Biology Department,
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Agarose Gel Electrophoresis

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Lab-3

Practical molecular biology

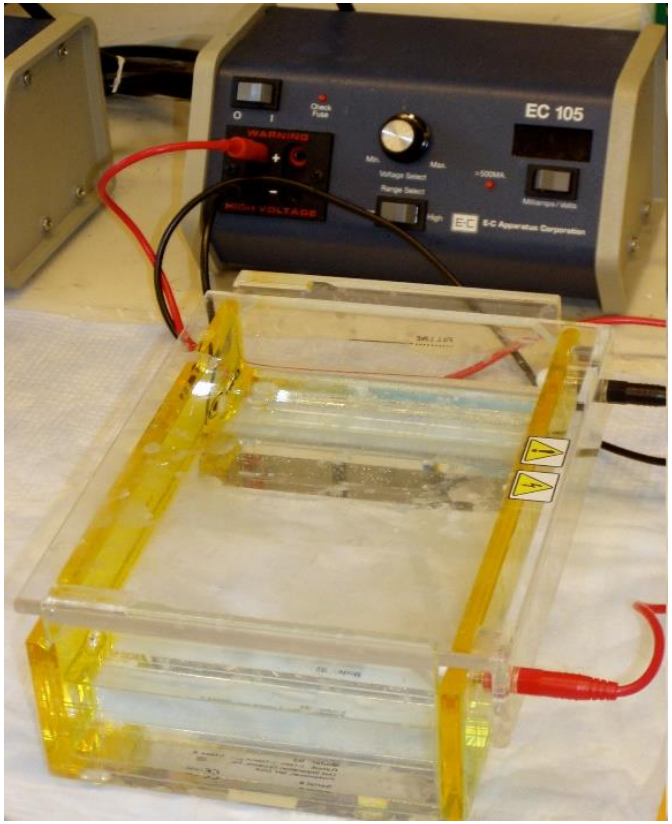
Aim

In this lecture, you will be to

- Learn the basics of how DNA fragments can be separated according to their size via gel electrophoresis.
- How organisms, crime, paternity, and genetic disease can be identified.

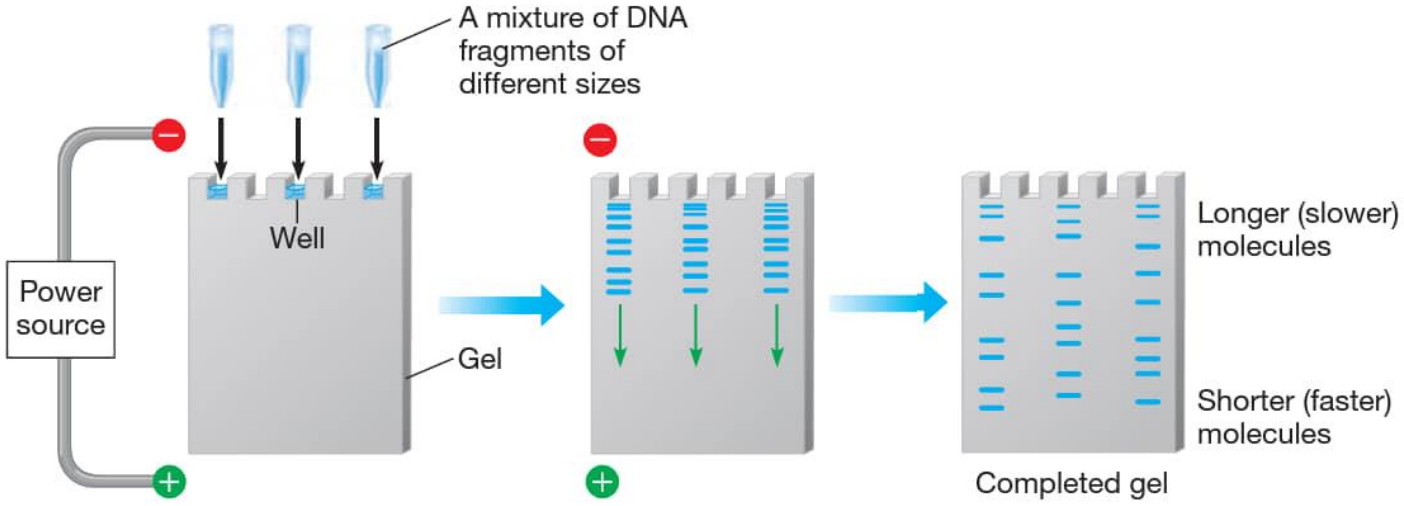
Gel electrophoresis

- Is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size
- A gel acts as a molecular sieve to separate DNA fragments differing in size.



