

Molecular methods

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MOLECULAR GENETICS

- Analysis and manipulation of DNA using biochemical and microbiological techniques
- Genetic engineering
- Restriction endonucleases
- DNA probes
- Blotting techniques
- Polymerase Chain Reaction

GENETIC ENGINEERING

- Genetic engineering or recombinant DNA technology
- Consists of isolation of genes coding for a desired protein from microorganism or cells of higher forms – their introduction into suitable microorganisms, in which genes would be functional – directing production of specific protein

GENETIC ENGINEERING

Uses

- Cloned human insulin
- Interferons
- Somatostatin growth hormone
- Vaccines – Hepatitis B, rabies

RESTRICTION ENDONUCLEASES

- Microbial enzymes that cleave double-stranded DNA
- Examples: Eco RI, Hind III, Taq I
- Restriction enzymes split DNA strands into fragments of varying length
- These are separated by gel electrophoresis stained with ethidium bromide and photographed

DNA PROBES

- Specific interaction in base pairing during DNA or RNA synthesis enables production of specific probes
- They are radioactive, biotinylated or otherwise labelled copies of cloned DNA
- Fragments 20–25 nucleotides long – containing unique sequences that can be used to detect homologous DNA by hybridisation

DNA Probes

USES

- Diagnosis of infectious diseases
- To detect microbe in cultures, body fluids, tissues or other material

ADVANTAGES

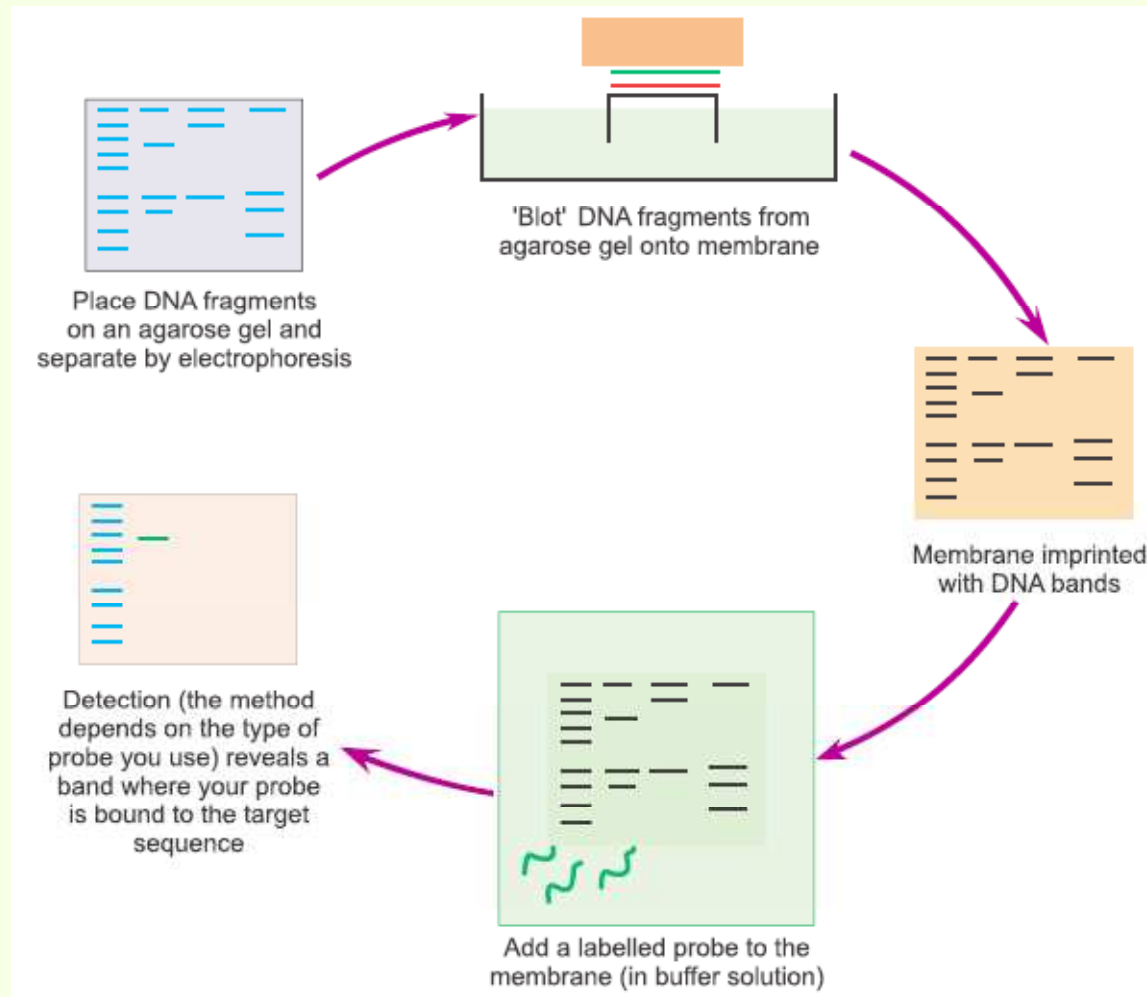
- High degree of specificity
- Ability to detect minute quantities of DNA
- Capacity to recognise difficult to culture microbes

