BACTERIAL GENETICS-II

Molecular Genetics

- Polymerase Chain Reaction
- Genetic engineering/Recombinant
- DNA probes
- Blotting technique

What is Genetic Engineering?

"Genetic engineering is the technology for modifying the genetic information in a plant, animal or human in order to produce some desired trait or characteristic"

Recombinant DNA technology

r DNA technology

- **The technology of preparing r DNA *in vitro* by cutting up DNA molecules and splicing them together fragments from more than one organism.**

Prospects for Genetic Engineering

- Transgenic Engineering
  - Putting genetic information from one type of plant or animal into another
- Cloning
  - Making genetic copies of an existing plant or animal

Transgenic Animals

Human genes have been inserted into:

- Bacteria
- Mice

- To produce various human proteins for treating diseases.

Cloning

- A “clone” is a copy of something.
- In genetics, a clone is a genetic copy of another organism.
- Clones occur naturally:
  - **Asexual breeding in plants & lower animals**

Genetic engineering/recombinant technology

- Isolate the gene coding for a desired protein from microorganisms/higher life & insert them into suitable microorganims in such a way that it can be expressed in the formation of specific (desired)protein
- Protein produced in large quantities at reasonable cost
- DNA cleaved by enzymes called restriction endonucleases

contd

- Desired DNA fragments are covalently bound to vectors-plasmids /temperate bacteriophages
- The recombinant DNA molecule sealed by a ligase & introduced into a bacterial host E.coli or yeast.

Applications of genetic engineering

- Production of vaccines
- Production of antigens in diagnostic kits
- Production of proteins used in therapy.
- Transgenic animals
- Gene therapy

DNA Probe

- Definition: “ short sequence of nucleotide bases that will bind to specific regions of a target sequence of nucleotides”
- Biotinylated or radio labelled or fluorescent labelled piece of ss DNA or RNA for detection of homologous nucleotide by hybridisation method

Size of the probe

- Size of a nuleotide- 14-40 bases; average-20 bases needde for a probe
- Short probes – Hybridisation is faster-minutes,

Limited specificity-non-specific hybridisations
Difficult to label

- Long probes-Reaction time longer- hours

More stable at high temperatures and low salt concentrations

Probe Labels

- The label has to be attached or incorporated to the nucleic acid probe
- Radio labels- radio active istopes 32P, 36S, 125I, 3H- More sensitive
- Non-radio active labels-biotin, enzymes
- Chemiluminescence- chemicals that can emit light
- Fluorescence- chemicals that can fluoresce under UV light
- Antibodies- Antigenic group is attached and detected by using monoclonal antibodies

BLOTTING TECHNIQUES

Definition

- Visualization of specific DNA , RNA & protein among many thousands of contaminating molecules requires the convergence of number of techniques which are collectively termed BLOT transfer .

Types of blotting techniques

- 1) Southern blotting (to detect DNA)
- 2) Northern blotting (to detect RNA)
- 3) Western blotting (to detect protein)

Southern Blotting

- In 1975 Edward Southern developed this technique that is widely used to detect fragments of DNA .
- This requires

  1) Separation of DNA or DNA fragments by agarose gel electrophoresis .

  2) DNA fragments are blotted onto a strip of nitrocellulose or a nylon membrane.

  3) Identification by hybridization with a labeled ,complementary nucleic acid probe.

Applications

- Southern blots are used in gene discovery, mapping , evolution & development studies , diagnostics & forensics .
- Deletions / insertions .
- pointmutations / polymorphisms .
- Structural rearrangements .
- Allow for determination of molecular weights of restriction fragments .
- Presence of particular bit of DNA in the sample.

Northern blotting

- Northern blotting is a technique for detection of specific RNA sequences .
- Developed by James alwine & George stark.
- RNA molecules have defined length & much shorter than genomic DNA it is not necessary to cleave RNA before electrophoresis .
- RNA is more susceptible to degradation than DNA .
- RNA sample are separated based on size by gel electrophoresis .

contd

- RNA is blotted on to a nylon positively charged membrane .
- The membrane is placed in a hybridization buffer with a labeled probe (usually DNA)
- Labeled probe is detected by autoradiography
- Expression patterns of sequences of interest in different samples can be compared .

Applications

- A standard for direct study of the gene expression at the level of mRNA .
- Detection of mRNA transcript size .
- Study of RNA splicing – can detect alternatively spliced transcripts .
- Study RNA half life

Western blotting

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

Contd

- SDS PAGE technique is a prerequisite for western blotting .
- Protein sample is subjected to electrophoresis on SDS polyacrylamide gel .
- Electroblotting transfers the separated proteins from the gel to the surface of nitrocellulose membrane .

contd

- Blot is incubated with generic protein (such as milk protein)which binds to any remaining sticky places on the nitrocellulose .
- An antibody which is specific for the protein of interest (the primary antibody Ab 1) is added to the nitrocellulose sheet & reacts with the antigen . Only the band containing protein of interest binds the antibody forming a layer of antibody molecules .

