MB504 – METHODS IN MOLECULAR BIOLOGY

FINAL TERM MOST IMPORTANT QUESTIONS

PREPARED BY: *SIR MUGHAL* (ADMIN, BS BIOLOGICAL SCIENCES)

GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2lSh1Gu

1. What do you understand by term Polymerase Chain Reaction?

Polymerase Chain Reaction (PCR) is a laboratory technique used to make millions of copies of a specific DNA segment. It allows the amplification of a targeted DNA sequence from a small amount of starting material by repeatedly copying it through cycles of heating and cooling, using a DNA polymerase enzyme and specific primers. PCR is widely used in research, diagnostics, forensic science, and genetic testing.

2. Explain the principle of DNA ligase.

Principle of DNA Ligase:

DNA ligase is an enzyme that facilitates the joining of DNA strands by catalyzing the formation of a phosphodiester bond between the 3'-hydroxyl end of one nucleotide and the 5'-phosphate end of another. This process is essential during DNA replication and repair, where DNA fragments (such as Okazaki fragments on the lagging strand) need to be joined together to form a continuous DNA molecule. DNA ligase requires ATP (in eukaryotes) or NAD^+ (in some bacteria) as a source of energy to drive the bond formation. In molecular biology, DNA ligase is widely used to join DNA fragments during cloning and recombinant DNA technology.

3. Write advantages of RAPD.

Advantages of RAPD (Random Amplified Polymorphic DNA):

- RAPD does not require prior knowledge of the DNA sequence, making it useful for studying organisms with unknown genomes.
- It is a quick and simple technique that uses short, arbitrary primers to amplify multiple DNA fragments simultaneously.
- RAPD can detect a high level of polymorphism, which helps in assessing genetic diversity.
- It requires only a small amount of DNA and standard PCR equipment.
- The method is cost-effective compared to other DNA fingerprinting techniques.
- RAPD is useful for population genetics, phylogenetic analysis, and species identification.
- It allows analysis of multiple loci in a single reaction, increasing efficiency.

4. Differentiate between Southern blotting and Northern blotting.

Southern Blotting:

Southern blotting is a molecular biology technique used to detect specific DNA sequences within a complex mixture of DNA. The process begins by digesting the DNA with restriction enzymes to cut it into smaller fragments. These fragments are then separated based on size using agarose gel electrophoresis. After separation, the DNA fragments are transferred from the gel onto a nylon or nitrocellulose membrane, where they are immobilized. A labeled DNA probe, complementary to the target sequence, is then hybridized to the membrane. The probe binds specifically to its matching DNA sequence, allowing the detection of the presence and size of the DNA fragment of interest. Southern blotting is commonly used for gene mapping, detecting mutations, and analyzing genetic structure.

Northern Blotting:

Northern blotting is a technique used to study gene expression by detecting specific RNA molecules within a mixture of RNA. In this method, total RNA is first extracted from cells or tissues and then separated by size through gel electrophoresis, typically using a denaturing agarose gel to preserve RNA integrity. The separated RNA is transferred onto a membrane and fixed in place. A labeled complementary probe is then hybridized to the membrane to bind to the target RNA sequence. This allows researchers to measure the presence, size, and abundance of specific RNA transcripts, providing insight into gene expression patterns under different conditions or in various tissues. Northern blotting is valuable in studying developmental biology, disease states, and gene regulation.

5. What is the purpose of using triple antibody ELISA and write it's another name used for it.

The purpose of using Triple Antibody ELISA is to increase the sensitivity and specificity of antigen detection. This method uses three antibodies: a primary antibody that binds to the target antigen, a secondary antibody that recognizes the primary antibody, and a tertiary antibody that is enzyme-linked and binds to the secondary antibody. This amplification through multiple antibody layers enhances the signal, allowing the detection of very low amounts of antigen.

Another name for Triple Antibody ELISA is Sandwich ELISA because the antigen is "sandwiched" between two antibodies—usually the primary capture antibody and a detection antibody—while the third antibody amplifies the signal.

