

Role of Nucleic Acid in Diagnosis of Molecular and Infectious Diseases

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Nucleic Acids:

- **Nucleic acids** are biological molecules essential for life, and include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).
- Together with proteins, nucleic acids make up the most important macromolecules; each is found in abundance in all living things, where they function in encoding, transmitting and expressing genetic information.

Diagnosis of Infectious Disease

- Recent developments have significantly altered the monitoring and diagnosis of infectious diseases
- There are two general methods for examining infectious disease:
- **Microbial phenotype** characterization
- **Nucleic acid techniques**

“Old School” Diagnosis:

Microbial Phenotyping (Examine physical characteristics)

- **Biotyping** (grow organisms on media)
- **Protein content** (of pathogen)
- **Bacteriophage** profiles (virus analysis)
- **Chromatography** (membrane lipids)
- **Antibiotic** (susceptibility testing)

“Old School” Diagnosis:

Challenges:

- **Time** – have to culture and grow pathogen
- **Lab Safety** – need to keep many pathogenic cultures alive in lab
- **Accuracy** – not always definitive
- **Limited Info** – no information on antibiotic resistance or virulence factors

Molecular Diagnosis:

- Can detect disease-causing agents ***without having to grow them*** (directly from sample)
- Can detect ***slow / hard to culture*** microbes
- Can ***amplify*** DNA to get more accurate results
- **ID sub-species** (excellent discrimination)
- ID genes that **impart drug resistance** (i.e. target the treatments)
- ***Fast results*** - automated systems available

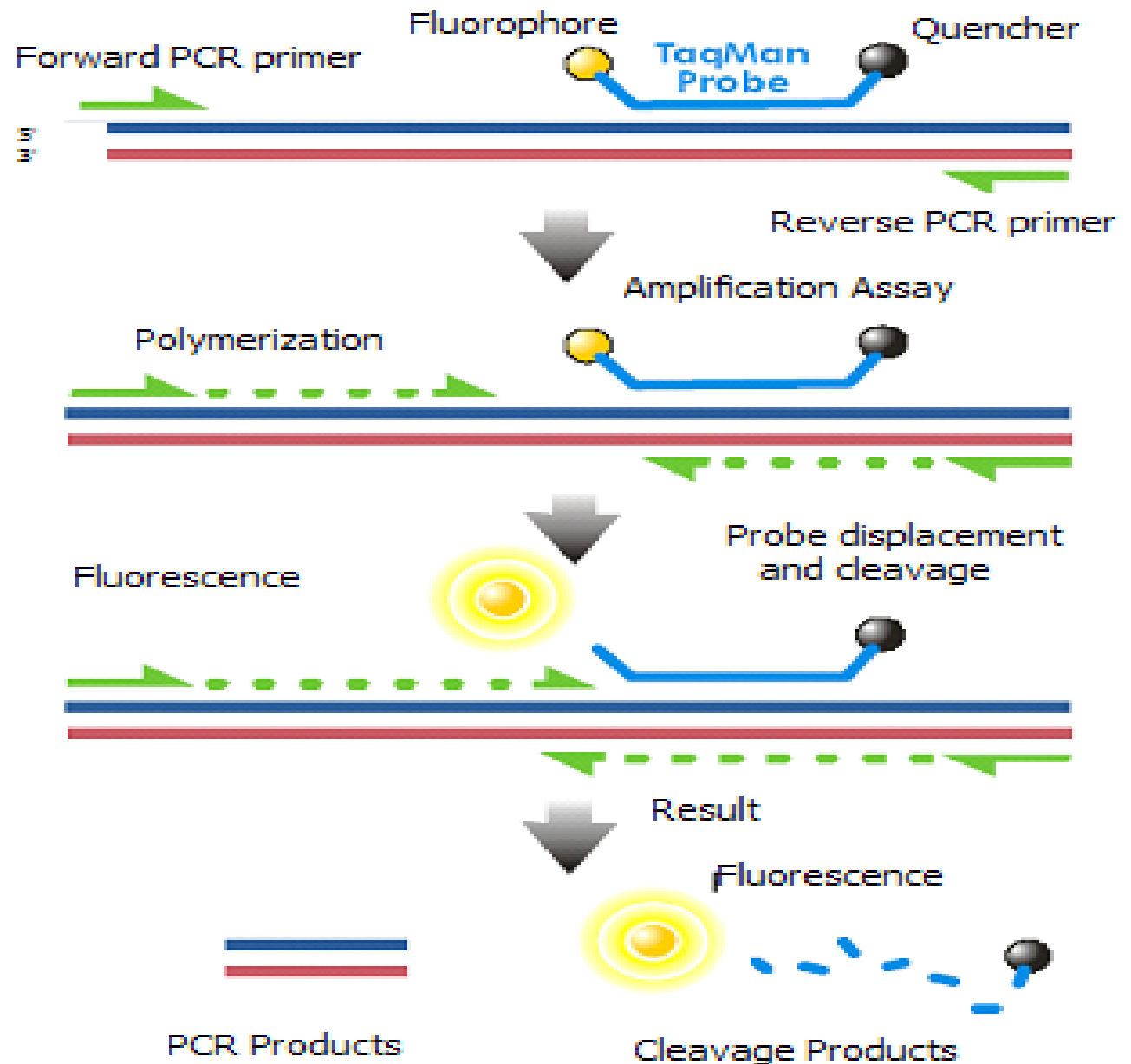
Molecular Diagnosis:

