CHANDIGARH UNIVERSITY

Discover. Learn. Empower.

INSTITUTE-UNIVERSITY INSTITUTE OF ENGINEERING

ACADEMIC UNIT-II

Computer Science Engineering

Subject Name-Biology For Engineers

Subject Code- 20SZT148

PCR AND ELECTROPHORESIS

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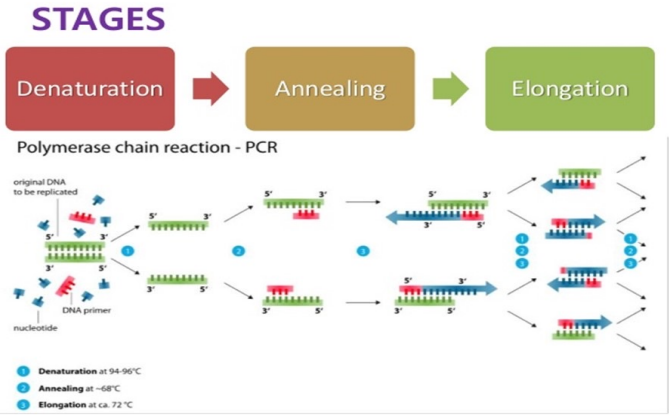
PCR AND ELECTROPHORESIS

Course Outcome

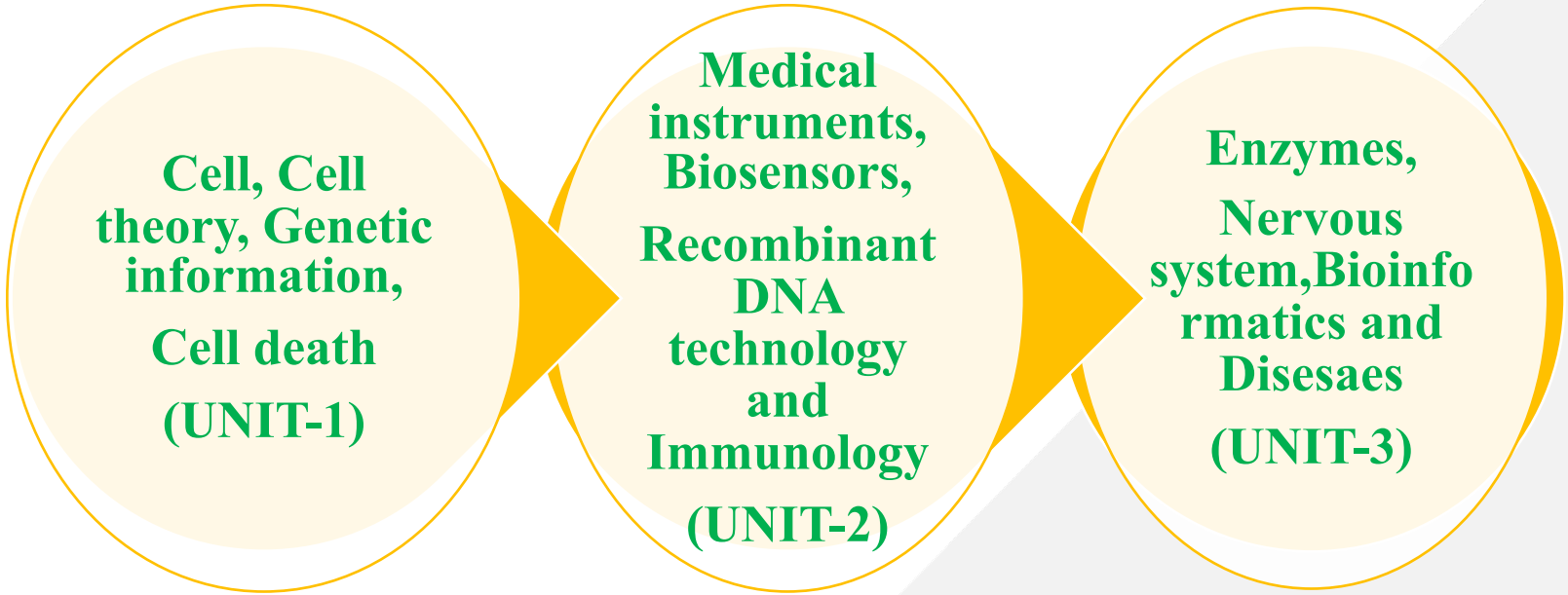
CO Number	Title	Level
CO1	It gives an idea about the about the basic cell biology.	**Understanding**
CO2	It deals with the idea of uses of biology in engineering.	**Understanding**
CO3	It provide knowledge about the uses of softwares in biology field.	**Remembering**



