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***Polymerase chain reaction:-***

The polymerase chain reaction (PCR) is a molecular biology technique to amplify a single or a few copies of a piece of DNA up to several orders of magnitude (1011-12 copies) of a particular DNA sequence. • This automated process bypasses the need to use bacteria for amplifying DNA.

***Procedure: -***

All the PCR components are mixed together and are taken through series of 3 major cyclic reactions conducted in an automated, self-contained thermocycler machine

***Denaturation***:

This step involves heating the reaction to 94oC for 15—30 seconds.

During this, the double strand DNA is denatured to single strand due to breakage in week hydrogen bonds.

***Annealing****:*

The reaction temperature is rapidly lowered to 54—60oC for 20—40 seconds.

This allows the primers to bind to their complementary sequence in the template DNA.

***Elongation***:

This step usually occur at 72—80oC

In this step the polymerase enzyme sequentially adds bases to the 3 each primer, exttinding the DNA sequence in the 5 to 3 direction

Under optimal conditions, DNA polymerase will add about 1000 bp/minute

***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 field of biology and applications

The diverse range of technologist that are possible due to PCR

PCR provide the possibility of personalize 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 identify tiny samples.

***Agarose gel electrophoresis:-***

chnique used to separate DNA fragments and other macro molecules by size and charge is called gel electrophoresis

***Process of gel electrophoresis:-***

***Gel electrophoresis step***

The broad steps involved in a common DNA gel electrophoresis protocol.

### ***1. Preparing the samples for running***

The DNA is isolated and preprocessed **(e.g. PCR, enzymatic digestion)** and made up in solution with some basic blue dye to help visualize the movement of the sample through the gel.

### ***2. An agarose TAE gel solution is prepared***

TAE buffer provides a source of ions for setting up the electric field during electrophoresis. The weight-to-volume concentration of agarose in TAE buffer is used to prepare the solution. For example, if a 1% agarose gel is required, 1g of agarose is added to 100mL of TAE.

### **3. Casting the gel**

The agarose TAE solution is **poured into a casting tray** that, once the gel solution has cooled down and solidified, creates a gel slab with a row of wells at the top.

### ***4. Setting up the electrophoresis chamber***

The solid gel is placed into a chamber filled with **TAE buffer.** The gel is positioned so that the chamber wells are closest to the negative electrode of the chamber.

### ***5. Loading the gel***

The gel chamber wells are loaded with the DNA samples and usually, a [DNA ladder](https://en.wikipedia.org/wiki/Molecular-weight_size_marker) is also loaded as reference for sizes.

### **6. Electrophoresis**

The negative and positive leads are connected to the chamber and to a power supply where the voltage is set. Turning on the power supply sets up the electric field and the negatively charged DNA samples will start to migrate through the gel and away from the negative electrode towards the positive.

### **7. Stopping electrophoresis and visualizing the DNA**

Once the blue dye in the DNA samples has migrated through the gel far enough, the power supply is turned off and the gel is removed and placed into an ethidium bromide solution. Ethidium bromide intercalates between DNA and is visible in UV light. Sometimes ethidium bromide is added directly to the agarose gel solution in step 2. The ethidium bromide stained gel is then exposed to UV light and a picture is taken. DNA bands are visualized in from each lane corresponding to a chamber well. The DNA ladder that was loaded is also visualized and the length of the DNA bands can be estimated. An example is given in the figure below.

***Thanks***