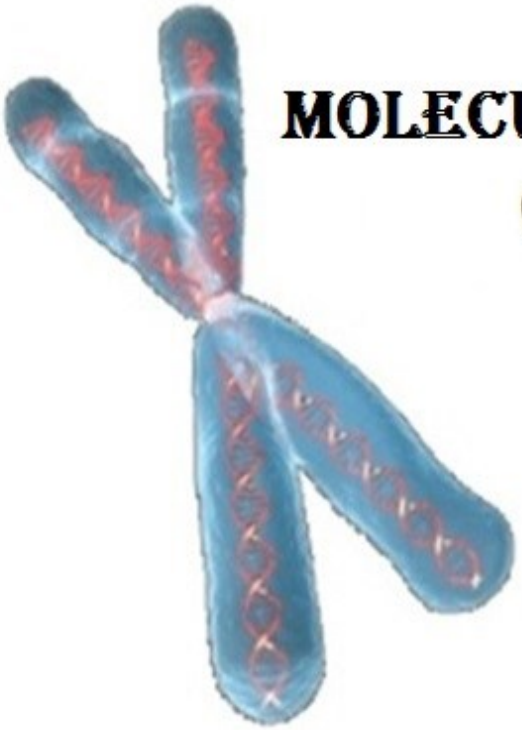
Lab. 5

زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

PRACTICAL



MOLECULAR GENETICS

Subject : AGAROSE GEL ELECTROPHORESIS

Department: Animal Resources

Stage : 2

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Agarose gel electrophoresis:

It is a technique that is used to separate nucleic acid as DNA that differ in size, charge. It is one of the most widely-used techniques in biochemistry and molecular genetics. The movement of particles under electric field in a fluid is called **electrophoresis**. DNA is forced to migrate through agarose matrix in response to an electric current. The phosphates on the DNA are negatively charged, and the molecule will therefore migrate to the positive (red) pole.

Factors that effect on the sample migration:

1. Size of DNA.
2. Gel concentration "pore size".
3. Strength of electrical field.
4. Electrophoresis Buffer pH: effect on electrical conductivity.
5. Present of Ethidium bromide

Requirement of gel electrophoresis:

1. Gel:

- **Agarose:**

It is a polysaccharide extracted from seaweed and is typically used at concentrations of 0.5% to 3%. The higher concentration of agarose is the "stiffer" the gel. Polymerized agarose is porous, allowing the movement of DNA through it. DNA fragments from about 0.1 kbp to 50 kbp can be separated in agarose.

2. Buffers:

Two buffers are used together:

- **Electrophoresis buffer:**

Usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).

- **6X Loading buffer:**

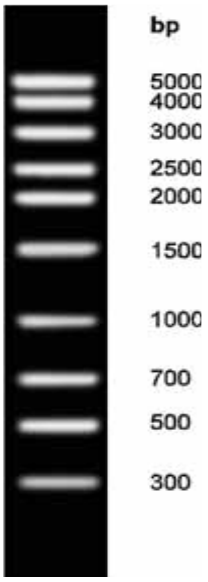
There are many names: Tracking buffer (Tracking dye) and blue juice. which contains something dense (glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes (bromophenol blue) which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.

3. Fluorescent dye:

It is important to visualize the separated DNA bands, usually ethidium bromide (EtBr) is used. EtBr is a fluorescent that intercalating between the bases of DNA and glows pink by UV light.

4. Samples:

Such as genomic DNA, PCR products and digestive products.



5. DNA Molecular Marker:

There are many names: DNA Molecular Marker, DNA ladder, or DNA standard. It is a mixture of DNA fragments of known sizes. The size of a fragment is measured by base pairs (bp).

6. Electrophoresis apparatus:

- **Tank:**
- **Tray:**

- **Power supply:**

It can be monitored and operated in current (amps), voltage (volts) or power (watts) mode. The black and red cords leading from the power supply are then attached to the tray in which the gel is run.

- **Combs:** It used to make wells on the gel to load different samples.

7. Detection system:

Transilluminator (Ultraviolet light box) and camera.

Preparation of the 1% agarose gel

1. Add 1 gm agarose to 100 ml of 1X TBE buffer.
2. Boil the agarose gel briefly (in microwave oven) and cool down to 55 °C (check the temprature that the tray tolerates).
3. Pour the gel gently into the tray and remove air bubbles by using a pasteur pipette or micropipette.
4. After the gel completely set, remove the tape and place the gel with the tray into the electrophoresis tank.
5. Pour a 1X TBE buffer into the electrophoresis tank,enough to cover the gel to a depth of about 4mm.
6. Remove a comb carefully. Ecpecially in case of small wells and a soft gel, then load samples.