Will be covered in this lecture

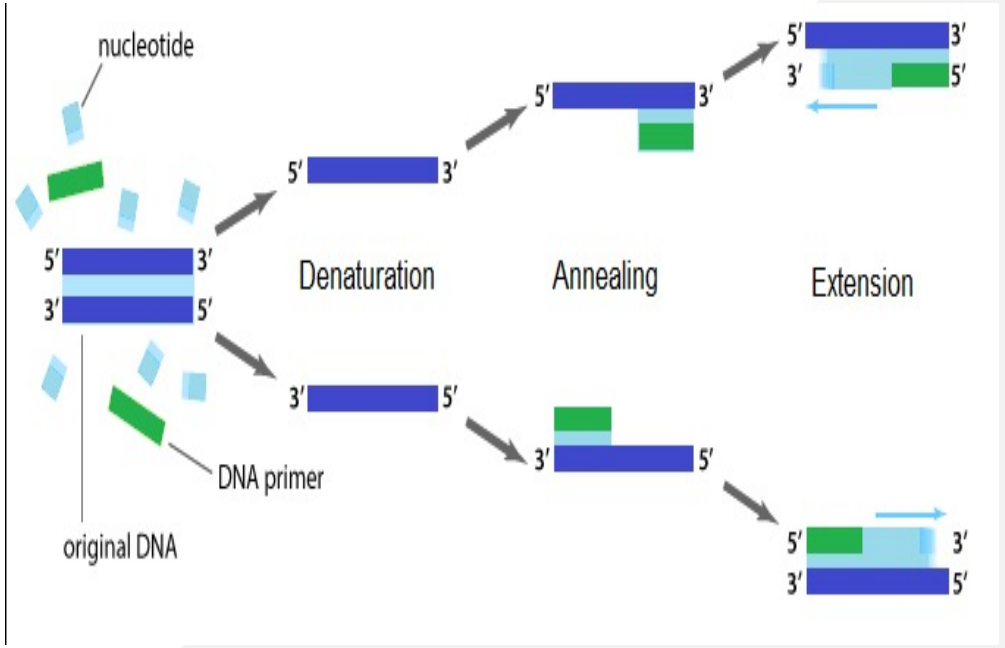
https://www.onlinebiologynotes.com/polymerase-chain-reaction-pcr-principle-procedure-steps-types-application/

BIOLOGY FOR ENGINEERS



PCR

•Sometimes called "molecular photocopying," the polymerase chain reaction (PCR) is a fast and inexpensive technique used to "amplify" copy small segments of DNA.Polymerase chain reaction (PCR) is an efficient and cost-effective molecular tool to copy or amplify small segments of DNA.



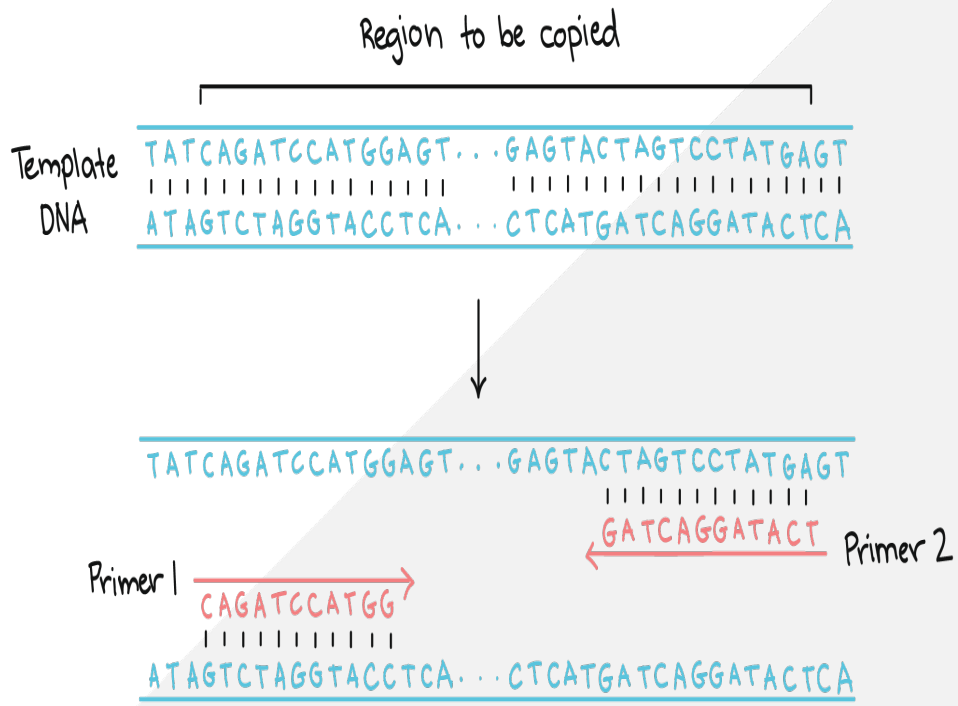
https://www.xxpresspcr.com/all-news/polymerase-chain-reaction-the-past-present-and-future/