- **Overall** - PCR and nucleic acid amplification technology has one enormous benefit: ***bacterial growth is no longer necessary to detect and characterize microorganisms!***
- "Amplicons"** (amplified products) are characterized by:
1. Nucleic acid probe hybridization (labeled probes)
 2. Analysis of fragments after restriction digestion
 3. Direct sequence analysis

Molecular Diagnosis:

- Hybridization probes:
- A hybridization probe is a fragment of DNA or RNA of variable length usually 100-1000 bases long which is used in DNA and RNA samples to detect the presence of nucleotide sequence (the DNA target) that are complementary to the sequence in the probe.
- Examples:
 - TaqMan probe
 - Molecular Beacon probe
 - LNA (Locked Nucleic Acid) probe
 - Cycling Probe Technology (CPT) etc.

TaqMan:



Molecular Diagnosis:

- Application of TaqMan:
- In gene expression assays
- Pharmacogenomics
- Human Leukocyte Antigen (HLA) genotyping
- Determine the viral load in clinical samples like HIV, TB, Hepatitis etc
- Bacterial identification
- DNA quantification
- SNP genotyping
- Verification of microassay results.

Molecular Diagnosis:

- Southern blotting:
- Based on DNA hybridization
- Developed by EM Southern in 1975.
- Used to detect a specific segment of DNA in the whole genome.
- Application:
 - mutant genes such as HbS, Cystic fibrosis DMD, PKU as well as presence of viral DNA (hepatitis virus B and C) Can be identified by this method.

Molecular Diagnosis:

Nucleic Acid Techniques:

- **Plasmid profiling** (when characteristic plasmids are available)
- **RFLP analysis** (restriction fragment length polymorphisms - use RE to produce characteristic gel fragments)
- **PCR** (amplify key sequences which distinguish target microorganisms)

Molecular Diagnosis:

