WELCOME

Gel Electrophoresis

- Gel electrophoresis is a process that can be used to separate DNA fragments.
- It uses an electric current to separate the pieces of DNA that are run through the gel.
- It can be separate DNA molecules from hundreds of nucleotides to hundreds of thousands of them.

Principle of gel electrophoresis

- Gel electrophoresis involves an electrical field.
- The molecules to be separated are pushed by an electrical field through a gel that contains small pores.
- The molecules travel through the pores in the gel at a speed that is inversely related to their lengths.
- This means that a small DNA molecules will travel a greater distance through the gel than will a larger DNA molecule.

- DNA and RNA are negatively charged molecules, they will be pulled toward the positively charged end of the gel.

- In case of proteins, first mix it with a detergent called sodium dodecyl sulfate. This treatment makes the proteins unfold into a linear shape and coats them with a negative charge, which allows them to migrate toward the positive end of the gel and be separated.

Types of gel electrophoresis

AGAROSE GEL	POLYACRYLAMIDE GEL
Poured horizontally	Poured Vertically
Separated large molecules	Separate small molecules
Non-toxic	Neurotoxin
Mostly used for DNA separation	Used for DNA or protein separation
Staining step :- before or after pouring the gel Ethidium bromide is mostly used	Staining step :- after pouring the gel Coommassie blue stain is mostly used

- Equipment Necessary

  - Gel casting
  - Comb
  - Gel box or electrophoresis chamber

- Necessary substance

  - Buffers as TAE
  - Gel powder
  - Ethidium bromide
  - Transilluminatary

How to pour and run a gel

1. For one gel, you will need a total of 30 ml of a 1% agarose solution.

2. Prepare 300 ml of 10* TBE buffer by measuring 30 ml into a 500-ml graduated cylinder. Then filling the cylinder to the 300-ml mark with dH^2O.

3. Pour 30ml of the 1* TBE into the flask with your agarose . The remaining TBE will be your running buffer, which carries the current from one electrode to the other.

4. Heat the agarose in the microwave on high for 15 seconds.

5. Insert a gel plate into a casting tray .

6. Allow the melted agarose to cool on the bench for two minutes while you set up the casting tray.

7. Pour the entire 30ml of melted agarose into the gel casting tray. Insert a clean comb near one end, and slide it up against the handles on the gel plate.

8. Let the agarose harden for at least 15 minutes. When the agarose has fully hardened, carefully remove the comb. Remove the gel plate, with the gel on it, from the casting tray and place it in the gel box.

9. Add running buffer until the gel is completely submerged if you look from the side, it should be covered by a few millimeters of liquid. The gel is now ready for use.

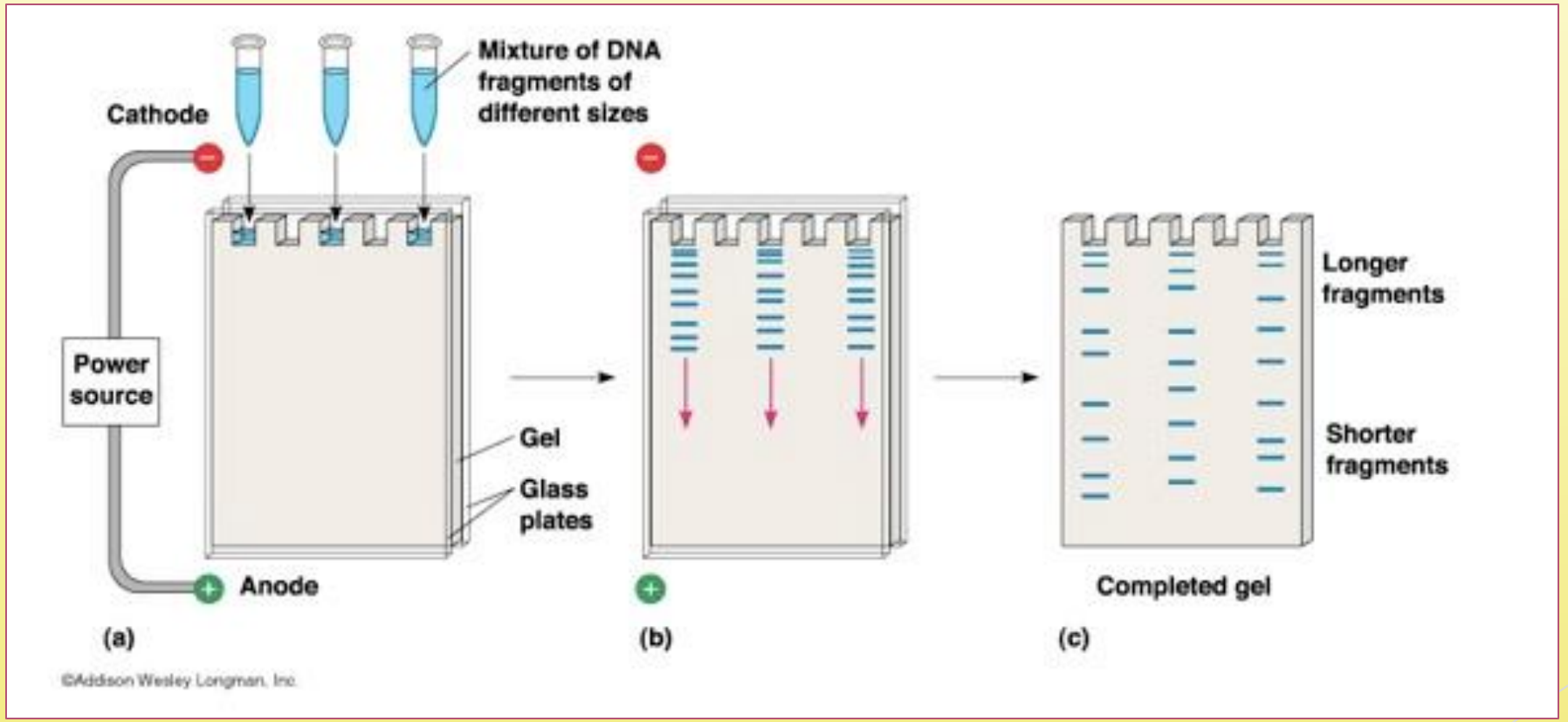
10. Use a micropipette and a clean tip to transfer each of your DNA samples to a well.

11. Place the lid on the gel box and fix the power cords over the two electrodes.

12. Normally, you would want to stop the gel when the bromophenol blue dye line is near the bottom edge of the gel but has not yet started to run off. Stop it sooner if you need to see small DNA fragments. Turn off the power supply and disconnect the leads.

13. Carefully remove the gel tray and gel from the gel box. Don't let the gel slide off the tray onto the UV light box of the photo documentation system.

Gel Electrophoresis Diagram



Application of gel electrophoresis

- In the separation of DNA fragments for DNA fingerprinting to investigates crime scenes.
- To analyze results of polymerase chain reaction.
- In the analysis of antibiotic resistance.
- In paternity testing using DNA fingerprinting.
- In the study of structure and function of proteins.
- In blotting technique for analysis of macromolecules.
- In study of evolutionary relationships by analyzing genetic similarity among population or species.
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- To analyze genes associated with a particular illness.
- In DNA profiling for taxonomy studies to distinguish different species.

THANK YOU