Experiment (4): Agarose Gel Electrophoresis

Aim:

- Evaluating the intactness of the extracted DNA by agarose gel electrophoresis.
- To separate and calculate the molecular size of DNA fragment by comparing the separated bands with known standard molecular weight marker.
- To quantify DNA fragment by comparing the separated band with known quantity of DNA.

Introduction:

Agarose gel electrophoresis is method for separation (by size), quantifying, purification of nucleic acids fragments mixture, and analysis of DNA restriction fragments. It is one of the most widely-used techniques in biochemistry and molecular biology. Agarose is a liner polymer composed of alternative residues of D-galactose and 3,6-anhydro-L-galactopyranose joined by α (1→3) and β (1→4) glycosidic linkages. Agarose and acrylamide matrices are used to separate DNA by gel electrophoresis. The choice of gel matrices and gel concentration depends on the size of nuclear acid molecules, as the concentration of the agarose or acrylamide determine the pores size:

w/v % Gel type	Size of DNA fragments (Kb = 1000 bp)
0.5 %	1 kb to 30 kb
0.7 %	800 bp to 12 kb
1.0 %	500 bp to 10 kb
1.2 %	400 bp to 7 kb
1.5 %	200 bp to 3 kb
2.0 %	50 bp to 2 kb

PAUSE AND THINK ➔ What is the relation between the concentration of the gel and the pores size?

Under physiological conditions, DNA is a negatively charged molecule due to the presence of phosphate groups in the backbone. Therefore, in aqueous media, under the influence of an electrical field, DNA molecules will move through an agarose matrix towards the positively charged anode, at a rate that is inversely proportional to the molecular weight. The electrophoretic migration rate of DNA through agarose gel depends on the following: size of DNA molecules, concentration of agarose gel, voltage applied, conformation of DNA, and the buffer used for electrophoresis.

Several buffers are used for agarose gel electrophoresis, but the most common are: Tris-acetate EDTA buffer (TAE) and Tris-borate EDTA buffer (TBE). The DNA mobility in TBE buffer is approximately two times slower than in TAE buffer. This is due to the lower porosity of agarose gel when agarose polymerizes in the presence of borate.

Since DNA is colourless, the loaded sample need to be tracked. This is achieved by using a loading dye solution. Finally, to visualize DNA (result), agarose gels are usually stained with ethidium bromide and illuminated with UV light.

PAUSE AND THINK ➔ How the DNA is visualized by ethidium bromide?

Identifying the size of a DNA sample is one of the common AGE uses and this accomplished through what called: DNA marker (Ladder). A DNA and RNA size markers contain a mixture of DNA (or RNA) fragments of known length, making them suitable for estimating the fragment length of concurrently run samples.

Principle:

Nucleic acids are separated by applying an electric field, so these negatively charged molecules will move through an agarose matrix towards the anode, and the biomolecules are separated by size in the agarose gel matrix, where the distance travelled by a DNA molecule is inversely correlated with its size.

Materials:

Agarose powder, 1X TBE buffer (89 mM Tris-base, 89 mM boric acid and 2 mM EDTA) prepared from 10X TBE, Ethidium Bromide (5 mg/ml), Gel loading dye (Glycerol and orange dye),1 kb and 100 bp DNA ladder, horizontal electrophoresis apparatus and power supply.

Protocol:

1. Measure the desired grams of agarose to make 1% agarose gel.
2. Heat the solution to boiling in the microwave to dissolve the agarose to produce a homogeneous mixture.
3. Add 4 µl of ethidium bromide CARFULLY to the dissolved agarose and mix .
4. Get a gel plate and a comb. Put the two dams into the slots on each side of the gel plate. Make sure that they fit tight. Pour the melted agarose onto the gel plate in the electrophoresis tray.
5. Place the comb in its place. Let the gel cool to room temperature.
6. Place the gel in the electrophoresis chamber.
7. Pour enough electrophoresis buffer (1X TBE) to cover the gel to prevent overheating of the gel.

8. Carefully remove the comb.
9. Prepare the DNA sample by mixing around 300 ng of DNA sample with 3-4 µl of loading dye.
10. Add 3 µl DNA ladder into the first well by using a micropipette.
11. Carefully place the prepared samples into adjacent wells
12. Electrophorese the samples at 95 V for 45 minutes. (Check the gel while it is running).
13. Carefully remove the gel, place it onto the UV light box and take a picture for the gel.

Results:

Picture of the gel.

References:

1. Surzycki S. basic techniques in molecular biology. Springer. (2000).