PCR

- The key ingredients of a PCR reaction are *Taq* polymerase, primers, template DNA, and nucleotides (DNA building blocks). The ingredients are assembled in a tube, along with cofactors needed by the enzyme, and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.
- Like DNA replication in an organism, PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. The DNA polymerase typically used in PCR is called ***Taq* polymerase**, after the heat-tolerant bacterium from which it was isolated.
- Like other DNA polymerases, *Taq* polymerase can only make DNA if it's given a **primer**, a short sequence of nucleotides that provides a starting point for DNA synthesis.

PCR

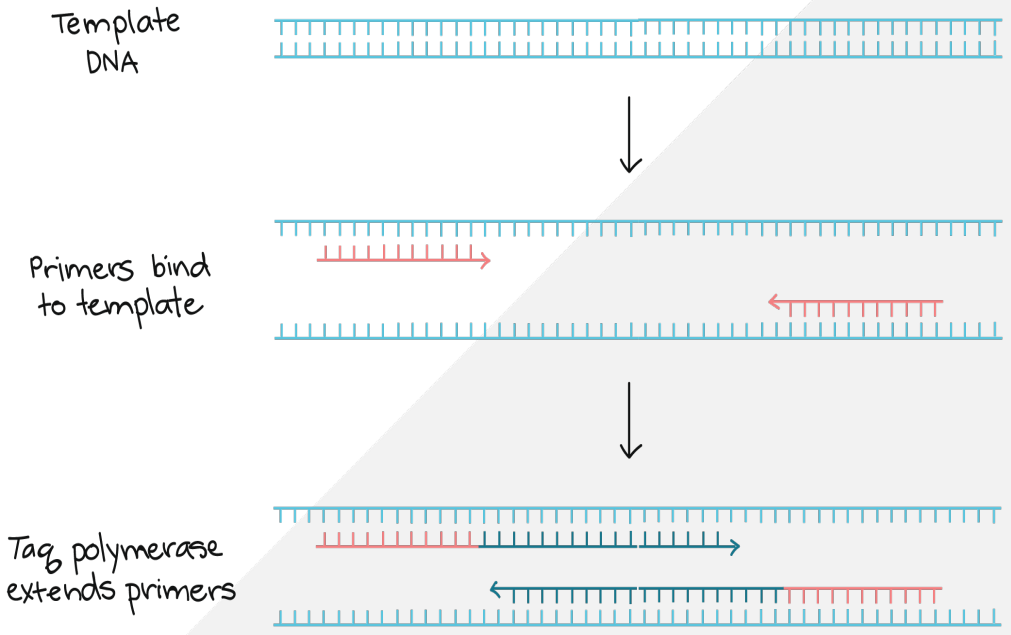
- PCR primers are short pieces of single-stranded DNA, usually around 202020 nucleotides in length. Two primers are used in each PCR reaction, and they are designed so that they flank the target region (region that should be copied). That is, they are given sequences that will make them bind to opposite strands of the template DNA, just at the edges of the region to be copied. The primers bind to the template by complementary base pairing.



https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/polymerase-chain-reaction-pcr

PCR

- When the primers are bound to the template, they can be extended by the polymerase, and the region that lies between them will get copied.



https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/polymerase-chain-reaction-pcr

