PROTOCOL FOR AGAROSE GEL ELECTROPHORESIS

1. Prepare gel solution as outlined under Recipes section.

2. Pour the gel when the agarose has cooled to about 55˚ C. Insert the proper comb for the particular gel rig. The gel should be allowed to cool until it has set (it will turn whitish and opaque when ready). The amount of agarose depends on the size of the gel rig. Gels should be fairly thin, approximately 1/4 to 1/2 inch.

3. Carefully remove the comb and place the gel in the gel rig with the wells closest to the cathode (black) end. Cover the gel with 1X TAE running buffer.

4. Cut a piece of parafilm and place a 5 µl drop of glycerol loading dye onto the waxy side for each sample to be loaded.

5. Keeping samples on ice, pipette up 5 µl of a sample, wipe the excess oil from the pipette tip with a Kimwipe and add the sample to one of the drops of loading dye.

6. Switch the pipette tip to another pipette set for 10 µl. Mix the sample and loading dye by filling and emptying the pipette a few times then load the mixture into a well.

7. Continue loading the rest of the samples, placing 5 µl of 1 Kb ladder at both ends of the series of samples and between every 10 samples.

8. Place the cover on the gel rig and run the samples towards the anode (red) end. For a small gel, we set the power pack to about 60 ma. For a large gel, we use about 120 ma. Milliamperage increases during the run, so check it periodically. Stop the run before the bromophenol blue loading dye front exits the gel.

9. Turn off the power pack, remove the gel and place it in a stain box with 40 µl ethidium bromide: 200 ml 1X TAE for approximately 45 minutes. NOTE: Ethidium bromide is light sensitive and must be stored in darkness.

10. Visualize with U.V. light (take proper precautions!) and photograph with a polaroid Photo documentation camera.

11. Dispose of the gel properly. (check to see how your facility handles disposal of ethidium bromide).

RECIPES SECTION FOR AGAROSE GEL ELECTROPHORESIS

A. 1.4% Agarose gel:

nanopure water.....	392 ml
50X TAE buffer.....	8 ml
electrophoresis grade agarose...	5.5 g

Heat to boiling.

Excess gel can be stored at room temperature and remelted for future use. To expedite gel runs, we make 1.6 l and divide into 200 ml aliquots in covered, 500 ml erlenmeyer flasks. These can quickly be remelted in a microwave oven as needed.

B. 0.5 M EDTA (pH 8.0):

mw= 336.2 g (anhydrous), 354.2 g (H_20), 372.24 g (2 H_20)

For 500 ml, start with approximately 450 ml water and adjust pH initially with NaOH pellets. Autoclave.

C. 50X TAE buffer:

Tris-borate.....	242 g
glacial acetic acid.....	57 ml
0.5 M EDTA, pH 8.0...	100 ml

Dilute to 1 L with nanopure water

D. Running Buffer (1X TAE):

Dilute 50X TAE to 1X and use the amount appropriate for the particular gel apparatus.

E. 1 Kb Ladder (1 µg/ml):

ladder stock.....	5 µl
1 M NaCl.....	44 µl
glycerol loading dye (6X)..	7.5 µl

We use Life Technologies 1 Kb Plus DNA Ladder.

F. Glycerol Loading Dye (6X):

bromophenol blue...	0.26 g
glycerol.....	30 ml

To 100 ml with nanopure water

G. 1 M NaCl

mw= 58.44 g/liter nanopure water

H. Ethidium Bromide:

10 mg/ml, store in a dark bottle at 4˚ C.

CAUTION! Ethidium Bromide is carcinogenic, so wear proper protection when handling.