Applications

- The confirmatory HIV test employs a western blot to detect anti HIV antibody in a human sample .
- Proteins from known HIV infected cells are separated & blotted on a membrane then the serum to be tested is applied in the primary antibody incubation step.
- Free antibody is washed away & a second anti human antibody linked to an enzyme signal can be added .

The three main steps of PCR

- The basis of PCR is temperature changes and the effect that these temperature changes have on the DNA.
- In a PCR reaction, the following series of steps is repeated 20-40 times

Note: 30 cycles usually takes about 2-3 hours and amplifies the DNA fragment of interest 1,000,000,000 fold

Step 1: Denature DNA

At 95°C, the DNA is denatured (i.e. the two strands are separated)

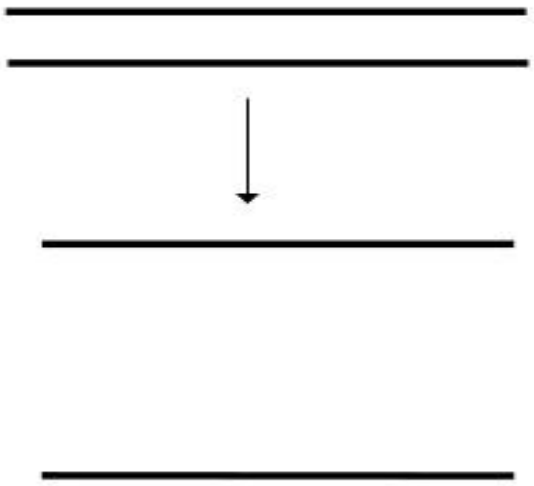
Step 2: Annealing (of Primers)

At 40°C- 65°C, the primers anneal (or bind to) their complementary sequences on the single strands of DNA

Step 3: Extension (of the DNA chain by DNA polymerase)

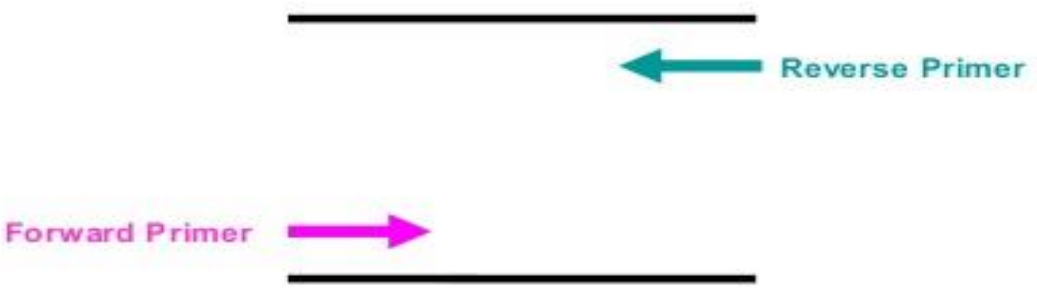
At 72°C, DNA Polymerase extends the DNA chain by adding nucleotides to the 3' ends of the primers.

Step: 1 Denaturation of DNA



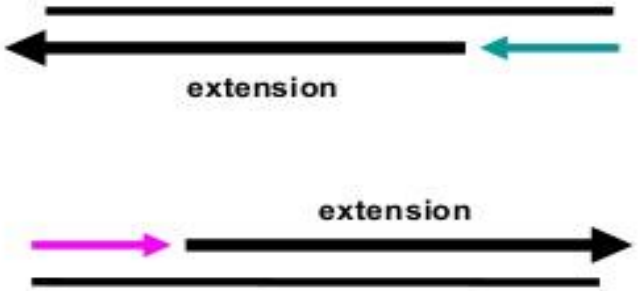
This occurs at 95 °C mimicking the function of helicase in the cell.

Step 2 Annealing or Primers Binding



- Primers bind to the complimentary sequence on the target DNA.
- Primers are chosen such that one is complimentary to the one strand at one end of the target sequence and that the *other* is complimentary to the *other* strand at the other end of the target sequence.

Step 3 Extension or Primer Extension



DNA polymerase catalyzes the extension of the strand in the 5-3 direction, starting at the primers, attaching the appropriate nucleotide (A-T, C-G)

Applications of PCR: Genetic Disease

- Primers can be created that will only bind and amplify certain alleles of genes or mutations of genes
    - This is the basis of genetic counseling and PCR is used as part of the diagnostic tests for genetic diseases.
- Some diseases that can be diagnosed with the help of PCR:
    - Huntington's disease
    - Cystic fibrosis
    - Human immunodeficiency virus

APPLICATIONS OF PCR IN DIAGNOSIS OF INFECTIOUS DISEASES

- Bacterial-

  Mycobacterial tuberculosis,Legionella, Chlamydia
- Viruses

  CMV, HSV,HBV,HIV
- Fungi

  Candida,Cryptococcus