PCR

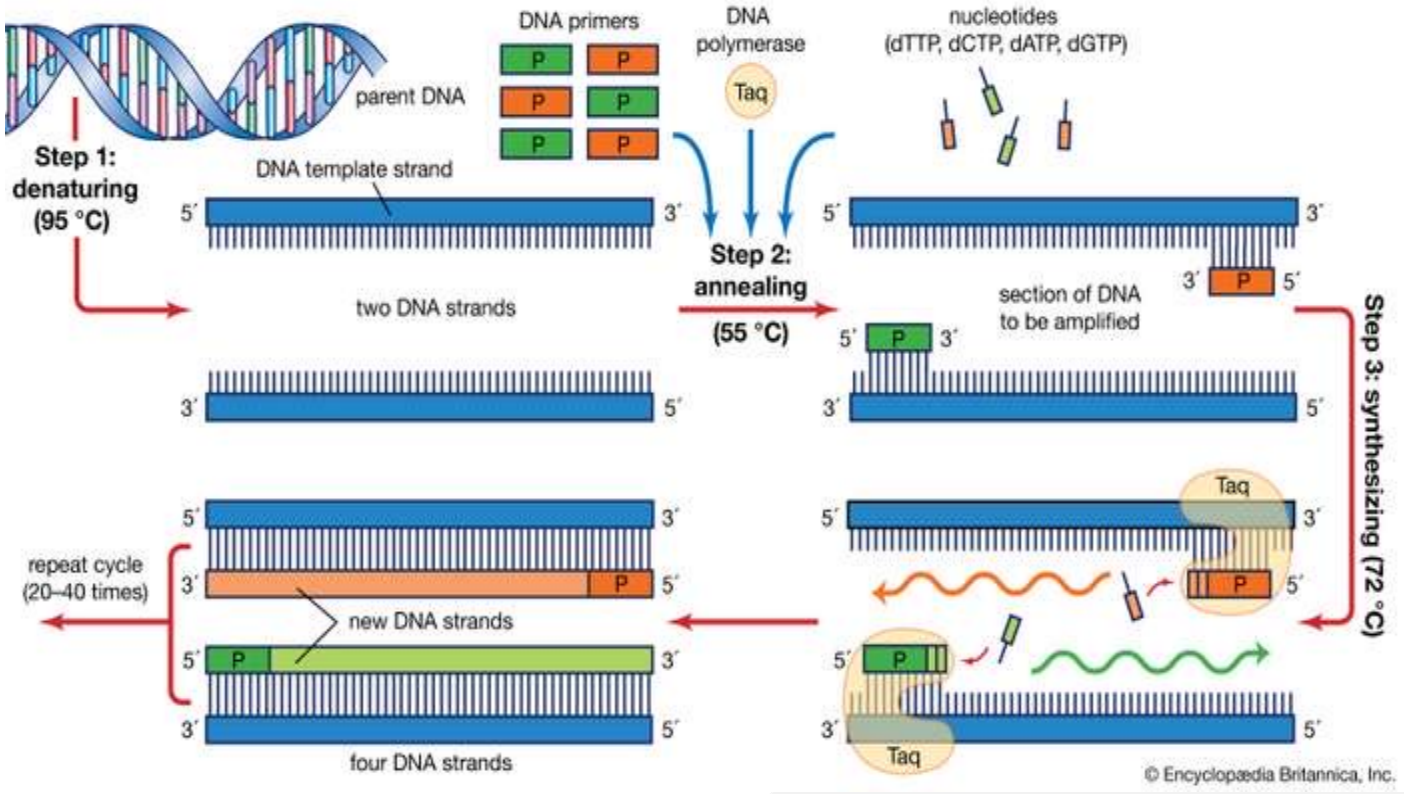
The basic steps are:

- **Denaturation** (96 °\text C96°C96, °, start text, C, end text): Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.
- **Annealing** (555555 - 656565°\text C°C°, start text, C, end text): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.
- **Extension** (72 °\text C72°C72, °, start text, C, end text): Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.

PCR

- This cycle repeats 252525 - 353535 times in a typical PCR reaction, which generally takes 222 - 444 hours, depending on the length of the DNA region being copied. If the reaction is efficient (works well), the target region can go from just one or a few copies to billions.
- That's because it's not just the original DNA that's used as a template each time. Instead, the new DNA that's made in one round can serve as a template in the next round of DNA synthesis.
- There are many copies of the primers and many molecules of *Taq* polymerase floating around in the reaction, so the number of DNA molecules can roughly double in each round of cycling.

PCR



https://www.britannica.com/science/polymerase-chain-reaction

ELECTROPHORESIS

- Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field. In the year 1807, Ferdinand Frederic Reuss was the first person to observe electrophoresis.
- He was from Moscow State University.
- Anaphoresis is the electrophoresis of negative charge particles or anions whereas cataphoresis is electrophoresis of positive charge ions or cations.
- Electrophoresis has a wide application in separating and analyzing biomolecules such as proteins, plasmids, RNA, DNA, nucleic acids.
- Charged macromolecules are placed in the electric field move towards the negative or positive pole based on their charge. Nucleic acid has a negative charge and therefore it migrates towards the anode.

