MB504 – METHODS IN MOLECULAR BIOLOGY

MID TERM MOST IMPORTANT QUESTIONS

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

1. A researcher has to synthesize complementary DNA using RNA as a template. Which type of PCR will he use? Write procedure.

Type of PCR: Reverse Transcription PCR (RT-PCR)

Procedure:

1. ***RNA Extraction:*** Total RNA is isolated from the sample using an RNA extraction method. The RNA should be pure and intact.
2. ***cDNA Synthesis:*** The RNA is converted into complementary DNA (cDNA) using reverse transcriptase. The reaction mixture includes RNA template, reverse transcriptase, dNTPs, primers (oligo-dT or random hexamers), and buffer. The mixture is incubated at 42–55°C for 30–60 minutes, then heated to 70°C to inactivate the enzyme.
3. ***PCR Amplification:*** The cDNA is amplified using PCR. The PCR mix contains cDNA, DNA polymerase, gene-specific primers, dNTPs, $MgCl_2$, and buffer. PCR cycles include:
    - Denaturation at 94–95°C
    - Annealing at 50–65°C
    - Extension at 72°C

2. Differentiate between Multiplex and Nested PCR.

Multiplex PCR and Nested PCR are two specialized forms of the polymerase chain reaction used for different purposes. Multiplex PCR allows the simultaneous amplification of multiple target DNA sequences in a single reaction by using more than one pair of primers. This technique is useful for detecting multiple genes or pathogens at once, saving time and resources. In contrast, Nested PCR is designed to increase the sensitivity and specificity of amplification. It involves two successive PCR reactions: the first uses one pair of primers to amplify a target region, and the second uses a new set of primers that bind within the first PCR product. This reduces non-specific amplification and is particularly useful when working with low amounts of DNA or in complex samples. While Multiplex PCR focuses on detecting multiple targets at once, Nested PCR focuses on increasing accuracy by reducing false positives.

3. Write applications of Symmetric PCR.

Applications of Symmetric PCR:

- Used in DNA cloning to amplify specific DNA fragments for insertion into vectors.
- Helps in genotyping to detect mutations, single nucleotide polymorphisms (SNPs), & other genetic variations.
- Used in clinical diagnostics to identify pathogens by amplifying target DNA regions.
- Applied in forensic science for DNA profiling and identification from crime scene samples.
- Supports gene expression analysis when combined with reverse transcription (RT-PCR).
- Essential in DNA sequencing to generate enough DNA for various sequencing techniques.
- Used in mutation detection for research and medical genetics.

4. Write applications of PCR in microbiology.

Applications of PCR in Microbiology:

- ***Detection of microbial pathogens:*** PCR is used to identify bacteria, viruses, fungi, and parasites in clinical and environmental samples by amplifying their specific DNA or RNA sequences.
- ***Diagnosis of infectious diseases:*** It helps in early and accurate diagnosis of diseases such as tuberculosis, HIV, COVID-19, and hepatitis.
- ***Identification and classification of microorganisms:*** PCR assists in identifying unknown microorganisms and classifying them based on genetic markers.
- ***Detection of antibiotic resistance genes:*** PCR can detect genes responsible for antibiotic resistance in bacteria, aiding in appropriate treatment decisions.
- ***Monitoring microbial contamination:*** It is used in food, water, and pharmaceutical industries to detect contamination by harmful microorganisms.
- ***Studying microbial diversity:*** PCR helps in analyzing microbial populations in different environments using techniques like 16S rRNA gene sequencing.
- ***Genotyping of microbial strains:*** It enables comparison of different strains of microorganisms for epidemiological studies and outbreak investigations.

5. Write a note on Specific Allele PCR and Suicide PCR.

Specific Allele PCR:

Specific Allele PCR is a type of PCR used to detect known single nucleotide polymorphisms (SNPs) or mutations in DNA. It uses allele-specific primers that only bind and allow amplification if a particular allele (variant) is present at the target site. This method is highly specific and is commonly used in genetic testing, mutation analysis, and genotyping. By designing primers that match either the normal or mutant allele, researchers can quickly identify the genetic makeup of a sample.

Suicide PCR:

Suicide PCR is a technique used mainly in ancient DNA or forensic studies to prevent contamination. It involves using primers that are unique and have never been used in the laboratory before, and are never reused. This ensures that any amplification seen in the reaction is from the target sample and not from

contamination by previous PCR products. Suicide PCR is particularly useful in situations where the DNA is highly degraded or present in very low amounts, and strict contamination control is essential.

6. Write a note on In Situ PCR.

In Situ PCR is a technique that combines the sensitivity of polymerase chain reaction (PCR) with the spatial resolution of histology. It allows the amplification of specific DNA or RNA sequences directly within fixed cells or tissue sections on microscope slides. This method enables researchers to detect and localize nucleic acids within the morphological context of the sample.

In Situ PCR involves fixing and permeabilizing the tissue or cells to allow the entry of PCR reagents, followed by amplification using standard PCR components. The amplified product is then detected using labeled probes, fluorescent dyes, or antibodies. It is especially useful for identifying the presence and location of pathogens, gene expression patterns, or genetic alterations in specific cell types within a heterogeneous tissue sample.

This technique is widely used in pathology, virology, cancer research, and developmental biology, providing both molecular and anatomical information from the same sample.

7. Explain type II and type IIs restriction endonuclease.

Type II Restriction Endonucleases:

Type II restriction endonucleases are enzymes that recognize specific, short, and usually palindromic DNA sequences and cut within or very close to these recognition sites. They require only magnesium ions (Mg^{2+}) for activity and do not need ATP or other cofactors. Because they cut at defined positions, they are widely used in molecular biology for DNA cloning, mapping, and analysis. The cuts produce either blunt or sticky (overhanging) ends, which facilitate the joining of DNA fragments.

