Bi 1X, Spring 2012

Assignment 1
There are three parts to this assignment. Complete all three.

Part 1: Growth curves

1. Briefly describe the experimental set up. What is the control in this experiment? Why did we normalize the amount of antibiotics added to be the same mole number? Do you think this method was best, or could you think of something better? (1-2 paragraphs)

2. How do Kanamycin, Ampicillin, and Rifampicin work? (1 or 2 sentences each)

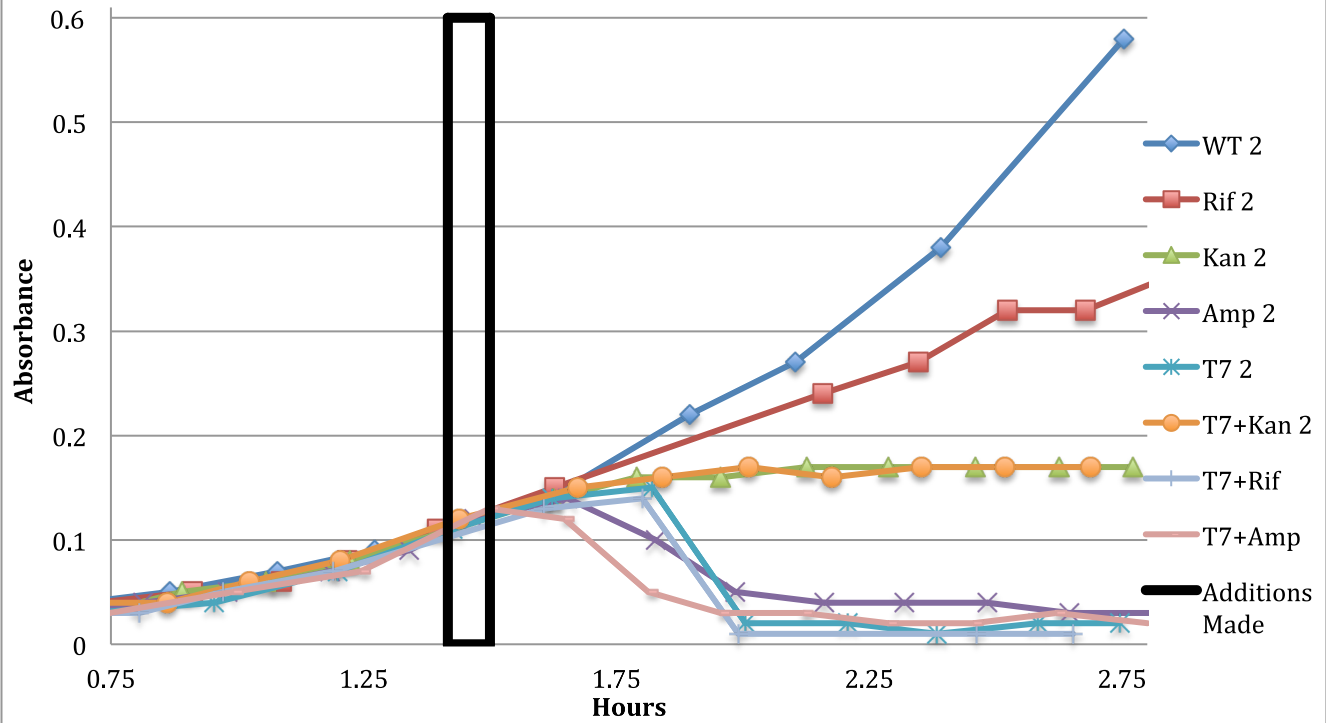
3. T7 is a bacteriophage. Describe briefly the T7 life cycle. It may be hard to find specific information on T7 itself, so it is ok to describe the general bacteriophage strategy. What host machinery is necessary for phage replication?(~1 paragraph)