6. Briefly describe the SNPs.

Single Nucleotide Polymorphisms (SNPs) are the most common type of genetic variation among individuals. They involve a change or substitution of a single nucleotide (A, T, C, or G) in the DNA sequence at a specific position in the genome. SNPs can occur in coding regions of genes, non-coding regions, or regulatory areas, and they may or may not affect how genes function. These variations contribute to individual differences in traits, susceptibility to diseases, and responses to drugs. SNPs are widely used as genetic markers in research, disease diagnosis, and personalized medicine.

7. What different reagents are used in conventional PCR and explain why.

In conventional PCR, several key reagents are used, each serving a specific purpose to enable the amplification of DNA:

1. ***Template DNA:*** This is the DNA sample containing the target sequence to be amplified.
2. ***Primers (Forward and Reverse):*** Short single-stranded DNA sequences that are complementary to the regions flanking the target DNA. They provide starting points for DNA synthesis.
3. ***DNA polymerase:*** An enzyme (commonly Taq polymerase) that synthesizes new DNA strands by adding nucleotides complementary to the template strand during PCR cycles.
4. ***dNTPs (Deoxynucleotide Triphosphates):*** The building blocks (dATP, dTTP, dGTP, dCTP) that DNA polymerase incorporates into the new DNA strand.
5. ***Buffer Solution:*** Provides the optimal chemical environment (including pH and salt concentration) necessary for enzyme activity.
6. ***$MgCl_2$ (Magnesium Chloride):*** Magnesium ions act as a cofactor essential for the activity of DNA polymerase and influence the fidelity and efficiency of the reaction.

8. Explain adjuvants, antibody affinity and antibody avidity.

Adjuvants:

Adjuvants are substances added to vaccines or immunogens to enhance the body's immune response to an antigen. They work by stimulating the immune system, increasing the effectiveness and duration of the immune reaction. Adjuvants can improve antigen presentation, activate immune cells, and promote the production of antibodies. Common examples include aluminum salts (alum) and oil-in-water emulsions.

Antibody Affinity:

Antibody affinity refers to the strength of the binding between a single antigen-binding site of an antibody and a single epitope on an antigen. It measures how tightly and specifically an antibody binds to its target. High affinity means the antibody binds strongly to the antigen, which is important for effective immune recognition.

Antibody Avidity:

Antibody avidity is the overall strength of binding between an antibody and an antigen when multiple binding sites are involved. Since antibodies (like IgG) have more than one antigen-binding site, avidity reflects the combined strength of all interactions. Avidity is influenced by both affinity and the number of binding sites, making it a measure of the functional binding strength in a real biological context. High avidity leads to more stable and effective antigen-antibody complexes.

9. Write the properties of probes used in Southern Blotting.

Properties of Probes Used in Southern Blotting:

- ***Specificity:*** Probes must be complementary to the target DNA sequence to ensure specific binding.
- ***Length:*** Typically 20–100 nucleotides long to balance specificity and binding strength.
- ***Labeling:*** Probes are labeled with radioactive isotopes (e.g., ^32P) or non-radioactive markers (e.g., biotin, digoxigenin) for detection.
- ***Stability:*** Probes should be stable under hybridization and washing conditions to maintain binding.
- ***Single-stranded:*** Probes are usually single-stranded DNA or RNA to allow hybridization with the target single-stranded DNA on the membrane.
- ***High Binding Affinity:*** Probes must bind strongly to the target sequence to produce a clear signal.
- ***Low Background Binding:*** Probes should minimize non-specific binding to reduce background noise in detection.

10. Differentiate the blotting technique on the basis of their detection method.

Southern Blotting: Detects DNA using labeled DNA or RNA probes that hybridize specifically to the target DNA sequence. Detection is done through radioactive signals (e.g., ^32P) or non-radioactive labels (e.g., chemiluminescence, colorimetric methods).