Type IIs Restriction Endonucleases:

Type IIs restriction endonucleases also recognize specific DNA sequences, but unlike Type II enzymes, they cut the DNA at a defined distance away from their recognition site, usually outside the recognition sequence. This property allows greater flexibility in molecular cloning because the cut sites can be positioned to create custom overhangs. Type IIs enzymes are commonly used in advanced techniques like Golden Gate cloning, which enables seamless assembly of multiple DNA fragments in a defined order.

8. Describe 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 main components: a restriction enzyme (restriction endonuclease) and a modification enzyme (methyltransferase).

The restriction enzyme recognizes specific DNA sequences (usually palindromic) and cuts the foreign DNA at or near these sites, thereby inactivating the invading genetic material. However, if the bacterial DNA were cut by these enzymes, it would be harmful to the bacteria itself. To prevent this, the modification enzyme methylates the host's own DNA at the same recognition sites, adding methyl groups to specific bases. This methylation protects the bacterial DNA from cleavage by the restriction enzyme because the enzyme cannot cut methylated DNA.

9. Define electrophoresis.

Electrophoresis is a laboratory technique used to separate charged molecules, such as DNA, RNA, or proteins, based on their size and charge by applying an electric field. When an electric current passes through a gel matrix, negatively charged molecules move toward the positive electrode, with smaller molecules traveling faster and farther than larger ones. This separation allows analysis, purification, or identification of the molecules.

10. What is the principle of QRT?

The principle of Quantitative Real-Time PCR (qRT-PCR) is based on the amplification of a specific DNA sequence while simultaneously measuring the amount of amplified product in real-time during each cycle of PCR. This is achieved by using fluorescent dyes or probes that emit fluorescence proportional to the amount of DNA generated. The increase in fluorescence is monitored after every cycle, allowing the quantification of the initial amount of the target nucleic acid. This technique is highly sensitive, specific, and enables both detection and quantification of gene expression or pathogen load.

11. What are advantages of PCR?

Advantages of PCR:

- PCR is highly sensitive and can amplify very small amounts of DNA.
- It is specific, allowing the targeting of particular DNA sequences using specific primers.
- PCR is rapid, producing results within a few hours.
- It requires only small amounts of sample.
- PCR can amplify DNA from various sources, including blood, tissue, and forensic samples.
- It allows detection of genetic mutations, pathogens, and gene expression.
- PCR is versatile and can be adapted for many applications, such as cloning, sequencing, and diagnostics.
- It can be automated and scaled up for high-throughput analysis.

12. Why we use DNTPs?

We use dNTPs (deoxynucleotide triphosphates) in PCR because they serve as the essential building blocks for new DNA strand synthesis. During the PCR process, the enzyme DNA polymerase reads the template DNA strand and incorporates complementary nucleotides to form a new DNA strand. The dNTPs—comprising dATP, dTTP, dGTP, and dCTP—are the substrates that provide the individual nucleotides (adenine, thymine, guanine, and cytosine) required to extend the growing DNA chain.

Each dNTP consists of a deoxyribose sugar, a nitrogenous base, and three phosphate groups. When DNA polymerase adds a dNTP to the DNA strand, it cleaves off two phosphates, providing the energy needed for the formation of the phosphodiester bond between nucleotides. Without dNTPs, the polymerase would have no material to synthesize the new DNA strand, and the PCR amplification would not occur. Therefore, dNTPs are crucial components that enable DNA replication and amplification during PCR.

13. Explain Hot Start PCR.

Hot Start PCR is a modification of the traditional PCR technique designed to improve the specificity and yield of the reaction by reducing non-specific amplification and primer-dimer formation. In Hot Start PCR, one or more key components of the PCR reaction—usually the DNA polymerase—are kept inactive or blocked at room temperature and only become active once the reaction mixture is heated to a high temperature during the initial denaturation step.

This activation method prevents the polymerase from extending primers nonspecifically at lower temperatures before the thermal cycling begins. There are different ways to achieve Hot Start, such as using chemically modified polymerases, antibodies that inhibit the enzyme, or physical barriers like wax beads that melt at high temperature.

14. What is the source of following 3 enzymes? Taq Polymerase, Vent, Pfu.

Here are the sources of the three enzymes:

- ***Taq Polymerase:***
  Derived from *Thermus aquaticus*, a thermophilic bacterium found in hot springs.
- ***Vent Polymerase:***
  Derived from *Thermococcus litoralis*, a heat-loving archaeon.
- ***Pfu Polymerase:***
  Derived from *Pyrococcus furiosus*, a hyperthermophilic archaeon found in deep-sea hydrothermal vents.

15. Write a note on Amplified Fragment Length Polymorphism (AFLP).

Amplified Fragment Length Polymorphism (AFLP) is a powerful molecular technique used to detect genetic variation by amplifying selective fragments of DNA. It combines the principles of restriction enzyme digestion and PCR amplification.

The process begins by digesting genomic DNA with specific restriction enzymes, which cut the DNA at particular sequences. Adaptors are then ligated to the ends of these fragments, providing known sequences for primer binding. A subset of these fragments is selectively amplified using PCR with primers complementary to the adaptors plus additional selective nucleotides. The amplified fragments are then separated by gel electrophoresis or capillary electrophoresis to generate a fingerprint pattern.

AFLP is highly sensitive and reproducible, allowing the detection of polymorphisms across the genome without prior knowledge of DNA sequences. It is widely used in genetic mapping, biodiversity studies, population genetics, and plant and animal breeding programs.

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,

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