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:

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

[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