Northern Blotting: Detects RNA using labeled complementary DNA or RNA probes. The detection methods are similar to Southern blotting, often involving radioactive or chemiluminescent labels to visualize RNA bands.

Western Blotting: Detects proteins using specific antibodies. The primary antibody binds the target protein, and a labeled secondary antibody (enzyme-linked or fluorescent) enables detection. Common detection methods include chemiluminescence, colorimetric reactions, or fluorescence.

11. Differentiate between WGA PCR, In Situ PCR and Suicide PCR.

WGA PCR (Whole Genome Amplification PCR):

WGA PCR is a technique used to amplify the entire genome from a small amount of DNA. It generates large quantities of DNA covering the whole genome, enabling downstream applications like genotyping or sequencing when starting material is limited. WGA does not target specific sequences but amplifies all genomic DNA uniformly.

In Situ PCR:

In Situ PCR combines PCR amplification with microscopy by performing PCR directly within fixed cells or tissue sections on slides. This method amplifies specific DNA or RNA sequences in their original spatial context, allowing visualization of gene expression or pathogen detection within cells, maintaining tissue architecture.

Suicide PCR:

Suicide PCR is a highly controlled PCR method used mainly to prevent contamination in sensitive applications like ancient DNA studies. It uses unique primers that have never been used before and are not reused, ensuring that any amplified product is from the target DNA and not from contamination. This helps avoid false positives.

12. Write features of radioactive substances in Southern blotting.

Features of Radioactive Substances in Southern Blotting:

- ***High Sensitivity:*** Radioactive labels, such as phosphorus-32 (^32P), allow detection of very small amounts of DNA due to their strong emissions.
- ***Strong Signal:*** They produce intense signals, making it easier to visualize low-abundance targets.
- ***Quantitative:*** The intensity of the radioactive signal can be quantified to estimate the amount of target DNA.
- ***Short Half-life:*** Many radioactive isotopes have relatively short half-lives, requiring careful timing and handling of experiments.
- ***Safety Concerns:*** Handling radioactive materials requires strict safety protocols to protect researchers and the environment from radiation exposure.
- ***Requires Special Equipment:*** Detection involves the use of X-ray film or phosphorimagers to capture the radioactive signals.
- ***Stable Labeling:*** Radioactive isotopes covalently attach to probes and remain stable during hybridization and washing steps.

13. What is DNA fingerprinting?

DNA fingerprinting is a technique used to identify individuals based on unique patterns in their DNA. It analyzes specific regions of the genome that vary greatly between people, such as short tandem repeats (STRs) or variable number tandem repeats (VNTRs). By comparing these patterns, DNA fingerprinting can establish identity, determine biological relationships, or link a person to a crime scene. It is widely used in forensic science, paternity testing, and genetic studies.

14. Write applications of Northern blotting.

Applications of Northern Blotting:

- ***Gene Expression Analysis:*** Northern blotting is commonly used to measure the presence and amount of specific RNA transcripts, helping researchers understand gene activity in different tissues or conditions.
- ***Study of RNA Processing:*** It helps detect different RNA splice variants and alternative transcripts.
- ***Verification of RNA Size:*** Northern blotting can determine the size of RNA molecules, confirming transcript length and processing.
- ***Diagnosis of Genetic Disorders:*** It is used to detect abnormal RNA expression patterns linked to certain diseases.
- ***Detection of Viral RNA:*** Northern blotting can identify and study RNA viruses in infected cells.
- ***Comparative Expression Studies:*** It allows comparison of gene expression levels between normal and diseased tissues or experimental treatments.

15. What are monoclonal antibodies?

Monoclonal antibodies are identical antibodies produced by a single clone of B cells or hybridoma cells. They recognize and bind to a specific, unique epitope on an antigen with high specificity. Because they are uniform and target only one specific site, monoclonal antibodies are widely used in diagnostics, research, and therapy, such as in cancer treatment, autoimmune diseases, and infectious disease detection. Their production involves fusing a specific antibody-producing B cell with a myeloma (cancer) cell to create a hybridoma that can be cultured indefinitely.

