

***Final Assignment for viva (lab)***

***Technology***

*Medical Lab Technology(MLT)*

***Subject***

*Molecular Biology*

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**Q1.What is PCR? Explain the procedure and uses of PCR.**

**[Answer]**

PCR was invented by Kary Mullis in 1983. He shared the Nobel Prize in chemistry with Michael Smith in 1993. Polymerase Chain Reaction (PCR) is a powerful method for amplifying particular segments of DNA, distinct from cloning and propagation within the host cell. This procedure is carried out entirely biochemically, that is, in vitro. Using PCR it is possible to generate thousands to millions of copies of a particular section of DNA from a very small amount of DNA.

**Types of PCR**

1. Real-time PCR
2. Quantitative real time PCR (Q-RT PCR)
3. Reverse Transcriptase PCR (RT-PCR)
4. Multiplex PCR
5. Nested PCR
6. Long-range PCR
7. Single-cell PCR
8. Fast-cycling PCR
9. Methylation-specific PCR (MSP)
10. Hot start PCR

**Procedure**

**1. Denaturation**

The DNA template is heated to 94° C. This breaks the weak hydrogen bonds that hold DNA strands together in a helix, allowing the strands to separate creating single stranded DNA. What happens in denaturation procedures discusses it below

* During this stage the cocktail containing the template DNA and all the other core ingredients is heated to 94-95⁰C.
* The high temperature causes the [hydrogen bonds?](javascript:void(%22Click%20to%20expand%20this%20glossary%20term%22)) Between the bases in two strands of template DNA to break and the two strands to separate.
* This results in two single strands of DNA, which will act as templates for the production of the new strands of DNA.
* It is important that the temperature is maintained at this stage for long enough to ensure that the DNA strands have separated completely.

**2. Annealing**

The mixture is cooled to anywhere from 50-70° C. This allows the primers to bind (anneal) to their complementary sequence in the template DNA. What happens in annealing procedures discusses it below.

* During this stage the reaction is cooled to 50-65⁰C. This enables the primers to attach to a specific location on the single-stranded template DNA by way of hydrogen bonding (the exact temperature depends on the melting temperature of the primers you are using).
* Primers are single strands of DNA or RNA. Sequence that are around 20 to 30 bases in length.
* The primers are designed to be complementary. In sequence to short sections of DNA on each end of the sequence to be copied.
* Primers serve as the starting point for DNA synthesis. The polymerase enzyme can only add DNA bases to a double strand of DNA. Only once the primer has bound can the polymerase enzyme attach and start making the new complementary strand of DNA from the loose DNA bases.

**3. Extension**

The reaction is then heated to 72° C, the optimal temperature for DNA polymerase to act. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template. What happens in extension procedures the final procedure of pcr discusses below:

* During this final step, the heat is increased to 72⁰C to enable the new DNA to be made by a special Taq DNA polymerase enzyme which adds DNA bases.
* Taq DNA polymerase is an enzyme taken from the heat-loving bacteria. Thermus aquaticus.
* 72⁰C is the optimum temperature for the Taq polymerase to build the complementary strand. It attaches to the primer and then adds DNA bases to the single strand one-by-one in the 5’ to 3’ direction.
* The result is a brand new strand of DNA and a double-stranded molecule of DNA.
* The duration of this step depends on the length of DNA sequence being amplified but usually takes around one minute to copy 1,000 DNA bases (1Kb).

**Uses of PCR:**

1. PCR is used in analyzing clinical specimens for the presence of infectious agents, including HIV, hepatitis, malaria, anthrax, etc.
2. PCR can provide information on a patient’s prognosis, and predict response or resistance to therapy. Many cancers are characterized by small mutations in certain genes, and this is what PCR is employed to identify.
3. PCR is used in the analysis of mutations that occur in many genetic diseases (e.g. cystic fibrosis, sickle cell anaemia, phenylketonuria, muscular dystrophy).
4. PCR is also used in forensics laboratories and is especially useful because only a tiny amount of original DNA is required, for example, sufficient DNA can be obtained from a droplet of blood or a single hair.
5. PCR is an essential technique in cloning procedure which allows generation of large amounts of pure DNA from tiny amount of template strand and further study of a particular gene.
6. The Human Genome Project (HGP) for determining the sequence of the 3 billion base pairs in the human genome relied heavily on PCR.
7. PCR has been used to identify and to explore relationships among species in the field of evolutionary biology. In anthropology, it is also used to understand the ancient human migration patterns. In archaeology, it has been used to spot the ancient human race. PCR commonly used by Paleontologists to amplify DNA from extinct species or cryopreserved fossils of millions years and thus can be further studied to elucidate on.

**Q2.Explain the process of agarose gel electrophoresis**

**[Answer]**

**Agarose gel electrophoresis:**

* Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA, RNA or proteins in a matrix of agarose.
* Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool.
* They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation

**Requirement and instrumentations of agarose gel electrophoresis:**

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

1. An **electrophoresis chamber** and **power supply**
2. **Gel casting trays**, which are available in a variety of sizes and composed of UVtransparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
3. **Sample combs**, around which molten medium is poured to form sample wells in the gel.
4. **Electrophoresis buffer**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
5. **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to “fall” into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
6. **Staining**: DNA molecules are easily visualized under an ultraviolet lamp when electrphoresed in the presence of the extrinsic fluor ethidium bromide. Alternatively, nucleic acids can be stained after electrophoretic separation by soaking the gel in a solution of ethidium bromide. When intercalated into double stranded DNA, fluorescence of this molecule increases greatly. It is also possible to detect DNA with the extrinsic fluor 1-anilino 8-naphthalene sulphonate.
7. **Transilluminator** (an ultraviolet light box), which is used to visualize stained DNA in gels.

**Steps involve in agarose gel electrophoresis:**

1. To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it.

* **The concentration of Agarose Gel**
* The percentage of agarose used depends on the size of fragments to be resolved.
* The concentration of agarose is referred to as a percentage of agarose to volume of buffer (w/v), and agarose gels are normally in the range of 0.2% to 3%.
* The lower the concentration of agarose, the faster the DNA fragments migrate.
* In general, if the aim is to separate large DNA fragments, a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended.

1. Ethidium bromide is added to the gel (final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis.
2. After cooling the solution to about 60oC, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.
3. After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells.
4. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer.
5. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied.
6. The current flow can be confirmed by observing bubbles coming off the electrodes.
7. DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge.
8. The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes like bromophenol blue and xylene cyanol dyes.

**Applications of Agarose gel electrophoresis:**

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA.

* Estimation of the size of DNA molecules
* Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
* Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis.
* The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with restriction enzymes, e.g. in restriction mapping of cloned DNA.
* Agarose gel electrophoresis is commonly used to resolve circular DNA with different super coiling topology, and to resolve fragments that differ due to DNA synthesis.
* In addition to providing an excellent medium for fragment size analyses, agarose gels allow purification of DNA fragments. Since purification of DNA fragments size separated in an agarose gel is necessary for a number molecular techniques such as cloning, it is vital to be able to purify fragments of interest from the gel.