Agarose Gel Electrophoresis Protocol for DNA

Reagents and Materials:

for preparation:  ● tank, tray, comb
  ● normal melting point agarose powder
  ● 10 x TBE buffer solution, gel stain (Eco Safe Nucleic Acid Staining Solution)
  ● microwave oven, Erlenmeyer flask, measuring cylinder, scales

for loading:  pipette, PCR tubes or tinfoil, power supply

for documentation: camera/ gel documentation system

Gel preparation

1. - Prepare sufficient electrophoresis buffer (1:10 dilution of TBE:distilled water)
   - Clean a plastic tray.
   - Position the comb 0.5-1 mm above the plate so that a complete well is formed when the agarose is added.

2. - Prepare agarose gel. For a 2% agarose gel:
    measure 2 g agarose in an Erlenmeyer flask
    add 100 ml 1x TBE buffer.
    - Scale the flask and note its weight on it.
    - Cover the flask with kimwipes/ parafilm and heat with microwave until the agarose dissolves. Measure it again and complete the evaporated liquid with distilled water.
    - Leave it to cool down to about 60 °C on the bench for several minutes but do not leave it too long so the agarose should not start to solidify.
    - Stain the agarose solution:
      5 µl ECO Safe Nucleic Acid Staining Solution / 100 ml gel
    - Mix the agarose solution well by swirling the flask. Pour the agarose into the mold. (3-5 mm thickness)

3. - After 30 minutes at room temperature carefully remove the comb.
    - Position the gel into the gel electrophoresis tank. Avoid bubbles!
    - Add enough TBE buffer to cover the gel to a depth of about 5 mm.

Loading

1. Mix the DNA samples with gel-loading buffer with pipettes:
   5 µl of buffer + DNA solution
   note: about 0.3 - 0.5 µg of DNA gives a visible band or 1/10 of PCR reaction volume
   Prepare marker DNA of known size: 6 µl from Promega Marker
2. Load the mixtures slowly into the slots. Avoid making bubbles!

3. Attach the electrical leads so that DNA can move toward the anode (red lead). Apply a voltage of 1-5V/cm. Run the gel until the gel-loading buffer stain has migrated the appropriate distance (normally until the bromophenol blue dye front migrated ¾ of the way down the gel).

Documentation:

1. Turn off the current and remove leads.
2. Examine the gels: Carefully place it on an ultraviolet transilluminator and take a photo.

Technical appendix:

- Type of Agarose: normal melting point, molecular grade
- Percentage of agarose for resolving DNA fragment:

```
<table>
<thead>
<tr>
<th>% Agarose concentration in gel (w/v)</th>
<th>Efficient separation range for linear double stranded DNA molecules (Kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>5 - 60</td>
</tr>
<tr>
<td>0.6</td>
<td>1 - 20</td>
</tr>
<tr>
<td>0.7</td>
<td>0.8 - 10</td>
</tr>
<tr>
<td>0.9</td>
<td>0.5 - 7</td>
</tr>
<tr>
<td>1.2</td>
<td>0.4 - 6</td>
</tr>
<tr>
<td>1.5</td>
<td>0.2 - 3</td>
</tr>
<tr>
<td>2.0</td>
<td>0.1 - 2</td>
</tr>
</tbody>
</table>
```

[Table taken from Sambrook, Fritsch, Maniatis: Molecular Cloning (1989)]

- Agarose solution: The excess solution can be stored at 4 °C until later use.
- 10 x TBE solution (per liter): from molecular grade reagents
  
  + 108 g Tris
  + 55.65 g boric acid
  + 40 ml 0.5M EDTA (pH 8.0)

  stored at room temperature

- Gel stain: Eco Safe Nucleic Acid Staining Solution (Pacific Image Electronics)
- Marker DNA: use according to manufacturer
- Loading dye (6X concentration): - 0.25% bromophenol blue
  - 0.25% xylene cyanol
  - 30% glycerol in water

  Store at 4 °C