AGAROSE GEL ELECTROPHORESIS OF DNA - Horizontal and Vertical

Introduction:

Section I describes preparation and running of the gel. Section II describes preparation of samples and comments on gel capacity. Section III lists molecular weight markers. The molecuar weight versus mobility plots in section III may be used in selecting the appropriate agarose concentration for the molecular weight region of interest.

I. Preparing and Running gel

A. Vertical gel using Sugden apparatus

The Sugden apparatus is designed to circumvent some of the problems encountered in using agarose for vertical gels. The following discussion supplements the illustrations in Sugden et al. (1975) Analyt Biochem. 68: 36-46.

(1) **Materials:**

-Sugden apparatus
-Two bulldog clamps
-Two three fingered clamps
-Electrophoresis power source and leads
-Ethidium Bromide (Sigma)
-Agarose (ME grade) (SeaKem)
-10 x TAE (titrated to pH 7.8 with acetic acid)
    0.4M Tris base
    0.05 M Sodium acetate
    0.01 M EDTA

(2) **Procedure:**

a. Wash Lexam plate of Sugden apparatus with Joy detergent using a bottle brush. Wash glass plates with household cleanser using gloved hands. rinse thoroughly and wipe dry.

b. Assemble apparatus according to Sugden et al. with the following modifications.
    1. Use only one pair of bulldog clamps positioned so that the completed gel may be mounted on electrophoresis tanks without moving clamps.
    2. Use three fingered clamps to squeeze plates tightly against comb.

c. Combine 12 mls of 10 x TAE, 108 ml dd (Deionized, glass distilled) H_2O and 0.72-3.00 gms of agarose (0.6%-2.5%) in an erlenmeyer with stir bar.

d. Cover erlenmeyer mouth with aluminum foil and melt using a slow exhaust cycle on autoclave with timer set at five minutes.

e. Place solution on magnetic stir plate and allow to cool with stirring to 65°C.

f. With apparatus propped in inverted position (comb up) make a bead of agarose around entire junction of comb and gel plates.

g. When agarose in flask has cooled to 55°C stand the assembly upright and pour the gel. It is advisable to run five to ten mls of agarose down the inside on each side before filling form by pouring. This eliminates leakage from the sides.

h. Place remaining agarose in 65°C oven and allow gel to cool for 10 to 15 minutes. The liquid in the gel form will shrink and gel should be 'topped off' with more agarose solution.

i. Allow gel to solidify for at least two hours at room temperature. If exposed edge of gel is covered with Saran wrap, the gel may be stored at room temperature for several days.

j. Release three fingered clamps (gel will pull aay from the glass a bit at the top, but don't worry), remove comb gently and attach gel to tanks as described in Sugden et al. In addition, to prevent leakage, seal plate- tank junction with agarose and fill most extreme well on each side with agarose.

k. Fill tanks with 1 x TAE (this requires a bit more than a liter). If gel has been stored in cold room allow to equilibrate at room temperature before running.

l. Apply samples (see Sample Preparation below) and run at 10 mA constant current for sixteen hours (bromophenol blue approximately three quarters of the way down the gel) .

m. Immerse gel in solution of 0.5 ug/ml ethidium bromide in TAE for one hour. Photograph under ultra violent light using an orange filter on the camera lens.

B. Horizontal Gel

(1) Materials:

-horizontal gel apparatus
-tape for ends of casting tray
-electrophoresis power source and leads
-ethidium bromide 10mg/ml ***beware; powerful mutagen
-agarose
-10x TAE (see above) or TBE (TBE has a greater buffering capacity so it is the buffer of choice here)

(2) Procedure

a. Wash casting tray and dry well.
b. Tape across ends to seal casting tray.
c. Position comb above casting tray by clamping it to a rack.
d. Combine agarose with 1x TBE to desired concentration and volume e.g. 1% agarose gel (200 mls): 2 g agarose, 20 ml 10x TBE, to 200 mls w/H_2O.

e. Dissolve agarose by microwaving or stirring on a hot plate. Agarose will dissolve when it reaches the boiling point.
f. Cool to ~55°C, add ethidium bromide to 1 ug/ul (add 1/10th ul 10 mg/ml etBr per ml of agarose), then pour into casting tray.
g. Allow to harden undisturbed - takes about 30 min- 1 hr..
h. Remove tape from ends, place in horizontal gel apparatus, then cover with 1x TBE.
i. Load samples and run at constant voltage.

II. Sample Preparation

A. Procedure

1. To a phenol extracted restriction digest add 3.0 M sodium acetate (pH 4.5) to 0.3 M. Add three volumes of ethanol, mix and place at -20°C overnight.
2. Pellet precipitate by spinning at 10k in the HB-4 Rotor for 20 min at 0°C.
3. Decant supernatant, add 70% ethanol.
4. Pellet by spinning as in (2) for 10 min.
5. Repeat steps (3) and (4).
6, Decant supernatant and remove excess liquid using a cotton swab.
7. Allow sample to dry at room temperature for 10-30 min. (Note: overdrying makes resuspension of high molecular weight material impossible.)
8. Resuspend pellet in 20 ul of a buffer of the following composition: 8mM tris, 4 mM NaCl, 1 mM EDTA, 10% glycerol, 0.03% bromophenol blue, pH 7.4.
9. Heat at 55°C for five minutes and allow to cool passively to room temperature.
10. Apply to gel

***Note: the above steps are necessary if you wish to do careful restriction mapping. However, for routine gel purification of fragments for cloning, etc., DNA restriction digests can be loaded onto the gels directly after incubation, as long as you add 1/10th volume of loading buffer (50% glycerol; 0.1 mM EDTA; 0.1% bromophenol blue).

Comments on sample preparation

1. Volume, ionic strength, and salt composition must be matched when mobilities fragments run in different channels are to be compared.
2. An agarose gel (in TBE) of 3-5 mm thickness, run as described above begins to overload at approximately 03. ug/band/cm well width. This figure may be used to calculate maximum advisable input for simple mixtures (e.g., a restriction digest of a plasmid). For complex mixtures (e.g., a digest of Drosophila DNA) the gel system is not overloaded at 7 ug/cm well width.
3. The lower limit of detectability using ethidium bromide staining is 5-10 nanograms/band.

III. Molecular Weight Markers

Below are lists of the molecular weights of fragments I have employed at molecular weight standards. Typical molecular weight versus plots are included on the following pages.

1. lambda (uncut): 46.5 kb
2. lambda digested with EcoRl (Thomas and Davis, 1975): 21.0 kb, 7.2, 5.7, 5.3, 4.6, 3.3.
3. lambda digested with Hind III (Murray and Murray, 1975): 22.3 kb, 9.3, 6.4, 4.3, 2.3, 2.0, 0.47,. 0.09.
4. pMB9 digested with Hae III (Sim Gek Kee and Tom Maniatis, personal communication): 800 bp, 610, 550, 500, 420, 400, 240, 220 200, 175, 115, 100, 85, 80, 60, 52, 48, 25.

In addition bromophenol blue may be used as a crude standard. In TBE it migrates at 800-1000 bp on 1% gels and at 50-100 base pairs on 2.0% gels.

References: 1. Thomas and Davis. (1975). J. Mol. Biol. 91:315.
2. Murray and Murray. (1975). J. Mol. Biol. 98:551.