Gel electrophoresis

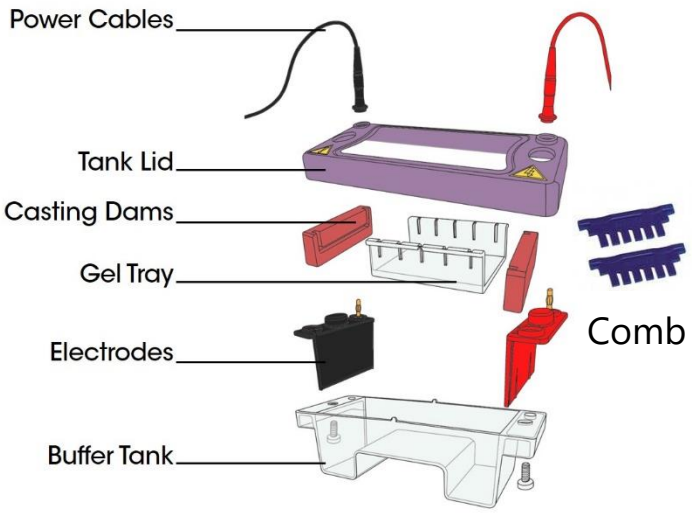
- DNA samples are loaded into wells at one end of a gel, and then an electric current is applied to pull them through the gel.
- DNA fragments are negatively charged, so they are attracted to the positive pole due to the phosphate group.
- Longer fragments move slower and shorter fragments move faster.
- **Band**, is a collection of DNA fragments of one particular length, can be detected from the gel and used for different purposes.

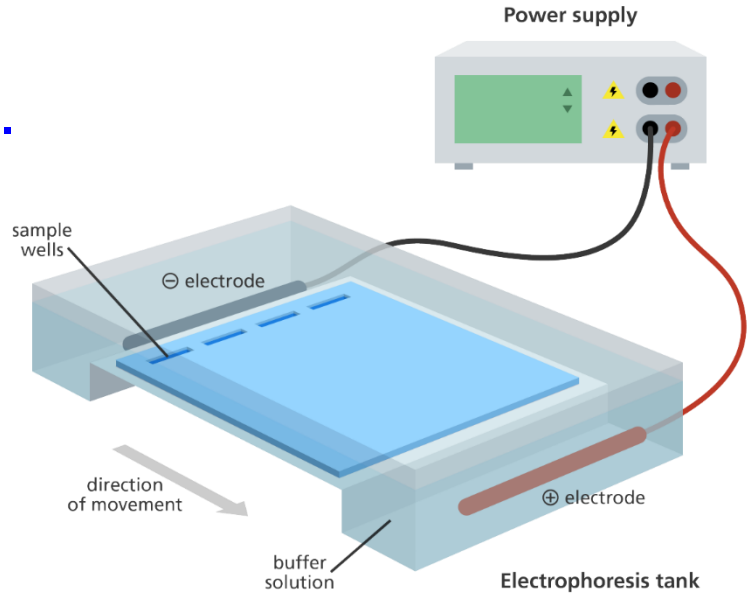


Requirements of Gel electrophoresis technique

A. Electrophoresis tool

- Electrophoresis tank (small or large size) with a **cathode (-)** usually black colour and **Anode (+)** usually red colour.
- Gel tray, Casting dams, and Comb.
- Tank lid.
- power supply.





Requirements of Gel electrophoresis technique

B. Agarose gel:

- is a polysaccharide, extracted from certain seaweeds (like red algae). It is a linear polymer made up of the repeating unit of agarobiose, which is a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose.
- Agarose gel is used as an electrophoresis medium due to?

It behaves like agar at 40 °C & gelling at about 4 °C.

Requirements of Gel electrophoresis technique

C. Electrophoresis buffer is used to run the gel and has

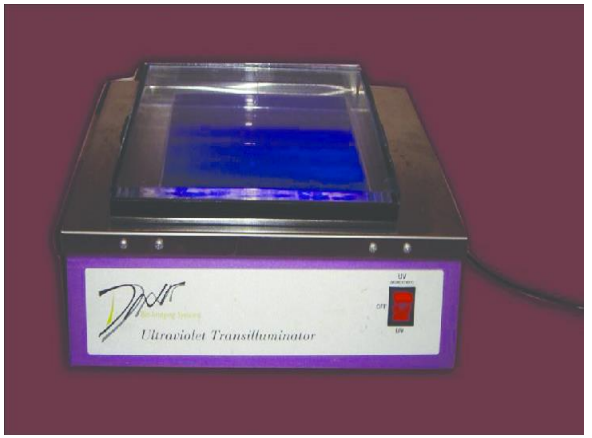
1. 1X TAE (Tris/Acetate/EDTA)
2. 1X TBE (Tris/Borate/EDTA).

