direct repeat locus in the *M. tuberculosis* genome.

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# **Spoligotyping in Tuberculosis**

Results can be obtained from a *M. tuberculosis* culture within 1 day. Thus, the clinical usefulness of Spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities

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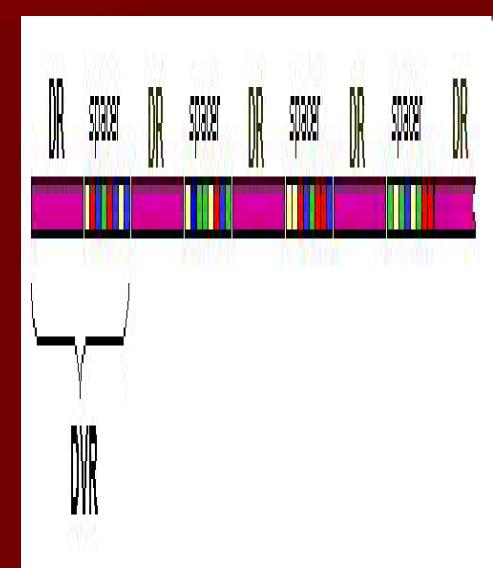
# Spoligotyping

- Spoligotyping is based on the polymorphism in the direct repeat (DR) locus of the mycobacterial chromosome. The DR locus is one of the most well studied loci of the MTC genome showing considerable strain-to-strain polymorphism The function of the DR locus in MTC bacteria is presently unknown.
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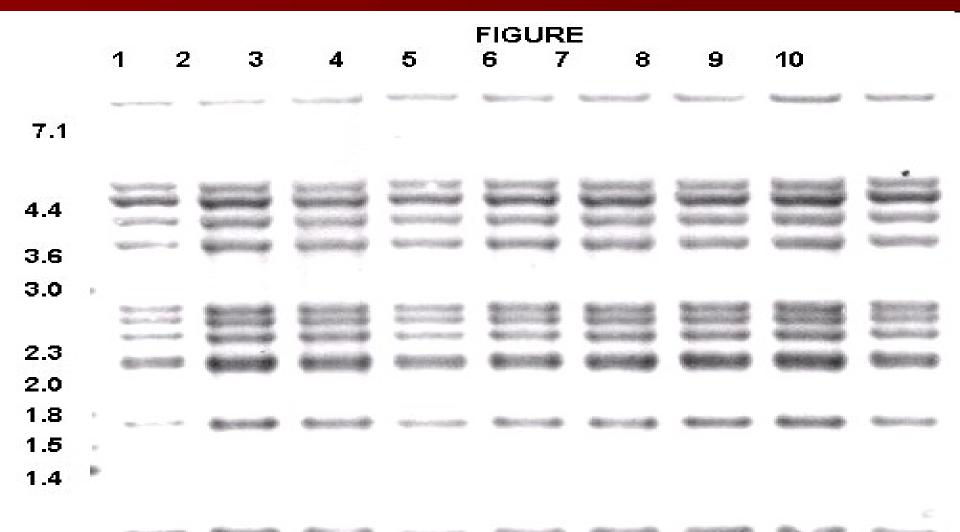
# **Spoligotyping in Tuberculosis**

 The well-conserved 36-bp direct repeats are interspersed with unique spacer sequences varying from 35 to 41 bp in size. Currently, 94 different spacer sequences have been identified of which 43 are used for MTC strain differentiation. Clinical isolates of MTC bacteria can be differentiated by the presence or absence of one or more spacers.

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# All documentation can be read and compared



