EXPERIMENT NO: 9 (B)

DATE:

AGAROSE GEL ELECTROPHORESIS

AIM:

To analyze the given nucleic acid using Agarose Gel Electrophoresis.

MATERIALS REQUIRED:

1. Agarose solution,
2. Ethidium bromide,
3. Electrophoresis buffer,
4. 6x gel buffer,
5. DNA sample,
6. DNA size standard.

PRINCIPLE:

Agarose gel electrophoresis used to analyze and quantitate nucleic acid. The Agarose for Agarose gel electrophoresis is purified from agar. Agarose is a linear polymer made up of repeating units of 1,3 –linked β D galactopyranose and 1, 4 linked 3,6 anhydro a L galactopyranose [ P-D –gal (1-4)-3,6 anhydro – a L Gal (1-3) -]n Agarose has an average MW of 12,000 and contains about 35-40 agarobiose units. Agarose in solution exist as left handed double helices. About 7 to 11 such helices form bundles which extend as long rods and appear to intertwine with one another, further strengthening the frame work of the gel. The cross links are held together by hydrogen and hydrophobic bonds. By changing the gel concentration the pre size can be altered. Higher the concentration of Agarose smaller the pre size and vice versa. Because of large pore size even at low concentration, Agarose gels are widely used for separation of DNA and RNA.
Effect of Agarose concentration on separation ranges

The following table describes the relationship between Agarose concentration and separation range of nucleic acid.

<table>
<thead>
<tr>
<th>AGAROSE CONCENTRATION (%)</th>
<th>SEPARATION RANGE (KB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>5 to 60</td>
</tr>
<tr>
<td>0.6</td>
<td>1-20</td>
</tr>
<tr>
<td>0.8</td>
<td>0.8 to 10</td>
</tr>
<tr>
<td>1.0</td>
<td>0.4 to 8</td>
</tr>
<tr>
<td>1.2</td>
<td>0.3 to 6</td>
</tr>
<tr>
<td>1.5</td>
<td>0.2 to 4</td>
</tr>
</tbody>
</table>

Factors which affect the rate of migration of nucleic acids in Agarose gels

Rate of migration of nucleic acids in Agarose gels depends mainly on four important parameters.

a. Agarose concentrations

Higher concentration of gels are used for the separation of lower molecular weight DNA and RNA fragments and vice-versa

b. Molecular weight

A duplex DNA fragment migrates at rates inversely proportional to the log Molecular weight. A plot of logM.W vs. Mobility gives a straight line

c. Conformation

Supercoiled DNA moves fastest followed by linear forms and relaxed open circular forms.

d. Applied Voltage

At low voltage (<5V/cm) the rate of migration is directly proportional to the applied voltage. However, if the voltage is increased, mobility of high molecular weight DNA fragments increased differentially.

e. Base composition and temperature

Base composition and running the gel between 4 and 30C do not change the nобilities
Preparation of stock solutions for DNA gel electrophoresis

To different buffer systems are widely used for separation of nucleic acids by agarose gel electrophoresis. Their composition are given in the table

**TBE buffer**

<table>
<thead>
<tr>
<th>Tris borate (1X TBE)</th>
<th>10X buffer/lit</th>
</tr>
</thead>
<tbody>
<tr>
<td>89mm Tris base</td>
<td>108.0g</td>
</tr>
<tr>
<td>89mm Boric acid</td>
<td>55.0g</td>
</tr>
<tr>
<td>25mm Na2-EDTA</td>
<td>9.3g</td>
</tr>
</tbody>
</table>

**TAE buffer**

<table>
<thead>
<tr>
<th>Tris acetate (1X TBE)</th>
<th>10X buffer/lit</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mm Tris base</td>
<td>302.5g</td>
</tr>
<tr>
<td>25mm acetic acid</td>
<td>71.4g</td>
</tr>
<tr>
<td>1mm Na2-EDTA</td>
<td>18.6g</td>
</tr>
</tbody>
</table>

Sterilize the stock solutions by autoclaving

**Preparations of ethidium bromide (stock solution)**

Weigh 10 mg ethidium bromide into a sterile tube and dissolve in 10 ml sterile distilled water. The stock is stored at 4C

**Preparation of sample loading dye Glycerol & bromophenol blue (6x)**

3ml glycerol (30%), 25mg bromophenol blue (0.25%) dH₂O to 10mL

**Preparation of agarose solution for casting the gel**

Dissolve the Agarose by placing the flasks in boiling water both cool to Luke warm. Cover the sides of a tray using cellotape and place the comb about 1 cm from the top of the tray.

Pour the Agarose with out making any bubles, cool it for 20 mins and take off the combs and uncover the cellotapes
The DNA sample (100 to 200 ng) is mixed with the loading dye (for 5 µl of DNA sample 1µl of 6x dye is used) and loaded in to the well carefully, using a pipetman or capillary tube. Once the sample is loaded in to the well, the cathode (Black negative terminal) is connected towards the top end of the gel and the anode (Red positive terminal is connected towards the bottom end of the gel. The maximum volume that can be loaded on to a well formed from a 1.5 mm thickness tooth of the comb is 30 µl. The electrophoresis is started by switching on the D. C. Powerpack. The gel is run at 5v/cm. As the bromophenol blue(the tracking dye) has moved 1 cm above the bottom end, the current is switched off, the power supply is disconnected and the gel along with the platform is stained in the plastic tray containing 0.5 µg/ml ethidium bromide in the sterile distilled water( use gloves when handling ethidium bromide). After about 30-45 min, the platform and the gel is rinsed with distilled water and by keeping the platform in a slanting position, the gel is gently pushed onto the UV Transilluminator. (As UV rays are dangerous for the eye, protect your eyes by wearing a UV face shield, goggles or using glass plate). Now the UV light is switched on and the DNA bands are seen and Photographed at f 5.6 for 10 seconds with an orange filter.

**PHENOL: CHLOROFORM EXTRACTION**

1. Mix the DNA solution with equal volume of phenol: chloroform (1:1 v/v).
2. Centrifuge at 10,000 rpm 5 min.
3. Transfer the aqueous phase to a fresh tube, add equal volume of chloroform, and centrifuge 10,000 rpm for 5 min.
4. Transfer the aqueous phase to a fresh tube and mix with 1/10th volume of 3 M NaOAc and 2.5 volume of ethanol. Leave at -20°C for 1 h for precipitation.
5. Centrifuge the samples at 10,000 rpm for 10 min; decant the supernatant.
6. Add 1 ml of 70% ethanol to the pellet, vortex and centrifuge at 10,000 rpm for 5 min.
7. Air-dry the pellet and dissolved in appropriate volume of 0.1X TE buffer.
RESULT:

After electrophoresis DNA bands can be visualized under UV light and they appeared as orange fluorescence.