Requirements of Gel electrophoresis technique

D. Ultraviolet (UV) transilluminator.

1. Transfer the gel to the UV transilluminator. You must wear safety glass, turn the UV transilluminator on and observe the stained DNA bands which can fluoresce bright orange.
2. Take a photograph of the gel by using a digital camera.

Note that: take care not to break the gel – it may be lifted while still in the tray, but it will slide out easily.



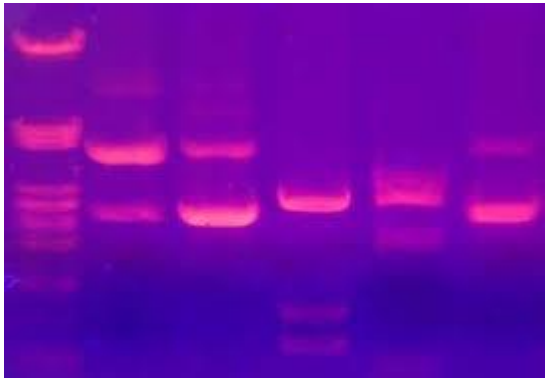
A photograph of the same DNA bands

DAN bands visualised under UV light

Requirements of Gel electrophoresis technique

D. Staining of Nucleic acid samples

- Ethidium bromide (EtBr): is a carcinogenic dye, dark red in colour and an intercalating agent used as a fluorescent tag (nucleic acid stain) in agarose gel.
- **– be careful when working with it).**
- DNA-EtBr is visualized at 300 or 360 nm. The absorbed **UV light** causes the EtBr to fluoresce at 590 nm in red-orange region of the visible spectrum.
- Add 0.5 µg of EtBr before casting to gel.



Requirements of Gel electrophoresis technique

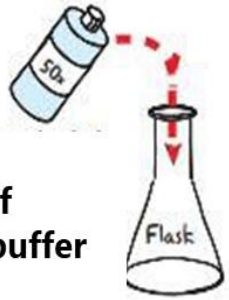
E. Gel loading dye

- Bromophenol blue (BPB) (1x) is mixed with a nucleic acid sample before loading it into a gel well.
- BPB has a high density (containing sucrose and glycerol) that enables the nucleic acid samples to drop and remain within the wells during sample loading.

Steps of Experiment

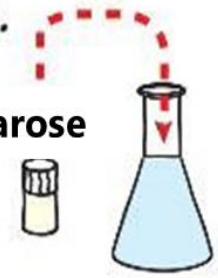
1. Preparation of agarose gel

1.



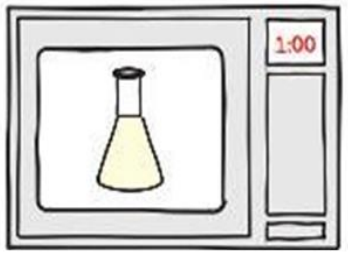
100ml of
1x TAE buffer

2.



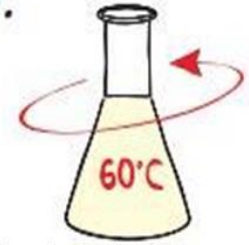
1g of agarose

3.



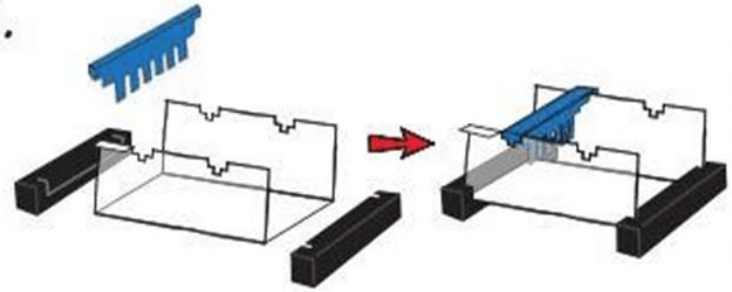
Heat the solution for 1.30mins
until it dissolved.

4.