16. Write methodology of Colony PCR.

Methodology of Colony PCR:

1. ***Sample Preparation:***
   Pick a small amount of bacterial colony from an agar plate using a sterile toothpick or pipette tip.
2. ***Template Preparation:***
   Either directly transfer the picked colony into the PCR reaction tube containing the PCR master mix, or first resuspend the cells in a small volume of sterile water or buffer. Sometimes, cells are heated briefly (e.g., 95°C for 5 minutes) to lyse them and release DNA.
3. ***PCR Reaction Setup:***
   Prepare the PCR mixture containing DNA polymerase, primers specific to the target sequence, dNTPs, buffer, $MgCl_2$, and the bacterial cells as the template.
4. ***PCR Amplification:***
   Run the PCR cycles:
   - Initial denaturation (e.g., 94-95°C for 2-5 minutes)
   - Denaturation (94-95°C for 30 seconds)
   - Annealing (50-60°C for 30 seconds) depending on primer Tm
   - Extension (72°C for 30 seconds to 1 minute, depending on amplicon size)
   - Repeat 25-35 cycles
   - Final extension at 72°C for 5-10 minutes
5. ***Analysis:***
   Run the PCR products on an agarose gel to check for the presence and size of the amplified DNA fragment, confirming whether the colony contains the desired DNA insert.

17. Explain radioactive and non-radioactive probes.

Radioactive Probes:

Radioactive probes are DNA or RNA sequences labeled with radioactive isotopes, commonly phosphorus-32 (^32P). These probes bind specifically to complementary target sequences during hybridization. The radioactive emissions allow sensitive detection of the hybridized probe using X-ray film or phosphorimagers. Radioactive probes offer high sensitivity and quantitative results but require careful handling, safety precautions, and have a limited shelf life due to isotope decay.

Non-Radioactive Probes:

Non-radioactive probes are labeled with molecules such as biotin, digoxigenin, or fluorescent dyes instead of radioactive isotopes. These labels are detected through enzymatic reactions producing colorimetric or chemiluminescent signals or by direct fluorescence. Non-radioactive probes are safer, more stable, and easier to handle than radioactive ones but may have slightly lower sensitivity. They are widely used in modern molecular biology for DNA and RNA detection.

18. What is Restriction Modification system?

The Restriction Modification (R-M) system is a bacterial defense mechanism that protects bacteria from invading foreign DNA, such as bacteriophages (viruses that infect bacteria). It consists of two key components: a restriction enzyme (restriction endonuclease) and a modification enzyme (methyltransferase).

The restriction enzyme recognizes specific DNA sequences and cuts foreign DNA at or near these sites, thereby inactivating the invading genetic material. To prevent damage to the host's own DNA, the modification enzyme methylates the bacterial DNA at the same recognition sites. This methylation protects the bacterial DNA from cleavage by the restriction enzyme.

Together, these enzymes allow bacteria to distinguish between self and non-self DNA, providing immunity against foreign genetic elements. The restriction modification system is also widely used in molecular biology for DNA manipulation and cloning.

19. Define Adjuvants with examples.

Adjuvants are substances added to vaccines or immunogens to enhance and prolong the immune response to an antigen. They help improve the effectiveness of the vaccine by stimulating the immune system, increasing antibody production, and promoting a stronger and longer-lasting immunity.

Examples of adjuvants include:

- ***Aluminum salts (Alum):*** The most commonly used adjuvant in human vaccines.
- ***Freund's adjuvant:*** Used mainly in experimental settings; includes Complete Freund's adjuvant (with killed mycobacteria) and Incomplete Freund's adjuvant.
- ***MF59:*** An oil-in-water emulsion used in some influenza vaccines.
- ***AS04:*** A combination of alum and monophosphoryl lipid A used in HPV vaccines.

