**Bio111 Week 11**

Before you come to lab

1) Read about isocitrate dehydrogenase (IDH) from an online source. What does it do? Where do you find the protein? How many different IDH genes do humans have? Where are the genes located?

2) Answer each of these four questions in two sentences or less.

A) What does a spectrophotometer do? (homeostasis)

B) What wavelength is maximally absorbed by NADPH? (homeostasis)

C) What is the substrate for IDH? What is the product? What are two the closely related coenzymes for IDH? What is the biological cofactor for IDH? (homeostasis)

D) Draw a graph showing the production of product for a typical enzyme reaction. (homeostasis)

 product

 production

 time

**Week 11 (Mo-vember 3th)**

Homeostasis Lab

1) You need to perform a standard enzyme reaction with the appropriate controls. The total volume of each well containing an experiment (on a 96-well plate) MUST be 200 µL. The volume of prepared enzyme should be 20 µL. The volume of substrate and coenzyme should be 10 µL each. On a blank sheet of paper, prepare a table so that you can perform each reaction condition in triplicate. You want one reaction condition to contain each reagent, and then you will need reaction conditions that demonstrate each reagent is required for the IDH enzyme to function. You will allow the reaction to run for 3 minutes while quantifying product formation every 30 seconds. Start the reaction by adding substrate last and only do this seconds before starting the spectrophotometer.

2) Once you have performed the experiment above, now set up a series of reaction conditions (each in triplicate) that determine the effect of changing enzyme concentration. Remember the final volume in each well must be exactly 200 µL.

You will analyze your data next week using Excel, so all you need at this time are the raw numbers.

Evolution In Lab

3) Analyze your results from the last evolution experiment and perform your final experiment. We have grown each frozen stock of bacteria in LB media without antibiotic. You will want to compare the evolved strain of bacteria with the original strain of bacteria. How well does each stain (evolved vs. original) grow in the presence of all five antibiotic disks? Remember to use the negative control disk too.