DNA GEL

Procedure:

1. Need ~1 L of 1X TAE buffer for the gel and for the running buffer. Maybe on shelf in 5S-19. If not, prepare a liter by taking the 50X stock (4°) and diluting it:
    - 20mL of 50X TAE, added to
    - 980mL of dH2O

2. Prepare a 1% Agarose medium gel by mixing:
    - 100mL of prepared 1X TAE
    - 1 gram of Agarose

3. Bring this mixture to a boil in the microwave (60-90 seconds)
4. Place on the stirrer and let stir until cool to the touch
5. Tape up open ends of horizontal gel casting tray. Place desired size comb (10 lane is typical) in tray
6. Add 5ul of Ethidium Bromide solution (1:10 dilution of stock [10 mg/ml]) to the agarose solution
7. Swirl and gently pour into casting tray, removing any bubbles
8. Let sit for an hour or until solidified. Remove tape from sides of casting tray and place in electrophoresis chamber. Fill electrophoresis tank with 1x TAE buffer until it covers the gel.
9. Prepare DNA Samples in 1.5 ml vials:
    - For plasmids: use 500 ng (0.5 ug) of DNA for each well. (Divide 0.5 ug by known DNA concentration ug/ul to determine volume needed). QS to 10 ul with 5x LB **WITH dye** and dH2O (see example table below)
    - For PCR products: may use entire volume or part of it. See PCR Protocol for details. Do not use dye.
    - Include an 8 ul aliquot of Promega DNA ladder + 2 ul of Promega 6x loading buffer (<u>not</u> same LB used for your DNA samples)

10. Each sample must have 5X Loading Buffer (in the freezer, small red box). Add dH2O as needed. The dH2O is a value that changes depending on the volume of DNA needed. The following examples are the amounts for each sample:

	DNA Ladder	**DNA #1**	**DNA #2**	**DNA #3**
Conc.	n/a	1.5ug/ul	.30ug/ul	.68ug/ul
ul of DNA	8ul	.333ul	1.66ul	.74ul
ul of 5X LB	2ul*****	2ul	2ul	2ul
ul of dH2O	n/a	7.7ul	6.34ul	7.26ul

*use Promega 6x LB for ladder

11. Load each sample into the wells, leaving wells on the outside empty (if possible)
12. Run at 100-125V for ~1.5 hrs (remember DNA **runs to red** elec. lead)
13. Take a photo of gel w/ Dr. Shewchuk's camera equipment