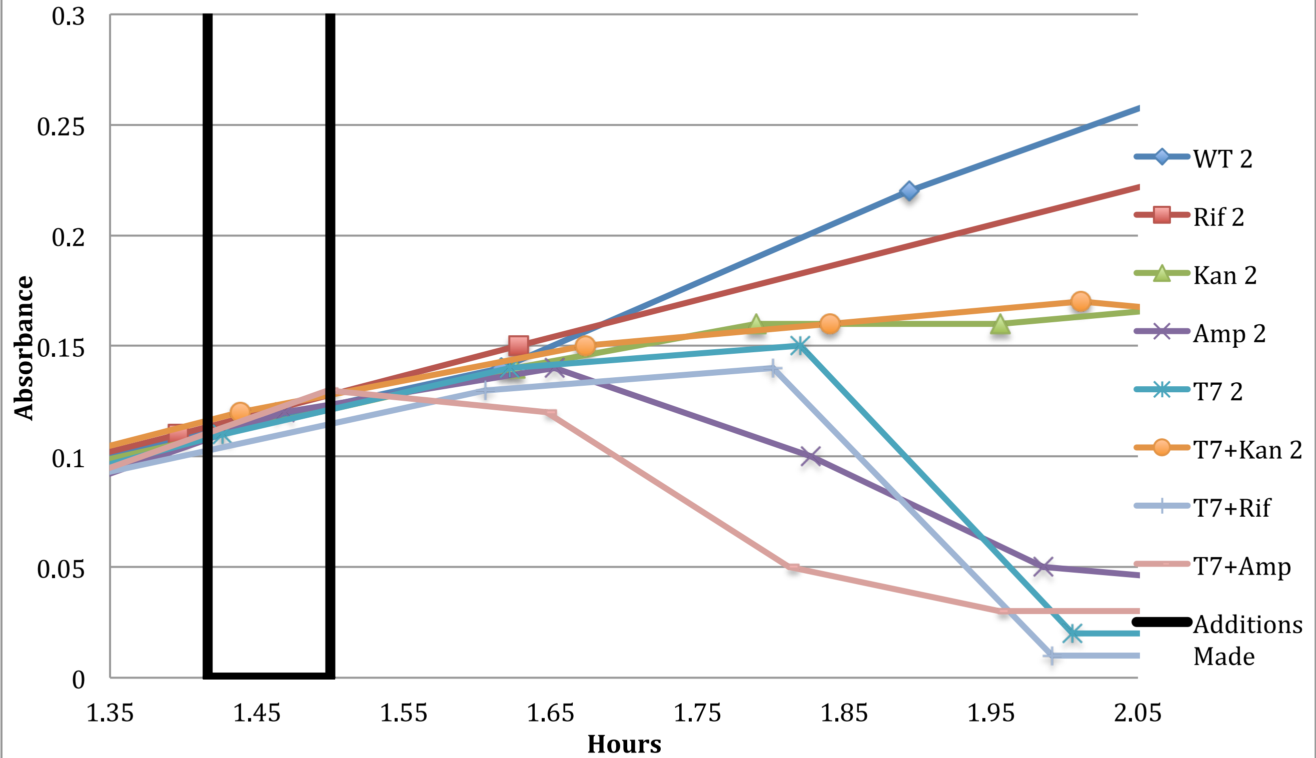
4. Look at the growth curves in the first plot below. The black box shows the time range in which the various perturbations were made. Excluding the possibility that Rif and Kan just are not as potent as Amp, come up with an explanation for the fate of these three conditions as compared to each other and wild type. How does their mode of action in the bacteria explain the curves? (Hint: It is very important to consider what it means for absorbance to drop off vs. plateau). (1-2 paragraphs)

5. Look at the second zoomed in plot, and compare Amp to T7. T7 has the steeper drop off, but there seems to be a lag before the *E. coli* population starts to fall. Why do you think there is a lag with T7 as compared to Amp? (1 paragraph)

6. Compare the Amp, T7 and Amp+T7 curves. Discuss the result of using Amp and T7 together. Does this make sense in the light of their mode of action? (1 paragraph)

7. Compare the Kan, T7 and Kan+T7 curves. Discuss the result of using Kan and T7 together. Does this make sense in the light of their mode of action? (1 paragraph)


E. coli response to various insults
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0.75
1.25
1.75
2.25
2.75
Hours
WT 2
Rif 2
Kan 2
Amp 2
T7 2
T7+Kan 2
T7+Rif
T7+Amp
Additions Made


E. coli response to various insults
Absorbance
0.3
0.25
0.2
0.15
0.1
0.05
0
1.35
1.45
1.55
1.65
1.75
1.85
1.95
2.05
Hours
WT 2
Rif 2
Kan 2
Amp 2
T7 2
T7+Kan 2
T7+Rif
T7+Amp
Additions Made

Comment on bacteria, antibiotic resistance, bacteriophages and evolution. You will get no credit for reading this and you wont be tested on it.