BLOTTING TECHNIQUES

SOUTHERN BLOTTING

- EM Southern
- DNA fragments obtained by restriction enzyme digestion – separated on gel transferred by blotting to nitrocellulose membrane
- DNA bound to membrane is denatured and treated with radioactive single-stranded DNA probes
- These probes hybridise with homologous DNA to form radioactive double-stranded segments that can be detected on X-ray film

SOUTHERN BLOTTING



Southern blotting

NORTHERN BLOTTING

- An analogous procedure used for analysis of RNA
- RNA mixture separated by gel electrophoresis
- Blotted and identified using DNA or RNA probes

WESTERN BLOTTING

- A similar technique for identification of proteins
- The protein antigen mixture separated by SDS-PAGE, blotted on nitrocellulose membrane and identified by radiolabelled or enzyme-labelled antibodies as probes

USE

- Ability to separately identify antibodies against different antigens of a pathogen

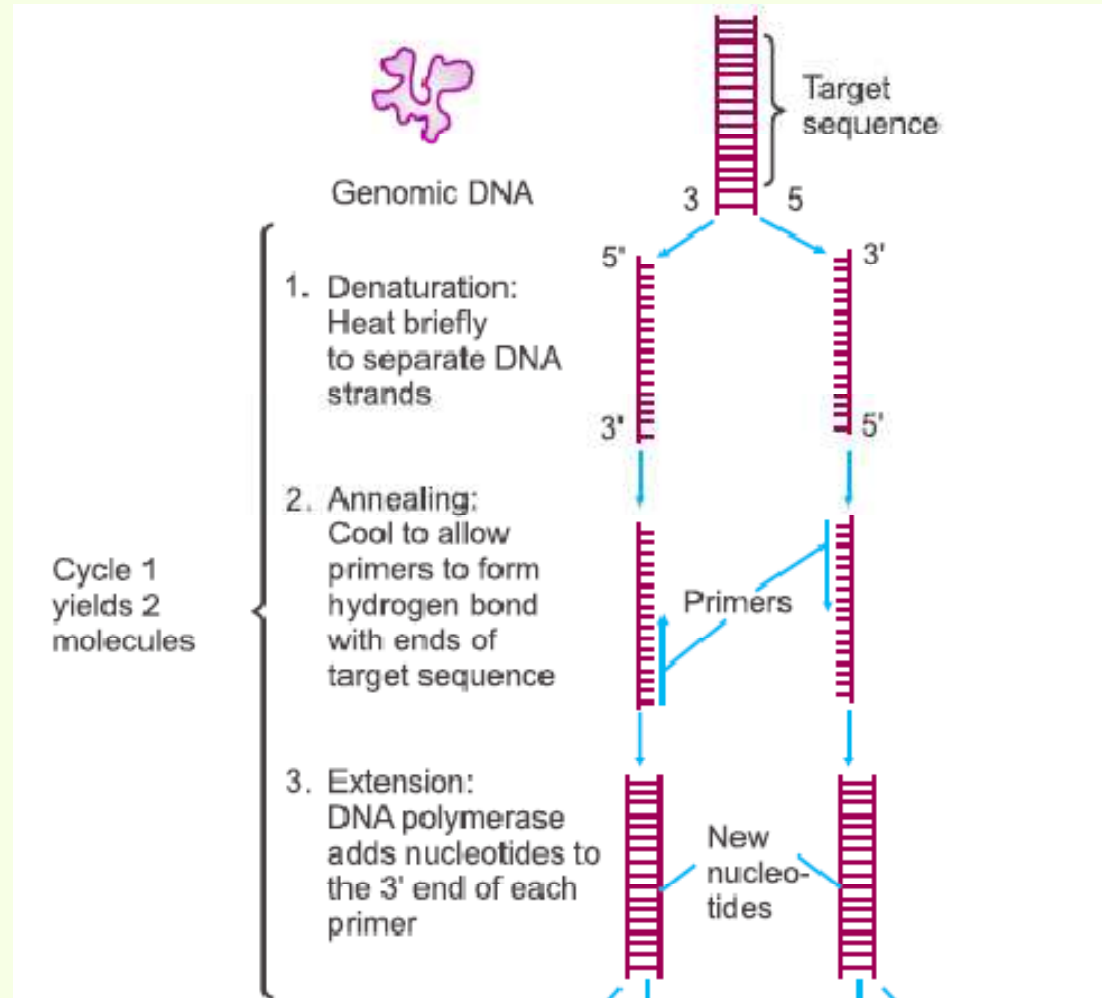
PCR

- Kary B Mullis – 1983
- Consists of sequential DNA replication, where products of the first cycle become template for the next cycle
- Makes available abundant quantities of DNA from small quantities of the same

