Agarose gel electrophoresis

1. Determine the suitable agarose percentage for electrophoretic analysis of your samples.
   a. use 0.7% for 1,000 to 10,000 bp fragments
   b. use 1% for 500 to 5,000 bp fragments
   c. use 2% for 100 to 1,000 bp fragments
   d. use polyacrylamide for fragments less than 100 bp

2. Weigh out a suitable amount of agarose
   a. Usually we use the mini-gels which take 30 mls of solution. For a 1% gel mix 0.3g agarose with 30 mls buffer, 2% = 0.6 g agarose with 30 mls of buffer, etc
   b. For most purposes use 0.5 x TBE (Tris-Boric Acid EDTA). However, if you are planning on extracting DNA from the gel slice 1 x TAE (Tris-Acetic Acid EDTA) is a better choice.
   c. Boil the solution in a microwave until the agarose is completely dissolved. This usually requires continuing to boil for 15-20" after the solution first comes to a boil.
   d. Cool to about 120˚ Fahrenheit, then pour into a gel tray with the gets raised (or sealed in another way.
   e. Add a comb with one more tooth than the number of samples you plant to run, since you need an extra well for the molecular weight marker.
   f. Allow the gel to harden (~10 min.).
   g. Remove the combs from the gel, being careful not to tear the wells. Release the "gates" from the gel bed and place it into the electrophoresis chamber. Add the same electrophoresis buffer used to make the gel to the chamber until it covers the gel completely.

3. Add 4 microliters of loading dye to each sample, if your total is 20 μl. Adjust volume up or down if you have more or less sample. The loading dye contains two tracking dyes that help us to load and follow the progress of the samples, and the fluorescent dye EZ-Vision to enable us to see and photograph the DNA.

4. Load 10 μl of sample into each well. Load 10 μl of a suitable molecular weight marker (with markers larger and smaller than the expected size range) into one of the outside lanes. Save your left-overs in case the first batch messes up.

5. Attach the electrophoresis chamber to the power supply. Start at 80 volts, then after 5 minutes increase to 120 volts. Electrophorese until the dark blue dye band is about 2/3 of the way down the gel.

6. After electrophoresis is completed, turn off the power supply and disconnect the chamber.

7. Photograph the gel using the gel documentation camera and the green filter. Usually 0.1 seconds and f1.2 are a good place to start, but increase exposure time if too dim and increase f stop if too bright.