**Gel Electrophoresis**
Last updated: December 10, 2010

Description: A gel is used to visualize the size of a DNA fragment. By cutting the band out and purifying it, we can also isolate a particular piece of DNA.

Total time: 1.5hr Lab time: 30min Wait time: 45min

1. Place dams and combs in gel in appropriate places. Dams should be on either end, and comb should be near top (and another one halfway, if two sets are desired). Make sure to use the appropriately sized well for your particular experiment (larger wells hold 20ul, small wells hold 10ul). Make sure combs are straight.
2. Gel preparation:
   Prepare 50ml 1.0% agarose. (Note: low % agarose gives better separation for large fragments (more dilute – more 'holes'); high % agarose gives better separation for small fragments)
   a. Wear gloves
   b. Weigh 0.5g agarose (100% = 1g/ml)
   c. Measure 50ml TAE buffer in 50ml graduated cylinder
   d. Dissolve agarose in buffer by microwave for 30sec intervals with GENTLE swirling. Repeat until boiling. Be careful! **AGAROSE SOLUTION WILL BE VERY HOT.** Use heat gloves.
   e. Once completely dissolved, use a glass pipette and squirt some agarose solution in the crevices of the dam. This will coat the edges of the gel to prevent leaks.
   f. Again, position the combs correctly and make sure they are straight. Do not place comb at the very top – leave some space (~1cm).
   g. Wait for agarose to cool until it is not scorching to touch.
   h. Use the special pipette (labeled) to add ~5ul ethidium bromide (EtBr) into the agarose solution.
   i. Once cool, pour agarose into gel. If there are bubbles in the gel, poke them out with a glass pipette.
   j. Allow agarose to solidify. Should take ~20mins
3. Loading prep
   a. For each tube of DNA, add **2-3ul of gel juice** (6x loading dye) – for seeing gel run
4. Once agarose has solidified, fill outside of gel with TAE buffer until gel is submerged in buffer.
5. Load **5-10ul (1 kb)** ladder in the first lane.
6. Load each well with of gel-ready DNA (10ul in small well, 20ul in large well). Make sure not to introduce bubbles into the well.
7. After each desired lane is loaded, hook up the cables. "DNA runs to red" (toward the positive end) – so hook up black wire at top, and red wire at bottom (gel runs from top to bottom).
8. Normal settings: 70-80V, 45min.