20. What is the effects of SNPs in coding region?

Effects of SNPs in the Coding Region:

Single Nucleotide Polymorphisms (SNPs) occurring in the coding region of a gene can affect the protein in several ways:

1. ***Silent Mutation:*** The SNP changes a nucleotide but does not alter the amino acid due to the redundancy of the genetic code. This usually has no effect on protein function.
2. ***Missense Mutation:*** The SNP causes a change in one amino acid in the protein sequence, which may affect the protein's structure or function. The impact can range from benign to harmful depending on the amino acid change and its position.

3. ***Nonsense Mutation:*** The SNP creates a premature stop codon, leading to truncated, usually nonfunctional proteins. This often results in loss of protein function.
4. ***Splice Site Mutation:*** If the SNP affects splice sites, it can alter mRNA splicing, leading to abnormal or nonfunctional proteins.

21. Write steps of DNA fingerprinting.

Steps of DNA Fingerprinting:

1. ***Sample Collection:***
   Collect biological samples containing DNA, such as blood, saliva, hair, or tissue.
2. ***DNA Extraction:***
   Isolate DNA from the collected samples using chemical or mechanical methods.
3. ***DNA Quantification and Purity Check:***
   Measure the amount and quality of extracted DNA to ensure it is suitable for analysis.
4. ***DNA Digestion:***
   Cut the DNA into fragments using specific restriction enzymes that recognize and cleave particular sequences.
5. ***Gel Electrophoresis:***
   Separate the DNA fragments by size through agarose or polyacrylamide gel electrophoresis.
6. ***Southern Blotting:***
   Transfer the separated DNA fragments from the gel onto a nylon or nitrocellulose membrane.
7. ***Hybridization with Probe:***
   Hybridize the membrane with a labeled DNA probe that binds to specific variable regions (like VNTRs or STRs).
8. ***Detection:***
   Visualize the hybridized fragments using autoradiography or chemiluminescence, producing a unique band pattern (DNA fingerprint).
9. ***Analysis:***
   Compare the band patterns between samples to establish identity or genetic relationships.

22. What are limitations of Western Blotting?

Limitations of Western Blotting:

- ***Time-Consuming:*** The procedure involves multiple steps (protein extraction, electrophoresis, transfer, antibody incubations) and can take several hours to days.
- ***Requires High-Quality Antibodies:*** The specificity and sensitivity depend on the availability of good-quality primary and secondary antibodies. Poor antibodies can lead to non-specific bands or weak signals.
- ***Semi-Quantitative:*** While Western blotting can indicate relative protein amounts, it is not highly accurate for precise quantification without additional controls.
- ***Low Throughput:*** It analyzes one or a few proteins at a time, making it less suitable for large-scale protein profiling.
- ***Sample Limitations:*** Requires relatively large amounts of protein compared to some newer techniques.
- ***Background Noise:*** Non-specific binding can cause background signals, making interpretation difficult.

- ***Protein Size Limitation:*** Very large or very small proteins may be difficult to resolve or transfer efficiently.
- ***Technical Variability:*** Results can vary based on gel quality, transfer efficiency, and antibody incubation conditions.

23. What is Ion-Exchange chromatography and its types?

Ion-Exchange Chromatography is a separation technique that separates molecules based on their charge. It uses a resin (stationary phase) with charged groups that attract and bind oppositely charged molecules (ions) from a mixture. Molecules are separated by changing the ionic strength or pH of the mobile phase, which affects their binding affinity to the resin.

Types of Ion-Exchange Chromatography:

1. ***Anion Exchange Chromatography:***
   The resin contains positively charged groups that bind negatively charged ions (anions). Common resins use groups like DEAE (diethylaminoethyl).
2. ***Cation Exchange Chromatography:***
   The resin contains negatively charged groups that bind positively charged ions (cations). Common resins use groups like CM (carboxymethyl).

Disclaimer:

For best results, it is advised NOT to rely on this file only. Go through video lectures, handouts and other sources as well.

Regards,

Sir Mughal,

BS Zoology, Virtual University of Pakistan

B.Sc (Honors), University of Karachi.