TYPES OF ELECTROPHORESIS

1. Horizontal and Vertical Gel Electrophoresis Systems:

- The equipment required for electrophoresis consists basically of two items, a power pack and an electrophoresis unit. Electrophoresis units are available for running either vertical or horizontal gel systems. Vertical slab gel units are commercially available and routinely used to separate proteins in acrylamide gel. The gel is formed between two glass plates that are clamped together but held apart by electrical spacers.
- The gel is cast on a glass or plastic sheet and placed on a cooling plate (an insulated surface through which cooling water is passed to conduct away generated heat). Connection between the gel and electrode buffer is made using a thick wad of wetted filter paper. Note, however, that agarose gel for DNA electrophoresis are run submerged in the buffer.

TYPES OF ELECTROPHORESIS

- The power pack supplies a direct current between the electrodes in the electrophoresis unit. All electrophoresis are carried out in an appropriate buffer, which is essential to maintain a constant state of ionization of the molecules being separated. Any variation in pH will alter the overall charge and hence the mobilities (rate of migration in the applied field) of the molecules are separated.

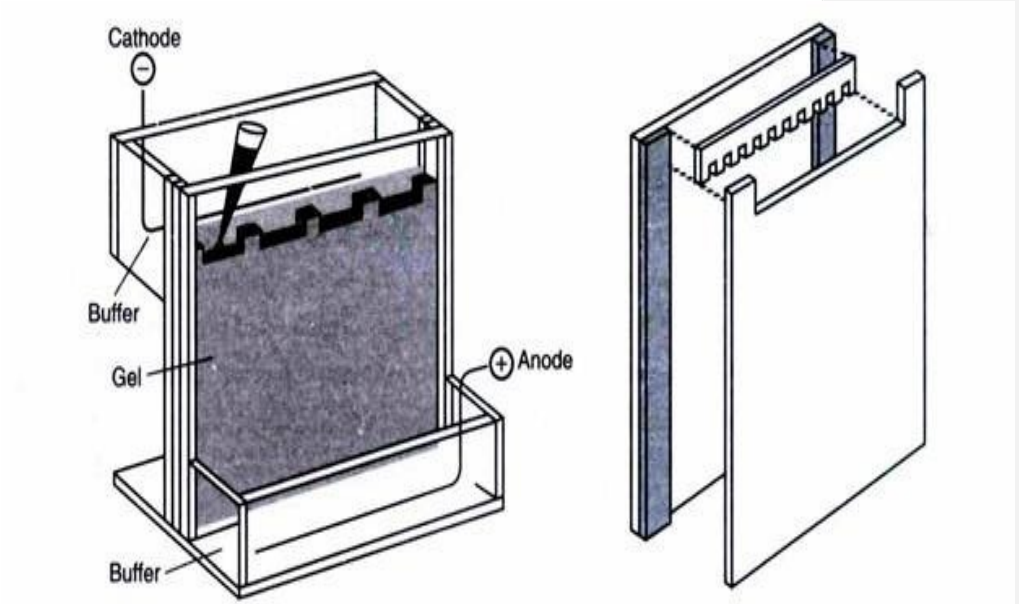


Fig. 8.1: A typical vertical gel apparatus

https://www.biologydiscussion.com/biochemistry/electrochemical-techniques/top-10-types-of-electrophoretic-techniques-used-in-biochemistry/12669

TYPES OF ELECTROPHORESIS

2. Agarose Gel Electrophoresis:

- Agarose is a linear polysaccharide (average relative molecular mass about 12000) made up of the basic repeat unit agarobiose, which comprises alternating units of the galactose and 3, 6- anhydrogalactose.
- Agarose is one of the components of agar that is mixture of polysaccharides isolated from certain seaweeds. Agarose is usually used at a concentration between 1% and 3%. Agarose gels are formed by suspending dry agarose in aqueous buffer, then boiling the mixture until a clear solution is formed. This is poured and allowed to cool to room temperature to form a rigid gel. The gelling properties are attributed to both inter- and intermolecular hydrogen bonding within and between the long agarose chains. This cross-linked structure gives the gel good anti-conventional properties.

TYPES OF ELECTROPHORESIS

•**Principles:-** Electrophoresis is a general term that describes the migration and separation of charged particles (ions) under the influence of an electric field.An electrophoretic system consists of two electrodes of opposite charge (anode, cathode), connected by a conducting medium called an electrolyte.

