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**Programme: BS (MLT) 4th semester**

**Instructor: Sir Fazli Zahir**

**Subject: Molecular biology Viva assign**

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

**Answer:-**

**PCR**

It is a technique used to amplify a segment of DNA of interest or produce lots and lots of copies. In other words, PCR enables you to produce millions of copies of a specific DNA sequence from an initially small sample – sometimes even a single copy. It is a crucial process for a range of genetic technologies and, in fact, has enabled the development of a suite of new technologies.

**Procedure**

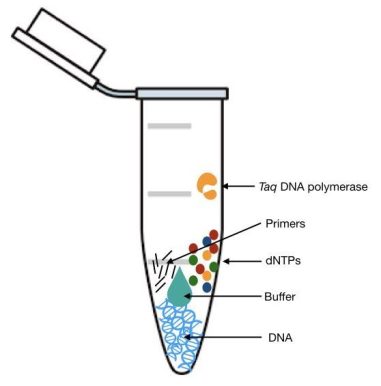
**Pre-preparation:**

For any molecular genetic experiment, pre-preparation plays an important role in getting good results.

* Before starting the reaction, one must have to be ready for doing the lab work, for that, wear a lab coat, gloves, a mouth cap, and a head cap.
* Clean the PCR reaction preparation area and arrange all other utilities nearby the reaction preparation.
* Now take reagents from the deep freeze and thaw all the reagents properly.

**Reaction preparation:**

Take a sterile PCR tube and start adding reagents as shown in the table.



Addition of different components while performing the PCR reaction. image copyright to ©Genetic Education Inc.

starts adding reagents in a sequential manner to reduce the chance of error.

If you have a ready to use mastermix, you can add it directly, this will save time and increases the efficiency of the reaction.

After the completion of reaction preparation, close all the tube caps and spin it properly, so that all the reagents mix well.

Now put the tubes in the PCR machine one by one in the pre-set PCR protocol. Remember: don’t waste time setting protocol during the PCR, set it before the reaction preparation, and immediately run the PCR.

Meanwhile start preparing the gel for agarose gel electrophoresis, because it will also take time for around 60 to 90 minutes.

**Post-preparation:**

* After completion of the PCR reaction, turn off the machine and collect all the tubes in an “orderly manner”.
* Rest tubes for some time in a freeze before doing agarose gel electrophoresis.
* You can also rest it for the next day, no problem with it.

**Uses Of PCR**

* polymerase chain reaction is used in
* Biotechnology.
* Microbiology.
* Environmental science.
* medical science.
* Dentistry.
* Anthropology.
* Food industry.
* Animal.
* Plant research.

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

## **Step 1: Gather Materials**

* **Stock Solution Chemicals**  
  1) 50X TAE solution  
  2) 1 liter plastic bottle  
  3) 250 milliliter flask  
  4) 2.5 g agarose  
  5) Distilled water
* **Casting Materials**  
  1) Gel plate  
  2) Gel form  
  3) Comb  
  4) Tape  
  5) Microwave
* **Electrophoresis Equipment**  
  1) Pipette aid  
  2) Pipette tips  
  3) Standard/Ladder  
  4) Samples with dye  
  5) Ethidium Bromide  
  6) Plastic pipette  
  7) Gel box  
  8) Gel box lid  
  8) Power supply  
  9) UV camera
* **Safety Equipment**  
  1) Gloves   
  2) Lab coat  
  3) Safety glasses  
  4) Oven mitts

## **Step 2: Prepare Stock Solutions**

* 1. **Prepare 1X TAE Stock Solution**  
     a. Pour 20 ml of 50X TAE solution into 1 liter plastic bottle  
     b. Bring final volume to 1 liter with distilled water  
     c. Gently shake solution  
       
     **2) Prepare 1X TAE + 1% Agarose**  
     a. Add 250 ml of 1X TAE solution in 250 ml flask  
     b. Add 2.5 g of Agarose   
     c. Boil in a microwave

## **Step 3: Cast the Gel**

1. **Place gel plate inside plate form.**TIP:  Although the gel form supposedly protects against leaks, it is not full proof. When attempting to caste a gel for the first couple of times, try to tape the edges as shown in the second picture.  
   **2) Turn front dial to tighten into place.  
   3) Attach comb to plate.  
   4) Boil 1X TAE + 1% Agarose solution in microwave.**This should take around 5 minutes.  
   **5) Pour hot solution into gel plate.**For best results, make sure solution is completely liquid before pouring into plate. Supply enough solution to adequately create wells on the plate.   
   **6) Wait for solution to solidify.**This should take around 5 to 10 minutes.

## **Step 4: Load Samples to Gel**

## **1)Loosen dial on the plate form** Remember to remove the tape if you used any.  **2) Slowly and carefully remove the comb**. CAUTION: Avoid puncturing or damaging the gel. **3) Place plate with gel into gel box as shown. 4) Slowly pour 1X TAE stock solution into gel box.**

## **5) Add enough solution to fill the chambers and supply a thin layer of solution above the gel.** If 1X TAE level does not adequately cover the wells, add more to gel box. **6) Pipette 10 µl of ladder into the leftmost well**

## **7) Pipette 10 µl of samples and dye into the following well using the same technique.**

## **Step 5: Process Gel**

**1) Using a plastic pipette, add several droplets of ethidium bromide to the front, middle, and back chambers of the gel box.**  
**2) Attach the lid to gel box.**  
Make sure to match up black electrodes with red electrodes.  
**3) Plug cords into power supply.**  
**4) Set desired voltage on monitor.**  
This depends on your gel, but a safe voltage to use is 90V.  
**5) Push the run button and let electrophoresis run for 20-30 minutes .**  
**6) Turn off power supply.**  
The gel is now ready to be photographed by a UV Camera.

**Step 6: Photograph Gel Using UV Camera**

**1) Unplug cords from power supply.  
2) Remove lid from gel box.  
3) Carefully remove plate and gel.**  
**TIP:** To remove excess liquid between the plate and gel, use a paper towel.  
**4) Place gel and plate onto a UV camera.  
5) Take a picture of the gel using the UV camera.**