# Agarose gel electrophoresis

## Solutions and reagents

- **Agarose**, ultrapure (USB #32827)

-**Electrophoresis buffer:**

TAE buffer (Tris, acetate, EDTA) 50X, 1L

| | |
|---|---|
| Tris | 242g |
| Acetic acid | 57,1ml |
| EDTA | 100ml |
| ddH2O | up to 1L |

Adjust pH to 8.3 with NaOH or acetic acid

Store at RT

TBE (5X)

| | |
|---|---|
| Tris | 54g |
| EDTA | 4.65g |
| Boric Acid | 24g |
| ddH2O | up to 1L |

Adjust pH to 8.3 with boric acid

Store at RT

- **Ethidium bromide** (EtBr), 10 mg/ml (20,000x)
Stock solution: 1 g EtBr in 100 ml $H_2O$. Stir on a mgnetic stirrer for several hours to dissolve. Wrap in aluminium foil or transfer to a dark bottle and store at room temperature. Dilute stock 1:20,000 for gels or stains solution.

**-** DNA **Loading buffer, 10x** (10ml)

| | |
|---|---|
| 50% glycerol | 5ml of 100% |
| 100mM Tris pH8 | 1ml of 1M |
| 100mM EDTA pH7.5 | 2ml of 0.5M |
| 0.1% Bromophenol blue | 1ml of 1% |

| | |
|---|---|
| 0.1% xylene cyanol | 200ul of 5% |
| H2O | 800ul |

**- DNA molecular weight markers**
1Kb DNA Ladder
λ/Hind III (to calculate DNA quantity)

## Method:

**Gel preparation**

1. Seal the gel casting platform or put the open ends with adhesive tapes and insert the gel comb.

2. In a glass bottle, Prepare the gel by adding the desired amount of electrophoresis-grade agarose to a volume of buffer (1x TAE or 1x TBE).

3. Melt the agarose, by boiling for several minutes, in a microwave oven.

CAUTION: when agarose come to a boil, handle the solution very carefully. Allow the solution to stand briefly at room temperature before handling

4. Remove the flask, swirl gently to resuspend any agarose particles. Continue this cycle of heating until all the agarose is completely dissolved.

5. Put the gel in an Erlen, if desired, add ethidium bromide solution to the gel and the electrophoresis buffer at a final concentration

CAUTION: ethidium bromide is a powerfull mutagen and is moderatly toxic. Glove should be worn when weighting it out.

6. Cool the agarose solution before pouring onto the gel platform to prevent warping of the gel apparatus. Make sure that no bubbles are trapped underneath the combs and all the bubbles on the surface of agarose are removed before the gel is set.

7. After the gel has hardened, remove the tape from the open ends of the gel platform and withdraw the gel comb, taking care not to tear sample wells.

8. Place the gel casting plateform containing the set gel in the electrophoresis tank. Add sufficient electophoresis buffer (1x TAE or 1x TBE) in the electrophoresis tank to cover the gel to a depht of about 1 mm, or adjust until the tops of the wells are submerged. Make sure no air pockets are trapped within the wells.

Caution, the percentage of gel depending of the fragment size

**Agarose, Non-denaturating**

| | | Approx dye migration rates |
|---|---|---|
| Gel % | Resolution Range | Bromo blue |
| 0,6 | 600 up to | About 300bp |
| 1 | 600 to 400 | |
| 1,5 | 500 to 150 | |
| 2 | 200 down to | |
| | (ds-DNA in bp) | |

**Sample application**

1. Prepare samples in a volume (typically 5-20 µl) that will not overflow the gel wells, by addition of loading buffer to a final concentration of 1X

2. load DNA molecular weight markers. Markers DNA of known size should be loaded into slots in both the right and the left sides of the gel.

3. Apply samples and weight markers with a pipetor into the sample wells by injecting them down through the thin layer of buffer covering the gel.

**Running conditions and DNA visualisation**

1. Be sure that the leads are correctly attached to the power supply. Set the voltage at the desired level, typically 125mV.

2. When the bromophenol blue dye from the loading buffer has migrate a distance judge sufficient for separation of the DNA fragments. Place the gel on a UV light source and be photographed directly. Alternatively, Soak the gel in ethidium bromide for 5 min and rinse in water before expose on UV.