Most bacteria pose no threat to people. Their physiologies are based on metabolisms that will not function inside the human body. There are a few bacteria however that do cause disease, either by design or by accident (meaning that some bacteria are obligate parasites, and others are not, but will grow inside humans if they find themselves there via a wound, etc.). It was not until the early 20th century that medicine began making serious strides towards combating bacterial infection with the discovery of antibiotics. The vast majority of antibiotics are natural compounds, produced by fungi or other bacteria for the express purpose of fending off competing bacteria.

The existence of natural antibiotics should come as no surprise. They are the result of organisms co-evolving with one another, famously summarized by Van Valen in 1973 by his Red Queen's Hypothesis, inspired by the line in Carroll's *Through the Looking Glass*: "Now here, you see, it takes all the running you can do, to keep in the same place." In biological terms this can be formulated as "for an evolutionary system, continuing development is needed just in order to maintain its fitness relative to the systems it is co-evolving with"(Wikipedia).

As an example consider a system of a bacteria and a fungus who are competing for the same resources. Evolution will drive each species to become more "fit", one measure of fitness being the ability to keep the other species out of the common resources. As time goes on the fungus will make new antibiotics, and the bacteria will evolve new mechanisms of resistance. They both will constantly be innovating, but in general neither species will ever "win".

Upon discovering natural antibiotics we were quite pleased, and began mass production of a few classes of compounds. We became more and more liberal with our application of antibiotics to everything, including pumping them into livestock to prevent infections in animals kept in truly unnatural conditions. This strategy has started to fail, and we are seeing the rise of multiple-drug resistant bacteria. So what went wrong?

Ultimately the problem is that we decoupled antibiotics from the evolutionary process. While we expose billions upon trillions of bacteria each year to our full arsenal of antibiotics, the antibiotic producers, various strains of yeast in gigantic industrial fermenters, are shielded from competition with the bacteria harboring antibiotic resistance, and have no impetus to develop novel antibiotics. We have tried to make up for this lack of natural innovation by artificially evolving antibiotics, making our own designer modifications to the naturally occurring antibiotics, but most of the evidence seems to be pointing towards us losing the race. We cannot artificially produce and screen enough compounds to combat the combined evolutionary power of bacteria.

It is not yet clear what the solution to this problem will be, but one increasingly attractive option is one that was demonstrated in the lab you just completed, that is, killing bacteria with bacteriophages. Phage therapy, as this technique is called, was championed in the early 20th century in countries of the former Soviet Union, and has been used to successfully treat infections in humans. Currently one of the only countries where this treatment is authorized is Georgia.

Phages offer a number of advantages over antibiotics including increased specificity (wont hurt non-targeted bacteria), built in evolution system (they've got a genome after

all) and virtually no cross-reactivity against human cells. After treating bacteria for a while with one phage they would certainly evolve some resistance, but you could imagine a process where you grow billions and billions of phages in a batch culture then screen them against cultures of the resistant bacteria. After enough iterations of this process you would probably isolate a phage that had a mutation to avoid the resistance, and we would recouple our anti-bacterial medicine to the evolutionary process and rejoin the race to stay in the same place.

Part 2: Matlab from week 1

1. Graticule: For all three magnifications of the graticule that you took images of turn in the image of the graticule, the plot of the pixel intensities along the line you used to calibrate the micron/pixel ratio (you should all have different plots!) and give the value of that ratio.
2. Take your images of pre-stained fluorescent slides and make an RGB photo out of them. Make sure you have added a scale bar using the ratio determined from the graticule at that magnification.

Part 3: Matlab from week 2

1. Using segmentation and masking which we will discuss in detail Monday night, get the intensity of the YFP signal from each of the photobleaching images you took. You know the time between images, so plot the intensity as a function of time. Fit this curve with an exponential and determine the half life of the YFP fluorophore under photobleaching conditions.
2. Using segmentation and masking calculate the area of cells in your frame, and plot the area vs. time. Fit the growth with an exponential curve and determine the doubling time of your bacteria. Compare this doubling time to an estimation of the doubling time in our spectrophotometric experiments.