- **Applications of gel electrophoresis**
- In the separation of DNA fragments for DNA fingerprinting to investigate crime scenes.
- To analyze genes associated with a particular illness.
- In DNA profiling for taxonomy studies to distinguish different species.

TYPES OF ELECTROPHORESIS

•Agarose gels are used for the electrophoresis of both proteins and nucleic acids.

• For proteins, the pore sizes of a 1% agarose gel are large relative to the sizes of proteins.

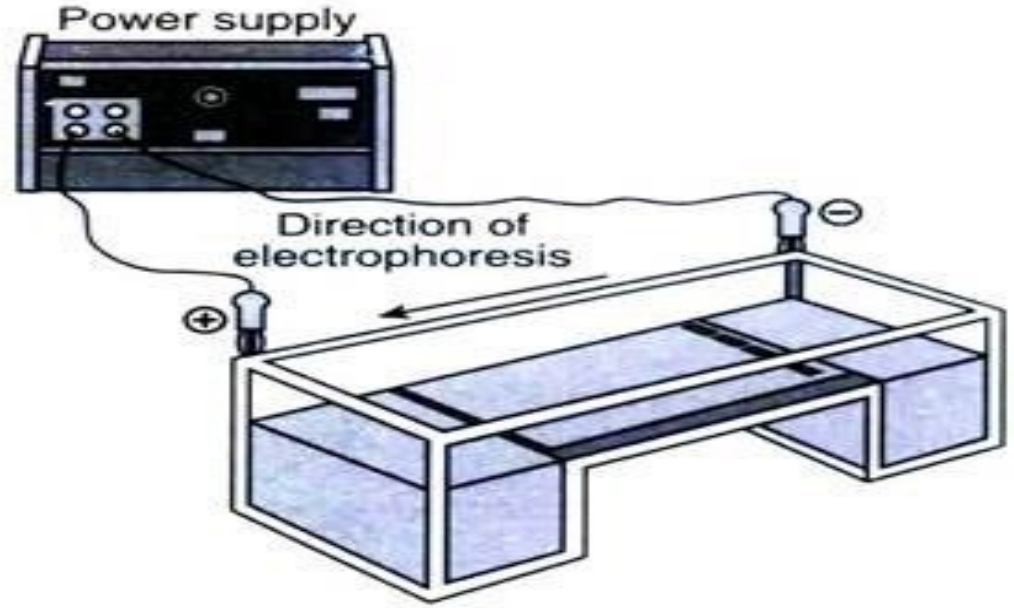
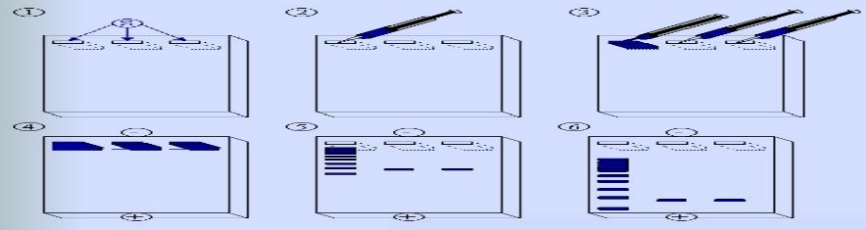


Fig. 8.9: A typical horizontal gel electrophoresis system

https://www.biologydiscussion.com/biochemistry/electrochemical-techniques/top-10-types-of-electrophoretic-techniques-used-in-biochemistry/12669