## Documentation can be read by computer and results can be interpreted

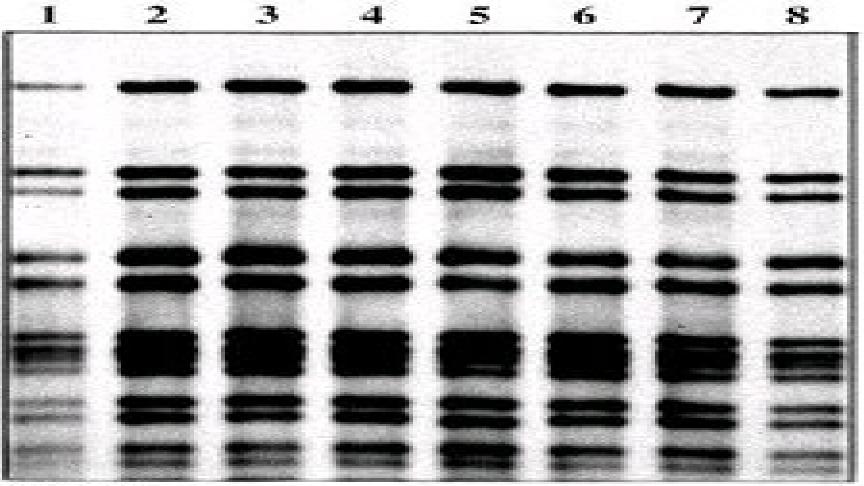
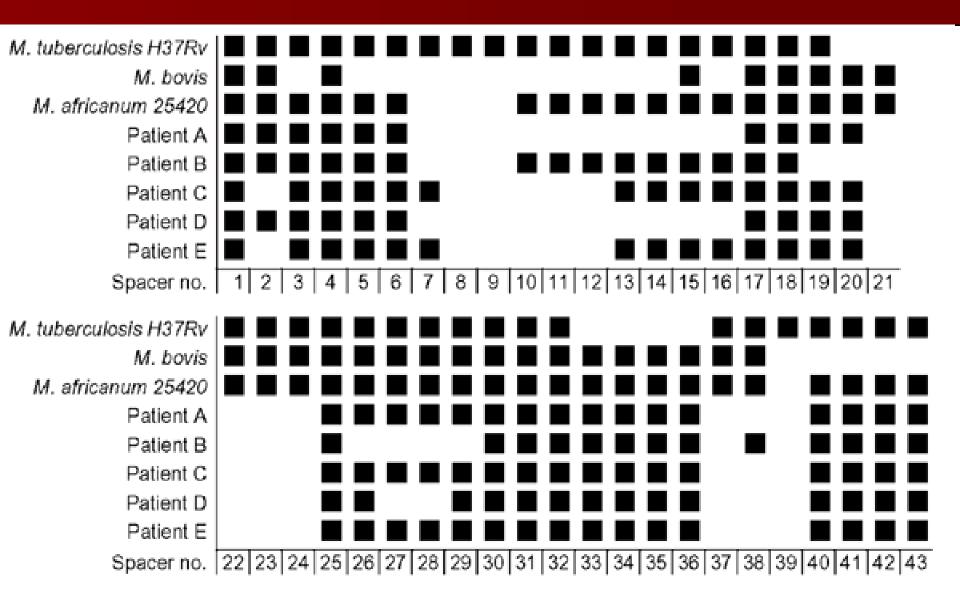


Figure 4.

# **Documentation of Finger printing for Records**

- Finger print means translating all the variable number of tandem repeats to visible records
- All VNTR is tested for restriction length polymorphisim which differ from species to species.
- All the obtained material is blotted to Nylon or Nitrocellulose membrane ( Southern Blotting )
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## **Results analyzed by Computer**



# DNA finger printing help the Progress of Biology

- Every one of our DNA is equal except for only about 0.10 %.
- DNA finger printing lies in uniqueness of those regions of DNA that do differ from person to person.
- Only 5 % of our DNA code rest donot code called in past as Junk DNA and contain repeated sequences of base pairs
- Called as Variable number of tandem repeats contain 20 to 100 base pairs and the same sequence is repeated one to 3 times in a row
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# Science of Molecular biology progress from RLFP to PCR

Isolation of sufficient DNA for RFLP analysis is time-consuming and labor intensive. However, PCR can be used to amplify very small amounts of DNA, usually in 2-3 hours, to the levels required for RFLP analysis. Therefore, more samples can be analyzed in a shorter time.

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# How much we differ from Ape DNA answers ?



#### **April 25th celebrates the DNA Model** *DNA continues to the Temple of Science*



# Madam, are you fiddling with GENES ?



## Be A GENE Genius Become Famous



#### Life is a Transition from Biology to Molecular Biology Are you Ready ?



### Created for Basic Understanding on Molecular biology

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