Several mins left to cool

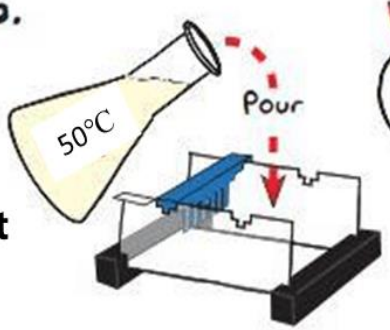
5.



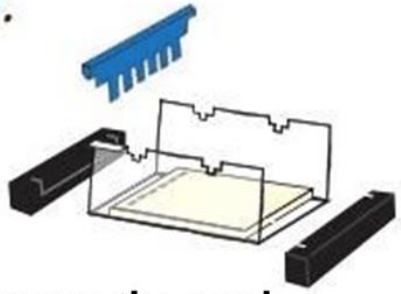
Seal the ends of
the clean tray with
the tape and put
comb vertically at
one end of the tray

6.

Add 5µl
Of EtBr/Red gel
and mix it up
and then pour it



7.



Remove the comb
and the tapes gently

Video

Steps of Experiment

2. Running an agarose gel

Put the tray into the gel tank

Add the running buffer (TBA 1X) thoroughly to the tank until 2millimetr

Before Loading the sample, Add 1 µl of **BPB** for every 5 µl of sample. Load slowly about **10 - 20 µl** (containing 100 – 200 ng) of the DNA

Running the gel
At 90 – 120 volts for 60mins. Bubbles should be generated at the cathode & anode

Caution! Turn off the power supplier before removing the gel.

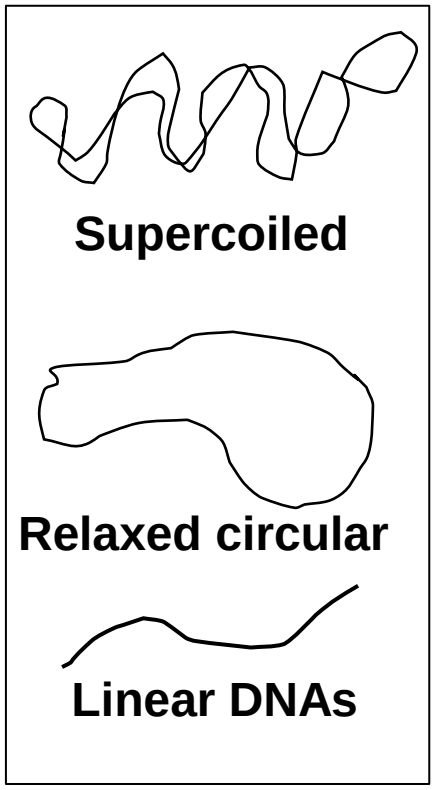
Video

Factors affect on the gel electrophoresis

1. If agarose is Partially dissolved.
2. If agarose is dissolved for along timed
3. If the tank is shacked or moved from a place to another.
4. If the top of the bottle is not tightened
5. Do not remove the comb before the gel is fully set.
6. Do not damage the well when loading your samples.
7. Make sure the air bubble is not produced inside the well
8. Do not Load the sample more quickly .

DNA conformations

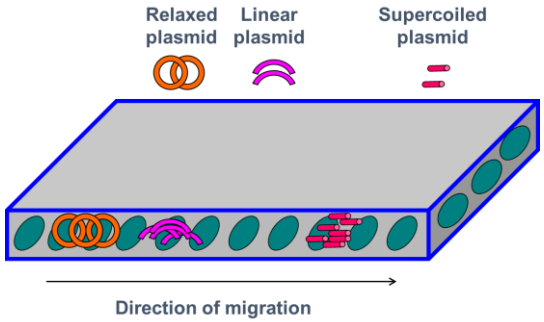
- Forms of bacterial DNA: Supercoiled circular, Relaxed circular & Linear DNAs of the same molecular size migrate through agarose gel at different rates according to the tightness of their conformation.
- Plasmid and bacterial chromosomes are mostly supercoiled in their native.
- Relaxed circular structures occur transiently during transcription and replication.



Factors affect on the DNA migration through agarose gel

1. The molecular weight of DNA**:** the Larger the fragments the slower the migration, why?

2. The conformation of DNA: Supercoiled DNA migrates faster than **linear** and **relaxed circular** is the slowest(supercoiled> linear> nicked or relaxed or open circular).



3. Agarose concentration**:** As agarose gel concentration increases, DNA goes slower.

4. Voltage Applied: The higher the voltage, the faster the DNA moves.

According to the data presented in the Figure, which suspect left DNA at the crime scene?