
# *Human Genetic*

## *Lap 3*

## Gel Electrophoresis

## *By*

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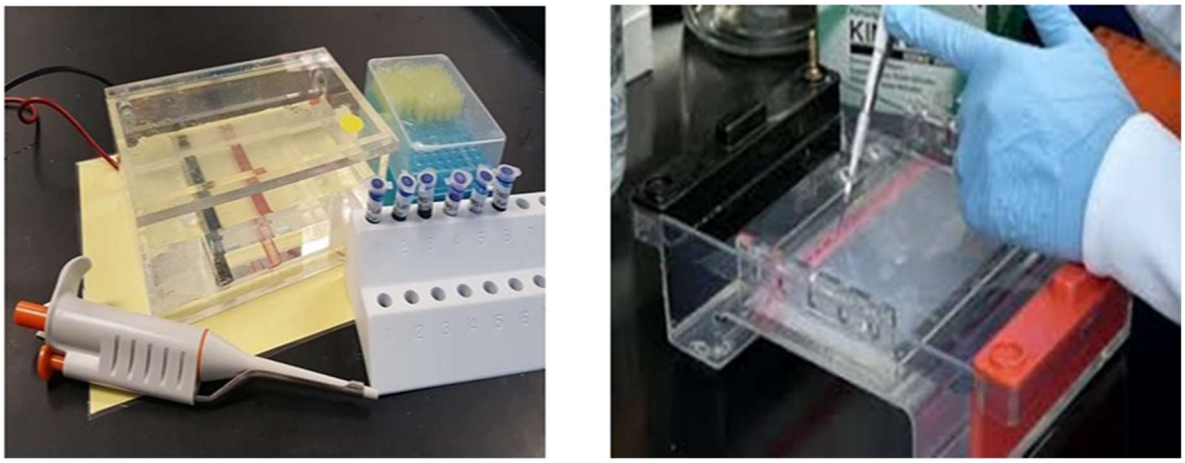
## Lap 3 Gel Electrophoresis

This technique separates and purifies fragments of DNA (single-stranded, double-stranded, and supercoiled) or RNA as well as proteins. The basic idea of **Electrophoresis** is to separate the molecules based on their intrinsic electrical charge. Since DNA carries a negative charge on each of the many phosphate groups making up its backbone, it will move towards the positive electrode during electrophoresis. Electrophoresis of DNA is usually used to separate the DNA into different sizes in addition to the chromosome, bacteria often contain plasmids; however, gel electrophoresis will separate the two different sized molecules of DNA.

**Principle:** by placing the molecules in wells in the gel and applying an electric field, the molecules will move through the matrix at different rates. Molecules with positive charge will migrate towards the cathode which is negatively charged. And molecules with negative charged will migrate towards the anode which is positively charged. DNA Gel electrophoresis is usually performed for analytical purposes, often after amplification of DNA via polymerase chain reaction (PCR).



**(Gel electrophoresis apparatus)**

## Materials

1- A microwave safe beaker or flask
2- Microwave oven
3- 1x or 0.5x TAE/TBE Buffer
4- Agarose
5- Ethidium bromide
6-Electrophoresis equipment
7-Gel loading dye (50 mM EDTA, 0.2% SDS, 50% glycerol, 0.05% w/v bromophenol blue)
8- A balance
9- Plastic Wrap
10- Thick gloves or potholders
11-Gel casting

**the equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:**

- An electrophoresis chamber and power supply.
- **Gel casting trays,** which are available in a variety of sizes and composed of UV transparent plastic.
- **Sample combs,** around which molten agarose is poured to form sample wells in the gel.
- **Electrophoresis buffer,** usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- **Loading buffer,** which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
- **Ethidium bromide,** a fluorescent dye used for staining nucleic acids.
- **Transilluminator** (an ultraviolet light box), which is used to visualize ethidium bromide-stained DNA in gels.

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## Protocol for Making an Agarose Gel

For preparation of 0.8% agarose gel

| Amount of 0.5%TBE | Agarose | Gel Red |
|---|---|---|
| 50 ml | 0.4 g | 1.5 ul |
| 75 ml | 0.6 g | 2.25 ul |
| 100ml | 0.8 g | 3 ul |

For preparation of 1.0% agarose gel

| Amount of 0.5%TBE | Agarose | Gel Red |
|---|---|---|
| 50 ml | 0.5 g | 1.5 ul |
| 75 ml | 0.75 g | 2.25 ul |
| 100ml | 1.0 g | 3 ul |

For preparation of 2.0% agarose gel

| Amount of 0.5%TBE | Agarose | Gel Red |
|---|---|---|
| 50 ml | 1.0 g | 1.5 ul |
| 75 ml | 1.5 g | 2.25 ul |
| 100ml | 2.0 g | 3 ul |

**1)** Prepare **1X** TBE (Prepared from the **10X** stock). This means that for every ml of 10X, you add 9 ml of deionized water. You need about 300 ml per gel run, but we usually make up 2 liters of it.