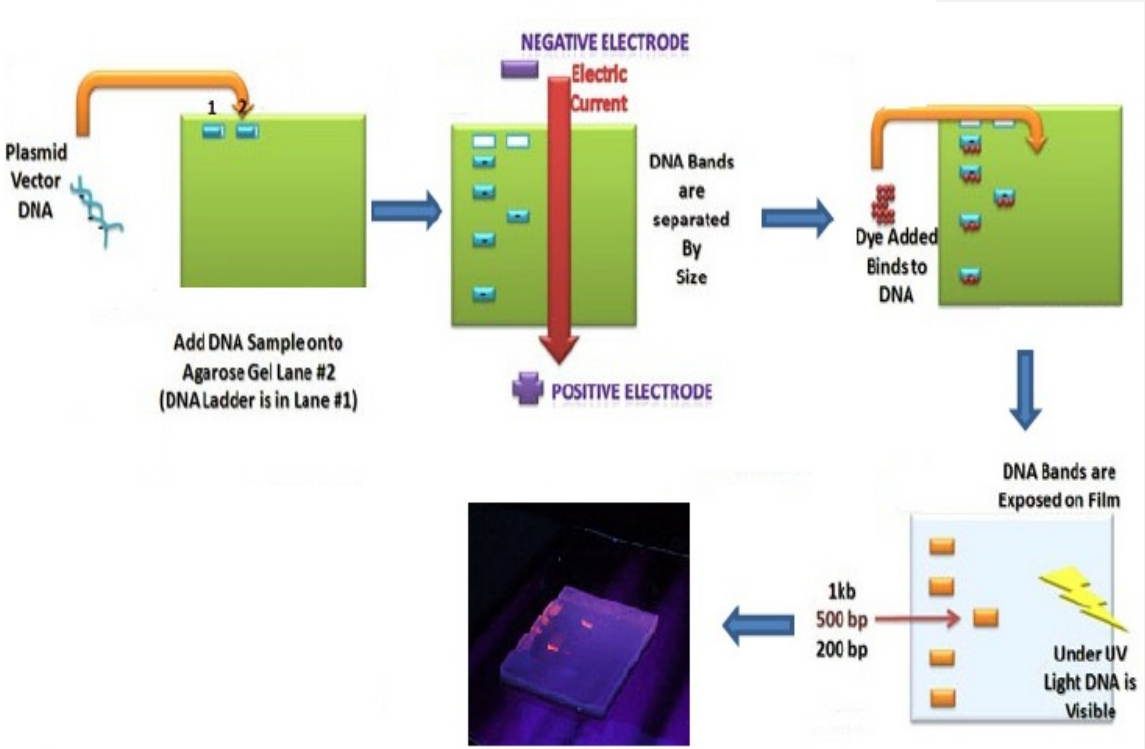
TYPES OF ELECTROPHORESIS

Gel Electrophoresis Procedure



- Step 1: The agarose gel with three slots/wells (S).
- Step 2: Injection of DNA ladder into the first slot.
- Step 3: DNA ladder injected. Injection of samples into the second and third slot.
- Step 4: A current is applied. The DNA moves toward the positive anode due to the negative charges on its phosphate backbone.

https://www.slideserve.com/fitzgerald-prince/pcr-gel-electrophoresis-and-southern-blotting



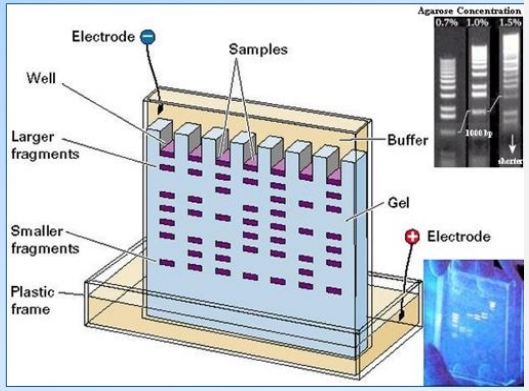
https://www.researchgate.net/figure/Agarose-gel-electrophoresis-method-modified-from_fig1_224829869

TYPES OF ELECTROPHORESIS

- To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it. Ethidium bromide is added to the gel (final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis.
- 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.

Steps Involved in Gel Electrophoresis

1. "Cut" DNA sample with restriction enzymes.
2. Run the DNA fragments through a gel.
3. Bands will form in the gel.
4. Everyone's DNA bands are unique and can be used to identify a person.
5. DNA bands are like "genetic fingerprints".



https://slideplayer.com/slide/4684514/

TYPES OF ELECTROPHORESIS

- After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer.
- 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.
- The current flow can be confirmed by observing bubbles coming off the electrodes.
- DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge.
- 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.

