Protocols for bacterial growth measurement

Measuring Cell Growth

This experiment will take ~3 hours.

Goals:
- Measure diauxic growth with carbon source shift – bacteria growing in a mixture of glucose and another sugar such as lactose.
- Determine the correspondence between OD_{600} and cell density.

Prior to your involvement:
1. M9 minimal media was made with a mixture of two carbon sources: glucose at 0.1 g/L and some other sugar (lactose/maltose/sorbitol) at 0.1 g/L. Groups will be assigned a particular mixture.

2. 100ml of each of these media was inoculated with 1mL of saturated wild type (MG1665) *E. coli* cultures and incubated on a shaker at 37C a few hours prior to the start of the class. This ensures that the so-called 'lag phase' has past, and that exponential growth has begun.

Procedure:
1. Every 5 – 10 minutes remove a 1mL 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 37degC. Don't forget to note the absolute time of day in your notebook (to compare to the time of the inoculation).

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 clock 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. The shift is expected to occur at an OD_{600} of ~0.25, so you may want to take more time points around this OD.

4. Every half hour or so, in addition to measuring the OD, take 100 ul from the culture and put it in 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 the 100 ul with beads or a sterile spreader. 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 37C. The next morning we will count colonies and determine the exact correspondence between OD_{600} and cell density.

Homework

1. Plot growth curve on a log scale. Identify all growth phases as discussed in class. Analyze your results in light of the class discussion on catabolite repression.
2. Identify the exponential growth periods and extract doubling times by linear regression. Do your values make sense?
3. Plot standard curve (OD_{600} vs. cell count). Is it linear? Are there errors? Explain.
4. Update your webpage.