Name = Abdul wadood

ID = 15213

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Submitted to = sir Fazli zahir

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Q1: what is PCR? Explain procedure and uses of PCR.

**PCR:**

* PCR is a technique that take a specific sequence of DNA of small amount and amplifies it to be used for further testing
* In vitro techniques
* 1983: Dr. Kary Mullis developed PCR
* 1985: first publication of PCR by Cetus corporation appears in science
* 1989: science declare Taq polymerase “molecule of the year”
* 1990: amplification and detection of specific DNA sequences using a fluorescent DNA binding dye, laying the foundation for future “real time” or “kinetic” PCR
* 1993: Dr. Kary Mullis share Nobel prize in chemistry for conceiving PCR technology

**Procedure:**

There are five steps of PCR.

* **Initialization**
* **Denaturation:**
* Temperature 92-94C
* Double stranded DNA melts
* **Annealing:**
* Temperature: 50-70 (dependent on the melting temperature of the expected duplex)
* Primer binds to their complementary sequences
* **Extension:**
* Temperature: 72C
* Time: 0.5-0.3 min
* DNA polymerase binds to the annealed primer and extend DNA at the 3 end of the chain
* **Elongation**

**USES:**

* The PCR is used to made billion of copies of a target of DNA
* It necessary tool in new molecular biology and transfer scientific research and diagnostic medicine
* In a wide range PCR is used in the field of biology and application
* The diverse range of technologist that are possible due to PCR
* PCR provide the possibility of personalized genome testing
* PCR is very important to knowing of criminals and the gathering of crime scene evidence such as hair, pollen, soil, blood and semen
* DNA fingerprint, identification of familial relationship, genomic DNA isolation
* PCR allows DNA to identified tiny samples.

**Q2: explain the process of agarose gel electrophoresis**

**Ans:**

Gel electrophoresis is the procedure used to separate biological molecule by size.

Electr0phoesis used an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode

The separation of these molecule is achieved by placing them in a gel with small pores and creating an electric field across the gel

Gel electrophoresis process because, from negative pole negative charged move away of the electric current and smaller molecule move faster than larger molecules.

When pole molecule running through gel size of separation is achieved

Each DNA molecule is has the same charge (-1), because DNA is formed by same 4 nucleotides and always carries a slightly negative charge regardless of its size

The purpose of gel electrophoresis is to identify, distinguish and visualize molecule have been process by previous method such as PCR

**Steps of gel electrophoresis:**

* **Casting the gel:**

The agarose TAE solution is poured into a casting tray that, gel solution is solidified and cool down, made a gel slab with a row of walls at the top

* **TAE gel solution is prepared:**

the agarose percentage used is determined by how big or small the DNA is expected to be

* **Prepare the sample for running:**

Made up a solution DNA is isolated and preprocessed with some basic blue dye to help visualize the movement of the sample through gel

* Setting up the electrophoresis chamber
* Loading the gel
* **Electrophoresis:**

Positive and negative leads are connected to the chamber and power supply where the voltage is set. Turning of the power supply is set up the electric field.

**Component of agarose gel electrophoresis:**

* Agarose
* DNA gel loading dye
* EtBr
* Powerpack
* Agarose gel electrophoresis buffer
* Agarose gel electrophoresis equipment