TYPES OF ELECTROPHORESIS

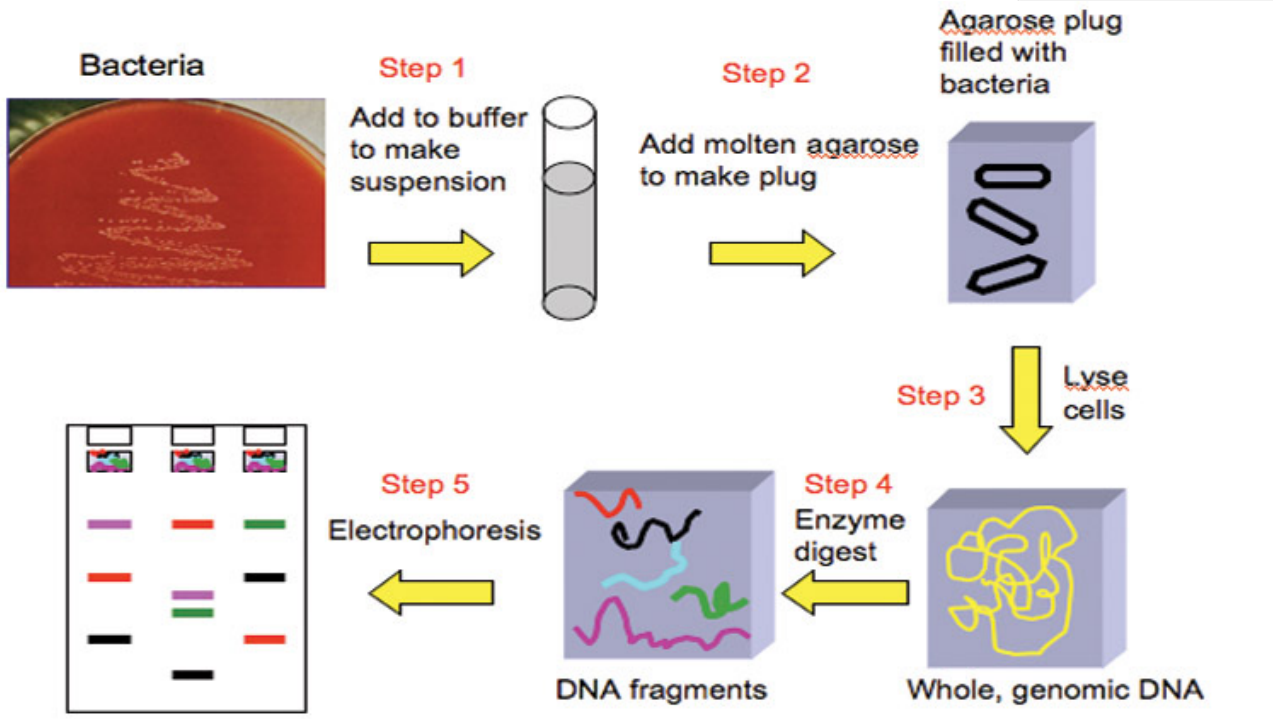
3.Pulsed-field Gel Electrophoresis:

- Manipulating and analyzing DNA are fundamental in the field of molecular biology. Indeed, separating complex mixtures of DNA into different sized fragments by electrophoresis was a well-established technique by the early 1970s. Typically, DNA was isolated intact and then treated with restriction enzymes to generate pieces small enough to be resolved by electrophoresis in agarose or acrylamide. Although this procedure still forms the core of DNA separation and analysis in today's laboratories, the rules of the separation have changed.

•With each reorientation of the electric field relative to the gel, smaller sized DNA will begin moving in the new direction more quickly than the larger DNA. Thus, the larger DNA lags behind, providing a separation from the smaller DNA.

TYPES OF ELECTROPHORESIS

- **Lysis:** First, the bacterial suspension is loaded into an agarose suspension. This is done to protect the chromosomal DNA from mechanical damage by immobilizing it into agarose blocks. Then the bacterial cells are lysed to release the DNA. The agarose-DNA suspension is also known as plug mold.



https://microbenotes.com/pulsed-field-gel-electrophoresis-pfge/

TYPES OF ELECTROPHORESIS

- **Digestion of DNA:** The bacterial DNA is treated with unusual cutting restriction enzymes so that it yields less number of larger size DNA fragments (in contrast to frequently used restriction enzymes used in RFLP which produces large number of smaller fragments).
- **Electrophoresis:** The larger pieces of DNA are subjected to pulse field gel electrophoresis by applying electric current and altering its direction at regular intervals (in contrast to the conventional agarose gel electrophoresis done to separate the smaller fragments where the current is applied in a single direction).
- **Analysis:** The fragments of different organisms generated by PFGE are compared to standards manually or by computer software like BioNumerics.

CONCLUSION

- Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field.
- Electrophoresis has a wide application in separating and analyzing biomolecules such as proteins, plasmids, RNA, DNA, nucleic acids.
- The types of gel most typically used are agarose and polyacrylamide gels. Each type of gel is well-suited to different types and sizes of the analyte.
- Polyacrylamide gels are usually used for proteins and have very high resolving power for small fragments of DNA

ASSESSMENT PATTERN

Assessment Pattern	Total Marks
1st Hourly Test	36
2nd Hourly Test	36
Surprise Test	12
Assignment (3)	10
Quiz	4
End Semester Examination	60

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THANK YOU

For queries
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