Plasmid Analysis

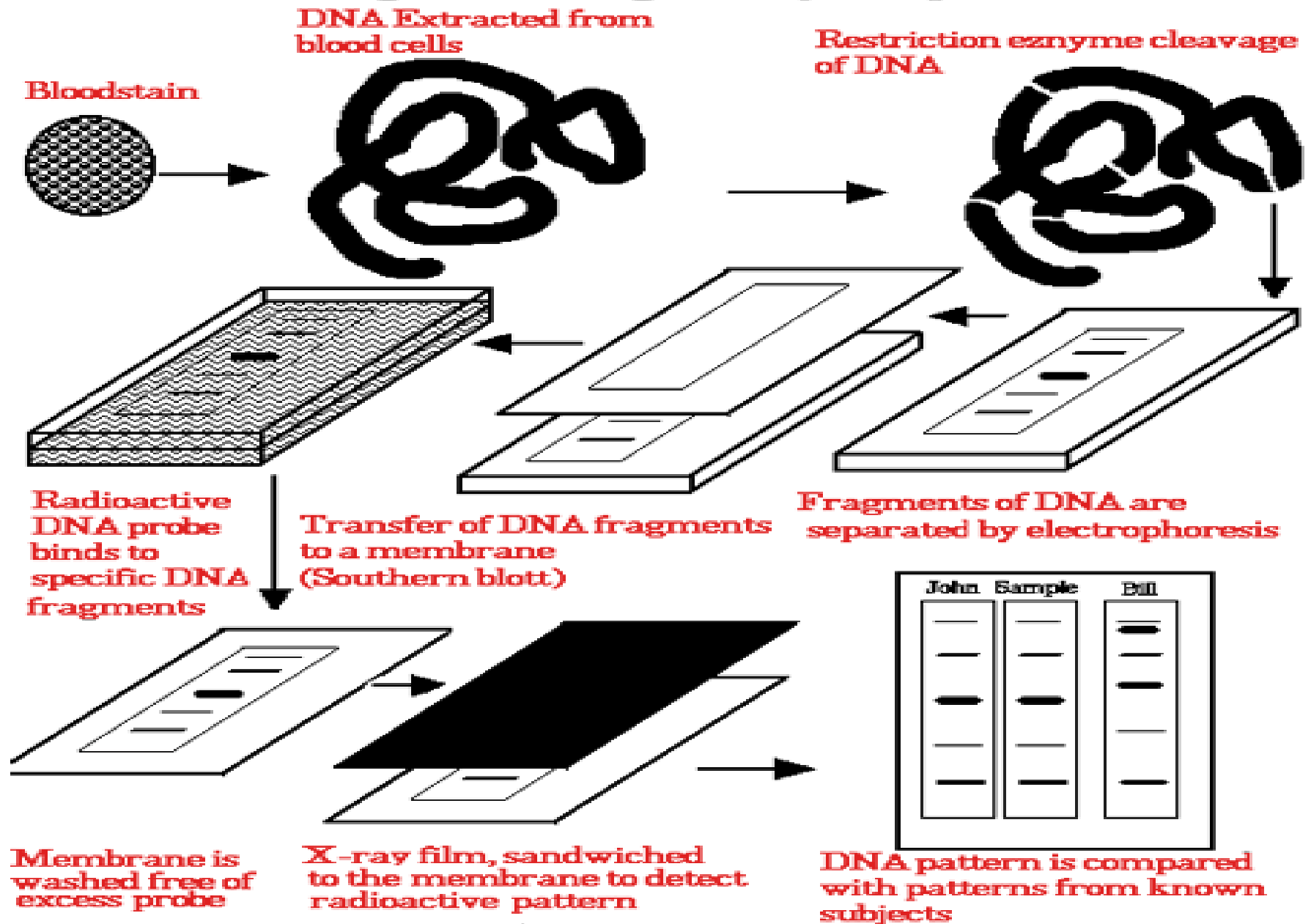
- Plasmids are small, self-replicating circular DNA molecules found in many bacteria
- Plasmids often code for **resistance to antibiotics** and certain **virulence** factors
- Widely-used for tracking resistance in **disease outbreaks / pandemics**
- Can track **transfer of resistance**: between hospitals, organisms, and countries
- **Weakness** - can transfer between microbes

Molecular Diagnosis:

Restriction Enzyme Pattern

- Cut DNA at specific location using natural enzymes: **restriction endonucleases**
- get characteristic fragments: “**RFLP's / restriction fragment length polymorphisms**”
- see fragments via electrophoresis
- very accurate – can ID between microbial strains

Restriction Fragment Length Polymorphism (RFLP)



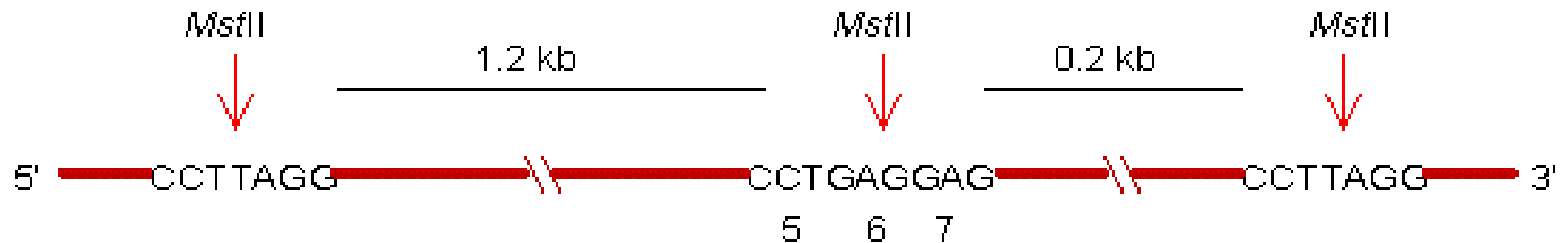
Molecular Diagnosis:

Uses of Restriction Enzyme Patterns:

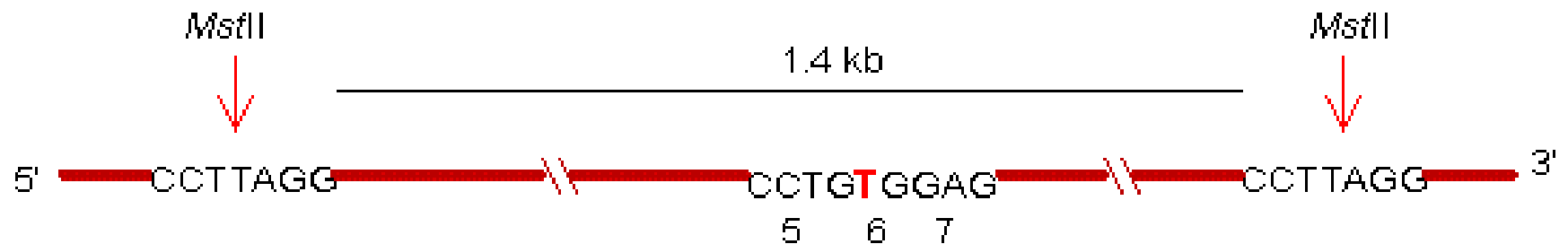
- Identification of bacterial populations.
- Epidemiology (spread of disease through population), pandemic science.
- Study of Tuberculosis in HIV-positive patients.
- Combined with DNA fingerprinting (southern blot, etc.) it is a very powerful tool.

Use of RFLP in Sickle cell disease:

Normal cell



Sickle cell

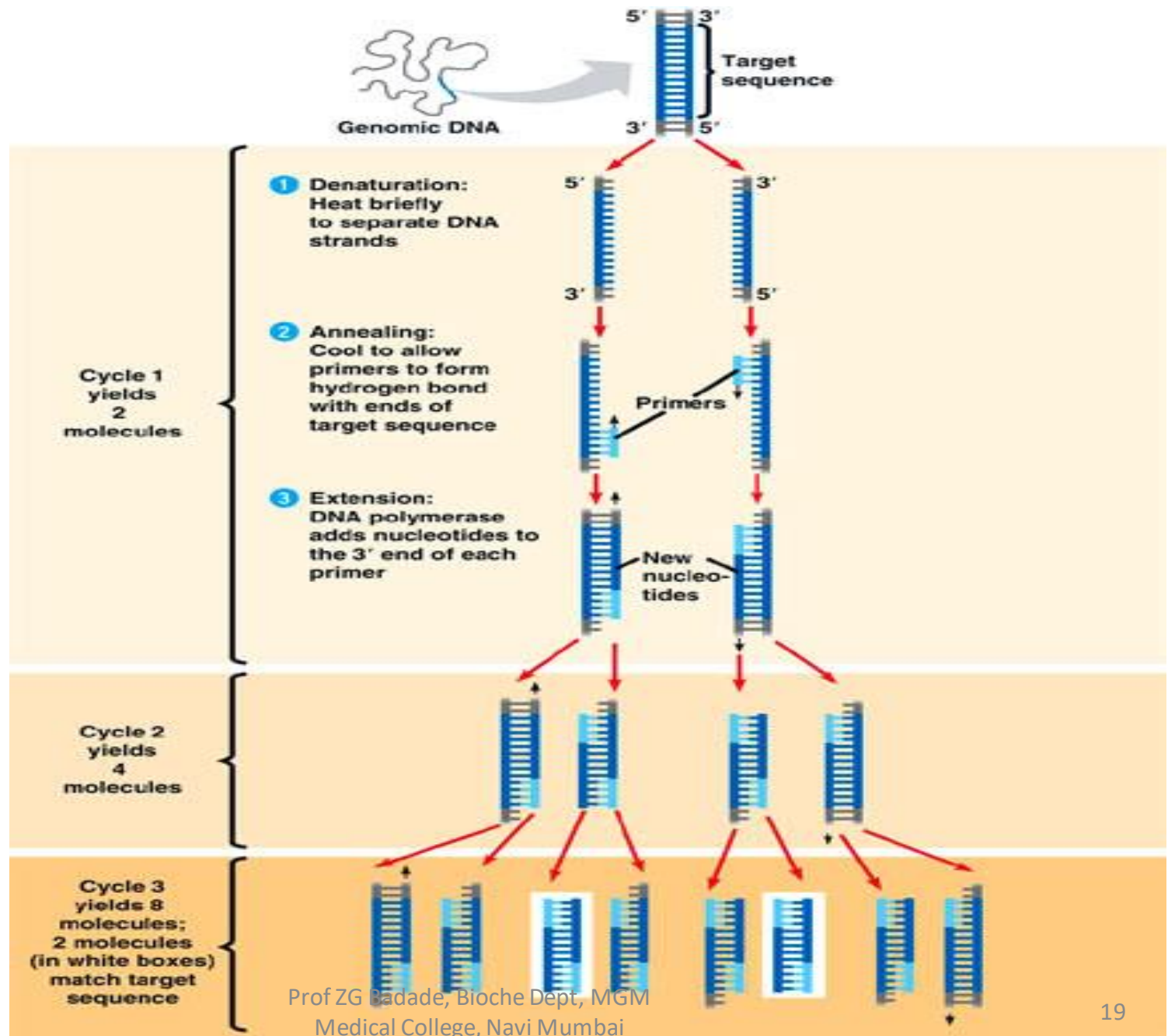


Molecular Diagnosis:

PCR: Polymerase Chain Reaction

- Making copies of a DNA sequence
- PCR is conducted in vitro (beaker / test tube)
- 20 cycles of PCR can allow for a 1,000,000 X amplification of DNA samples
- Typical PCR reactions use small (ng-mg) quantities of DNA and go through 30-40 Amplification cycles
- PCR has Revolutionized R&D in Biology / Medicine and helped refine Criminology and Law

PCR:



Application of PCR:

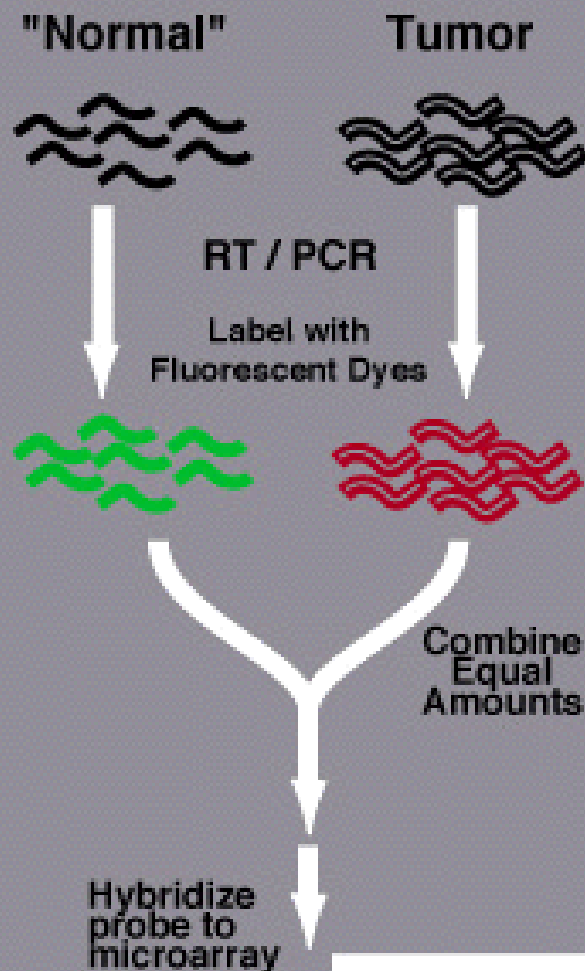
- Detection of infectious disease
 - AIDS, TB, CMV, H1N1 etc.
 - Lyme Disease-joint inflammation from tick bites
 - Detect 3 sexually transmitted diseases in one swab - herpes, papillomavirus, chlamydia.
 - Can diagnose even one bacteria or virus present in the specimen.
 - Latent virus can also be diagnosed
- Detection of variations and mutations in Genes.
 - Detects people with inherited disorders and carriers
 - Track presence or absence of DNA abnormalities characteristics to cancer
 - Prenatal diagnosis of genetic disorders
 - PCR combined with RE and southern blotting is used for mutation detection.
- PCR and the law
 - DNA Fingerprinting: can multiply small amounts of DNA found in blood samples, hair, semen, and other body fluid