**Note:** Failure to dilute the TBE will result in very slow migration of the samples and very high amperage (causing excessive heating of the gel).

**2)** For a 1% gel, add 0.3 g agarose to 30 ml 1x TBE.

**Note**: For analyzing smaller DNA (500 bp or less), use a 1.5% gel. To do this, use proportionately more agarose. A 1% gel is 1% weight/volume (w/v). *[for example, for a 1.5% gel, add 0.45 g agarose to 30 ml final volume]*

**3)** Heat the solution to boiling in the microwave to dissolve the agarose.

**Note:** You should not see any beads in the solution. If you have beads in the agarose, you will get distorted bands.

**4)** Add 1 µl of ethidium bromide to the dissolved agarose and mix.

Note: EtBr is a suspected carcinogen and must be properly disposed of per institution

regulations. Gloves should always be worn when handling gels containing EtBr. Alternative dyes for the staining of DNA are available; however EtBr remains the most popular one due to its sensitivity and cost.

**5)** Get a gel plate and a comb. Put the two dams into the slots on each side of the gel plate.

Note: Make sure that the comb is nearest to the black electrode (cathode), as the DNA migrates towards the red electrode (anode).

**6)** Let the gel cool to room temperature. This will take 20 minutes. It should look cloudy, and, if you touch it, the gel will feel cool or cold. Note: Do not pull the comb out too soon, as it causes the wells to collapse.

**7)** Carefully remove the dams and the comb.

**8)** For the large wells, use up to 10 µl of your samples. For the smaller wells, use 8 µl samples. Carefully place the samples into adjacent wells by using a pipette and a steady hand.

**9)** Electrophorese the samples at 100 V for 30 minutes.

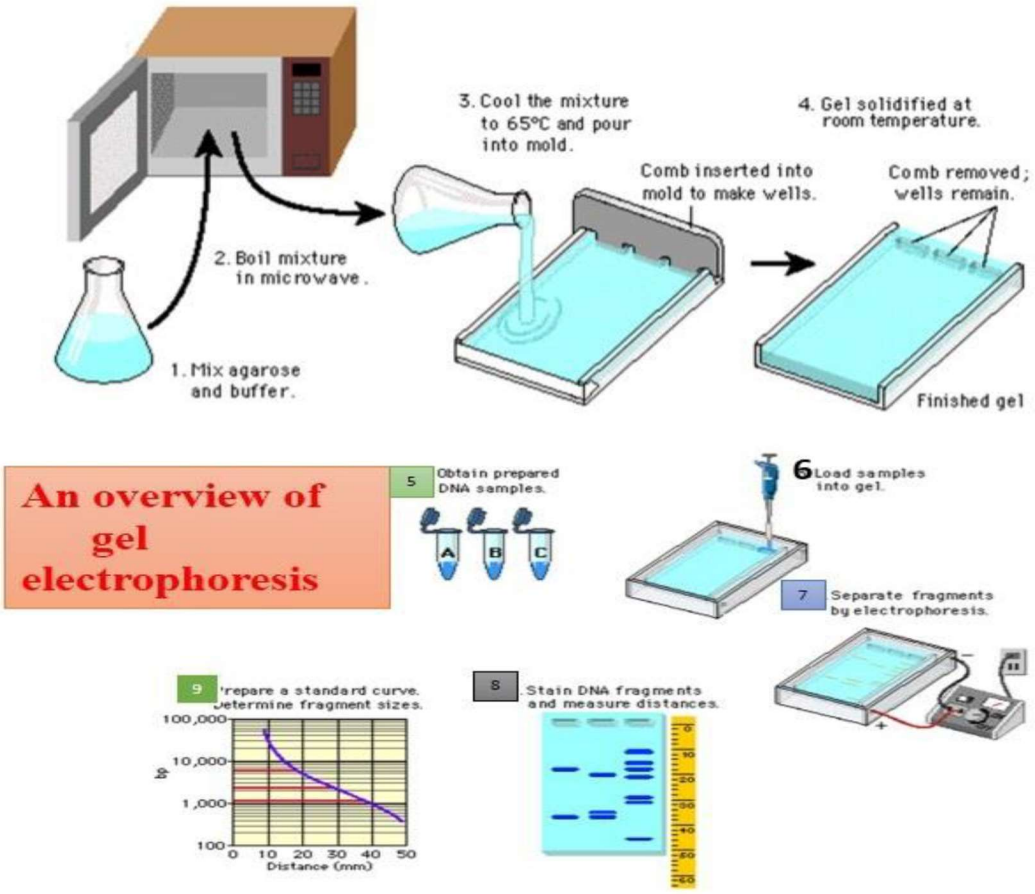
**10)** Wearing gloves (since ethidium bromide is present), carefully remove the gel from the box, put it onto the UV light box and take a look. If the gel looks Ok, take a picture.

