# Protocols for bacterial growth measurement

**Measuring Cell Growth**

This experiment will take about 3 hours.

Goals:
- Measure differences between the strains at differing levels of antibiotics.
- Determine the correspondence between $OD_{600}$ and cell density.

*Prior to your involvement:*
1. LB media was made with several amounts of Kanamycin: 0 μl, 3μl, 6μl, 9 μl, 12μl, 15 μl, 18μl, and 21μl.  Groups will be assigned a particular media for the class.

2. 100ml of each of these media was inoculated with .5 mL of saturated *E. coli* cultures and incubated on a shaker at 37°C 1.5 hours prior to the start of the class. This ensures that the so-called 'lag phase' has past, and that exponential growth has begun.  We will be using two strains, one carrying the T89 plasmid and one with the same plasmid chromosomally integrated: MG1655+T89(no fluo) and MG1655::T89(no fluo), respectively.

*Procedure:*
1. Every 5 – 10 minutes remove a .5 mL sample of your culture using a sterile pipette. Deposit this into a 1 ml cuvette and immediately bring to the spectrophotometer (the 'spec'). Try to minimize the time the incubator is open to keep the cultures at precisely 37°C. Don't forget to note the absolute time of day in your notebook (to compare to the time of the inoculation) as well as the time on the class stopwatch.

2. Carefully insert the cuvette into the spec in the correct orientation. Make sure the spec is set to read $OD_{600}$, and measure the absorbance, and record the exact time.
You should have been given a 'blank', i.e. a sealed cuvette with sterile media. Absorbance is always measured relative to the blank. Be sure to measure the absorbance of the blank with every culture measurement!

3. Properly dispose of the sample cuvette, and repeat these steps for the next ~3 hours.

4. At $OD_{600} \approx 0.1$ or your first measurement and at the half-way point of the class, measure the exact OD, take 100 μl and put it into labeled eppendorf.
    a. For the moment, let us assume $OD_{600}$ 0.1 = $10^8$ cells/ml. With that in mind, properly dilute your sample with LB so that 1mL contains $10^2, 10^3, 10^4$ cells ($10^2, 10^3, 10^4$ cells/ml). This will require serial dilution – try to be accurate in your pipetting!
    b. On three different LB plates, evenly spread 100μl of each dilution with beads, trying not to get too much on the lid of the dish.  Make sure the plates are dry before incubating them (leave them slightly open until they dry up).

c. Incubate the plates upside-down overnight at 37°C. Later, you will count colonies and determine the exact correspondence between $OD_{600}$ and cell density.

**Homework**

1. Plot the class growth curves on a log scale.
2. Identify the exponential growth periods and extract doubling times by linear regression. Do your values make sense? What can you infer about the metabolic costs of carrying a plasmid versus a chromosomally integrated gene?
3. Hypothesize about what the curve might look like (at three concentrations of antibiotic) for plasmid and chromosomally integrated genes that also included fluorescence, i.e. included and integrated T89 with fluorescence.
4. Plot standard curve ($OD_{600}$ vs. cell count) using the plates you made. Is it linear? Are there errors? Explain.
5. Update your webpage.