Agarose Gel Electrophoresis (for nucleic acids)

0.5X TBE buffer (Tris-Borate-EDTA)

54 g Tris Base
27.5 g Boric Acid
20 ml 0.5 M EDTA
-- dissolve the above in about 2 L of dH_2O
-- fill to **10 L** with dH_2O (mix this in a 10 L plastic carboy)

6X Loading Dye (without bromophenol blue)

Volume	*Reagent*	*Final Concentration*
6 mL	Glycerol	60 %
1.2 mL	0.5 M EDTA solution	60 mM
0.009 g (approx.)	Xylene cyanol (just a dye)	0.09 % (w/v)
2.8 mL	ddH_2O	

-- Mix the above reagents.
-- Transfer 1 mL aliquots in to microfuge tubes and store them in the fridge or freezer

Agarose Gels

		Amount of Agarose*		
		Gel Percentage		
	Volume of 0.5 X TBE	*0.8%*	*1.0%*	*1.2%*
Big Gel	100 mL	0.8 g	1.0 g	1.2 g
Small Gel	50 mL	0.4 g	0.5 g	0.6 g

*NOTE that agarose is NOT the same as agar.

1. Prepare gel casting frame with desired comb.

2. Suspend agarose in the appropriate amount of TBE in an Erlenmyer flask.

3. Microwave on HIGH for 50-70 seconds to dissolve agarose. Watch for boil-overs—they are messy.

4. Swirl solution to make sure agarose is completely dissolve, then gently swirl the flask under a stream of cold water for 60 seconds to cool the gel down to about 50-60 degrees.

5. Pour gel in to casting frame. Allow 1 hour for gel to solidfy.

Running the Gel

1. Dilute loading dye in to your DNA samples (ie, 2 µL of dye in 10 µL sample)

2. Fill the gel tank with fresh 0.5X TBE (using old TBE can make your gel run funny).

3. Submerse the cooled gel completely in the tank. Add more TBE if necessary.

4. Load samples, including the ladder

5. place lid on the gel, and connect power leads. Take note of polarities (red-to-red, black-to-black), and REMEMBER THAT DNA RUNS FROM BLACK TO RED (NEGATIVE TO POSITIVE).

6. Run the gel at less than 110V for as long as you need to (about 1 hour).

Staining with Ethidium Bromide

NOTE: Please do not add ethidium bromide to your gel before pouring it. This is a bad way to stain you gel because it A) gives you a crappy looking gel afterward and B) uses tons and tons of ethidium bromide, which is a nasty chemical.

1. Wear gloves—ethidium bromide is carcinogenic.

2. In one of our Tupperware containers, add **200 mL TBE** and then add **10 µL of a 1% ethidium bromide** solution. (Note that we buy ethidium bromide pre-diluted to 1%). *This staining solution is good for a least a week.*

3. Place gel in staining solution. Rock gently for 20-30 minutes.

4. Remove gel from staining solution, place in second Tupperware container, rinse briefly with water, then visualize and photograph gel on the UV light box.

REMEMBER THAT ETHIDIUM BROMIDE IS BAD. Keep it contained, use as little as possible.