Note: Wear UV protective glasses or cover the light box with the UV protective shield when the UV light is on. The UV can cause skin cancer.

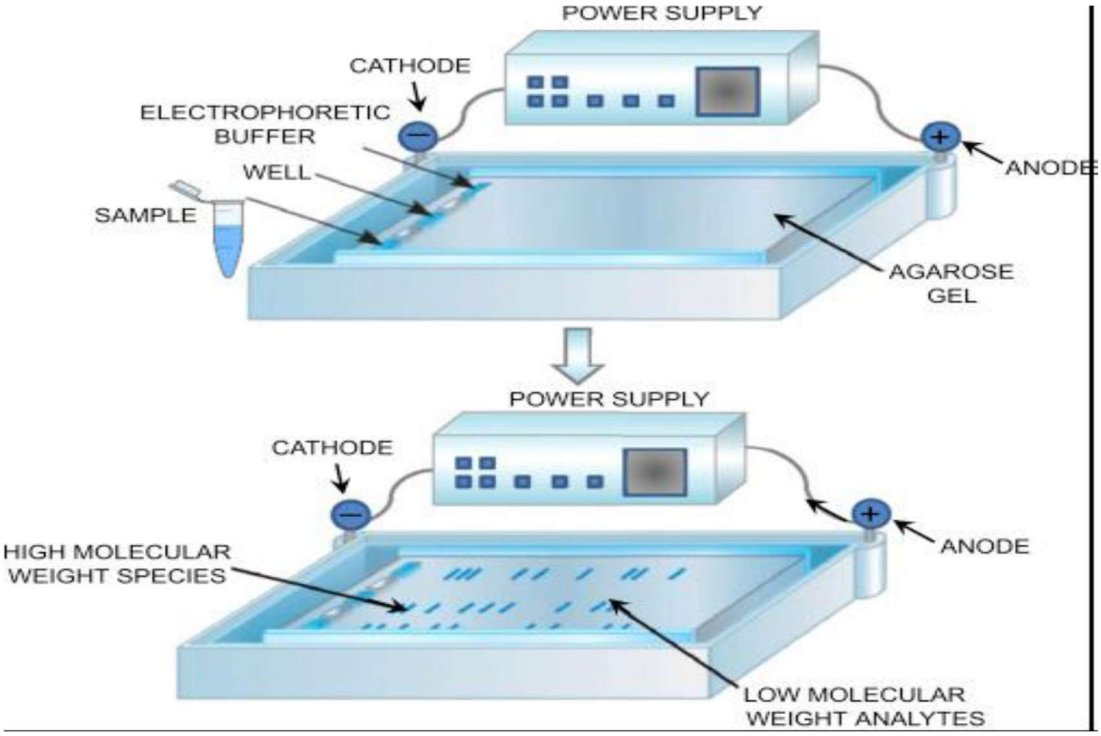
## Factors affecting migration

1. **DNA molecular weight:** The length of the DNA molecule is the most important factor shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel
2. **Agarose:** In general lower concentrations of agarose are better for larger molecules because they result in greater separation between bands that are close in size **(**1% gels are common for many applications.)

3. **Buffer:** tris acetate EDTA (TAE) provides the best resolution for larger DNA.
4. **Visualization:** The most common dye used to make DNA bands visible for agarose gel electrophoresis is ethidium bromide.




**(Overview of gel electrophoresis)**




**UV-trans illuminators:** is apparatus used to view DNA or RNA that has been separated by electrophoresis through agarose gel.

**Principle:** after the electrophoresis, the agarose gel is stained with a fluorescent dye which binds to nucleic acid. Exposing the stained gel to a UV light source causes the DNA dye to fluoresce and become visible.

**What is agarose?**
**What is the purpose of ethidium or SYBR-Safe?**
**Why is it important to pour thin gels?**
**What is the difference between agarose gels and polyacrylamide gels? Why do we use agarose gels for DNA and polyacrylamide gels for proteins?**