USES

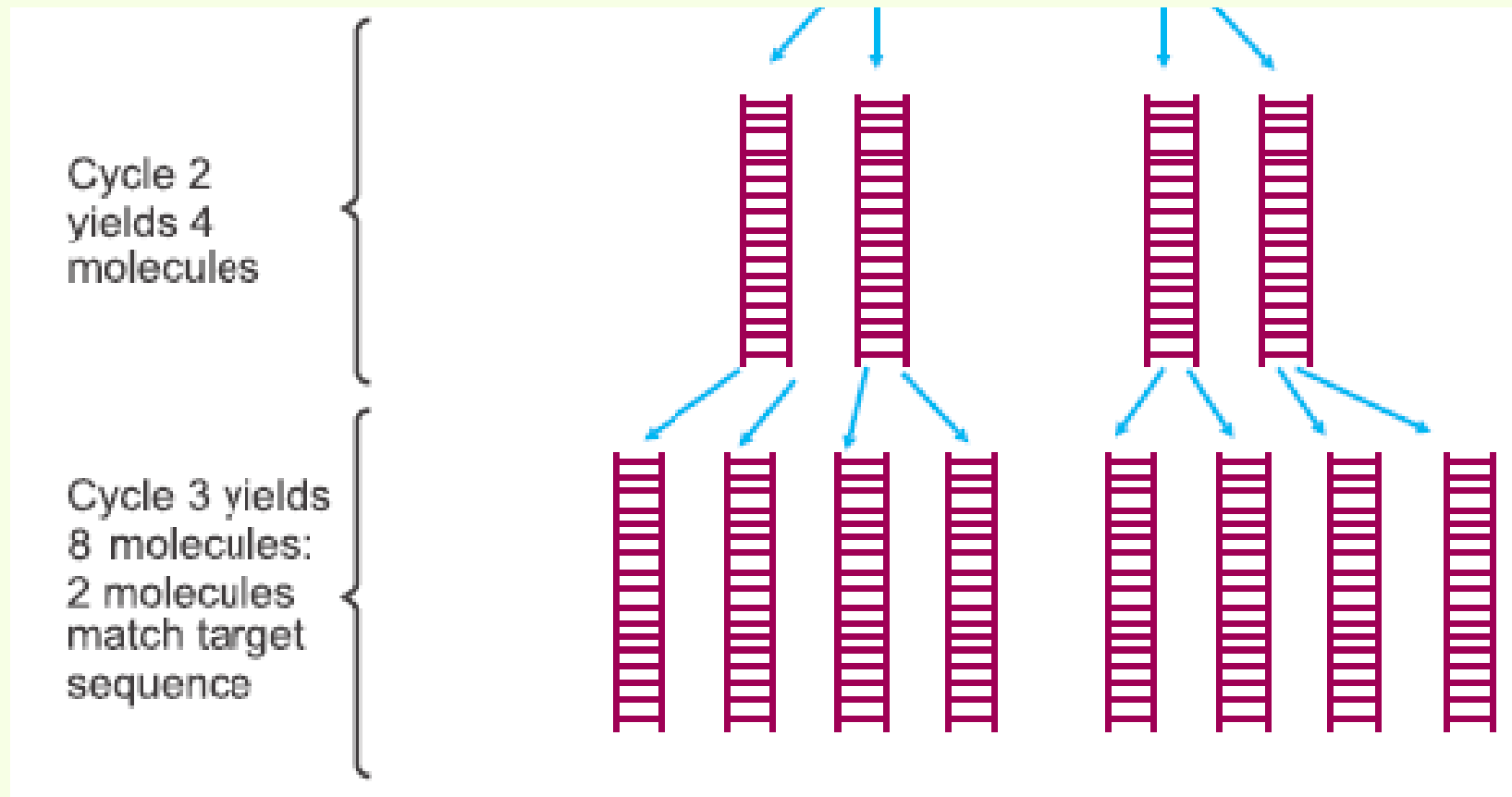
- Diagnosis of infections, genetic diseases, neoplastic diseases and forensic investigations

PCR



Amplification of genomic DNA by PCR *(Continues...*

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Amplification of genomic DNA by PCR