

✚ **Final viva Assignment (BS-MLT 4th)**

✚ **Course Title: Molecular Biology**

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

○ **What is PCR?**

PCR is the shorthand for a simple but very useful method in molecular biology called polymerase chain reaction. This is a technique used to amplify any part of the DNA of interest or to make many copies. In other words, PCR enables you to initially create millions of copies of a specific DNA sequence from a small sample - sometimes even a single copy. This is an important process for a range of genetic technologies and, in fact, has enabled the development of a suite of new technologies.

Procedure of PCR,

○ **Pre-preparation:**

Pre-preparation for any molecular genetics experiment plays an important role in achieving good results. Someone must be ready to do the lab work before starting the reaction. Wear a lab coat, gloves, hat, and headgear. Clean the PCR reaction preparation area and Arrange all other utilities as soon as the reaction is ready. Now take the reagents from the deep freezer and melt all the reagents properly.

○ **Reaction preparation:**

Take a sterile PCR tube and start adding reagents. If you have a master mix ready to use, you can add it directly, it will save time and increase reaction efficiency. After completion of reaction preparation, turn off all tube caps and rotate it well, so that all reagents mix well. Now put tubes in PCR machine one by one in PCR protocol. Remember: don't waste time setting during PCR, set it before reaction preparation, and run PCR immediately. Meanwhile start preparing the gel for agarose gel electrophoresis, because it will also take time for around 55 to 85 minutes.

○ **Post-preparation:**

After the PCR Reaction is complete turn off the machine and assemble all the tubes "in an orderly manner". Agaros gel rests in a frozen tube for some time before electrophoresis. You can also rest it for the next day, no problem.

○ **Uses,**

*PCR has a multitude of uses;

*Genetic Testing: to screen for and detect DNA mutations.

*Tissue typing: before organ transplant to test for compatibility.

*Genetic fingerprinting at crime scenes.

*paternity testing,

*DNA sequencing.

*DNA cloning.

*Creating large volumes of DNA for other work.

*Genetic mapping.

Q2. Explain the process of agarose gel electrophoresis.

○ **What is gel electrophoresis?**

Gel electrophoresis is a technique commonly used in laboratories to separate charged molecules like DNA, RNA, and proteins, according to their size.

○ **The process of agarose gel electrophoresis,**

A) An agarose and buffer solution is poured into a plastic tray. A comb is placed into the tray on one end.

B) The agarose polymerizes into a gel as it cools. The comb is removed from the gel to form wells for samples.

C) DNA samples colored with a tracking dye are pipetted into the wells.

D) The tray is placed into a chamber that generates electric current through the gel. The negative electrode is placed on the side nearest the samples. The positive electrode is placed on the other side.

E) DNA has a negative charge and will be drawn to the positive electrode. Smaller DNA molecules will be able to travel faster through the gel.

F) One well, called a DNA ladder, will contain DNA fragments of known sizes. This ladder is used to determine the sizes of other samples.

THE END