Molecular Diagnosis:

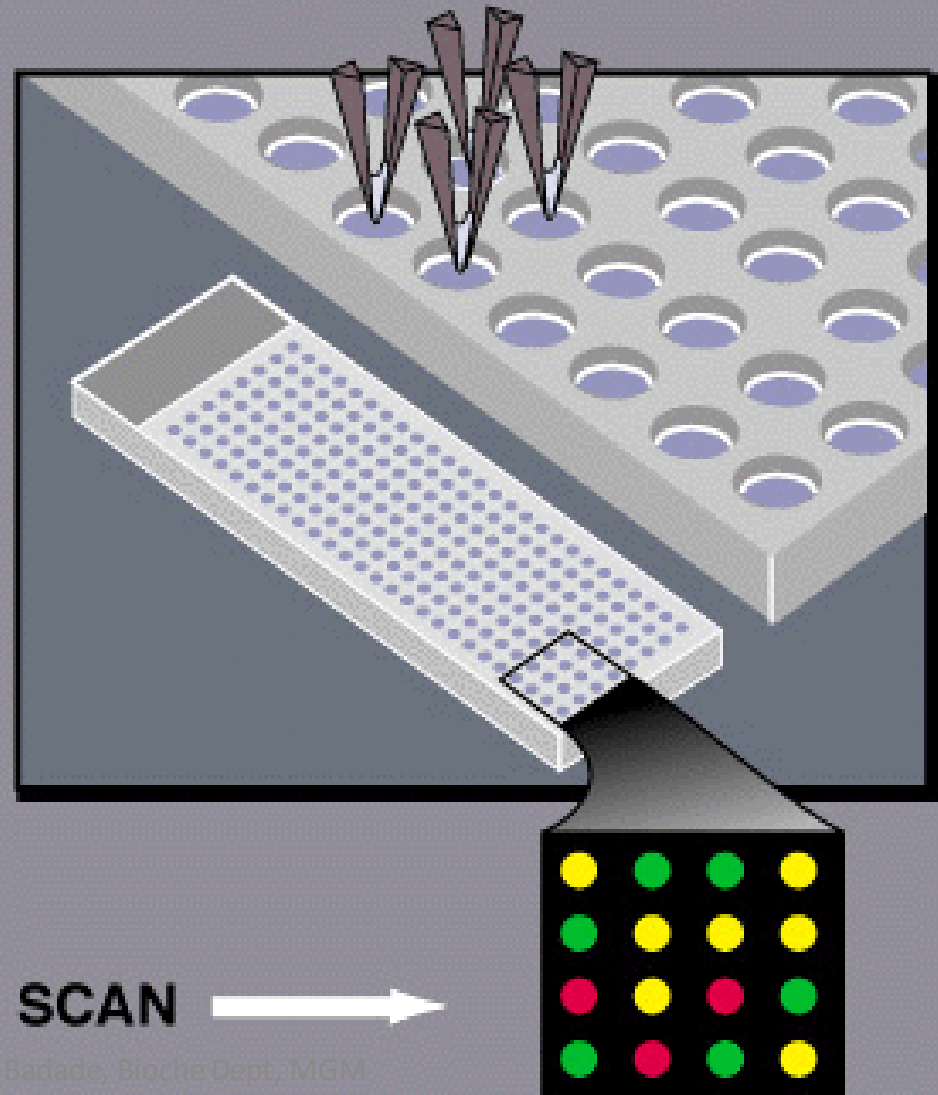
Nucleic Acid Probes - MicroArray

- Can identify organisms at, or below species level
- Can detect fastidious organisms directly (bacteria, viruses, mycobacteria, fungi and parasites)
- Commercial kits available: Gen-probe, Microprobe, Digene etc. (all FDA approved)
- Procedures are well-standardized
- Use short, synthetic DNA probes for well understood characteristic sequences

Prepare cDNA Probe



Prepare Microarray



Summary - Molecular Diagnosis:

- Many different nucleic-acid based methods
- Don't have to culture / grow organism (excellent for dangerous / fastidious organisms)
- Can detect disease-causing gene mutations (in humans, etc.)
- Can track drug resistance
- Fast, sensitive, and improving all the time
- Limit - contamination and amplification of contaminants