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**Lab 3**

**Gel Electrophoresis for DNA/ RNA analysis**

What is Gel electrophoresis?

Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g. length in base pairs) for visualization and purification.

Electrophoresis

uses an electrical field to move the negatively charged DNA toward a positive

electrode through an agarose gel matrix.

The gel matrix allows shorter DNA fragments to migrate more quickly than larger ones. Thus, you can accurately determine the length of a DNA segment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

**What the materials are using in electrophoresis?**

1- Buffer TBE (**Tris/Borate/EDTA**) or TAE (**Tris-acetate-EDTA**) **(or TPE**

**Tris-Phosphate EDTA)**.

2- Agarose

3- Loading dye buffer (6X): Bromophenol blue or orange G + glycerol and/ or

xylene cyanol FF

4- Fluorescent dye: Ethidium bromide or gel red, gel green or Sybr safe.

**What components are using in electrophoresis?**

1-Power supply

2-Cell

3-Tray or gel bed

4-Comb

5- Ultraviolet cabinet

**Factors effects on mobility of DNA fragments**

Several additional factors have important effects on mobility of DNA fragments in agarose gels:

1. Agarose concentration, 1% gels are common for many applications.

(Agarose gel electrophoresis can be used for the separation of DNA

fragments ranging from 50 base pair to several megabases (millions of

bases) using specialized apparatus. Increasing the agarose concentration of

a gel reduces the migration speed and enables separation of smaller DNA

molecules).

2. Voltage (The higher the voltage, the faster the DNA moves. But voltage is

limited by the fact that it heats and ultimately causes the gel to melt

High voltages also decrease the resolution (above about 5 to 8 V/cm).



**3. Electrophoresis Buffer**

The two most popular types of buffers for running agarose gels are Trisacetate

with EDTA (TAE) and Tris-borate with EDTA (TBE). Because both

buffers have a near-neutral pH, the DNA in the buffers has a net negative

charge and migrates toward the anode (+) end of the gel apparatus.

**4. Effects of Ethidium Bromide.**

**Procedur :**

1- Prepare 1% agarose gel by adding 1 gm of agarose to 100 ml of 1x TBE

buffer, heat until the agarose completely dissolved then allow the gel to

cool to 45-50 °C.

2- The next step, adds 5μl of Ethidium bromide (final concentration 0.5 μg/mL),

mix well and pour carefully in the tray (always avoid air bubbles formation

between the teeth of the comb). Allow the gel to set at room temperature for

30-40 minutes.

3- Remove the comb carefully from the gel and place the tray in the gel tank,

after that add a sufficient 1xTBE buffer to the tray (should cover the gel).

4- The gel is ready to load samples (e.g amplified PCR products) and run your

gel for 1 hour and a half (7 Volts/ cm).

5- DNA ladder is used to determine samples size (e.g PCR product size), and

then PCR products are visualized by UV light at 336 nm, and are

photographs by using digital camera.

