You may use the textbook, your class notes and lab notebook, the course web site, or any other source of information to answer these questions. You are allowed work together with other students on these answers, or ask for help or information of any kind from anyone else. The only stipulation is that you MUST physically write (or type) your answers yourself, in your own words.

Name : _____ Date : _____

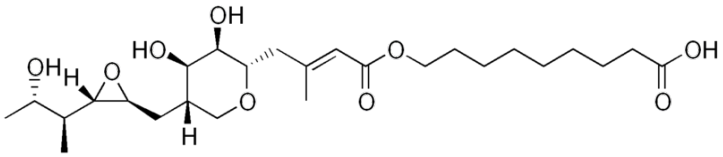
Experiment 1:

Pseudomonas fluorescens produces the antibiotic mupirocin, an analog of isoleucine. *P. fluorescens* is resistant to mupirocin; otherwise it couldn't produce it without killing itself. Another isoleucine analog antibiotic is furanomycin, produced by *Streptomyces threomyceticus*. Because *P. fluorescens* is resistant to one isoleucine analog, you suspect that it might also be resistant to the other. **Design a quick-and-dirty experiment to compare the resistance to mupirocin and furanomycin in *P. fluorescens*.**

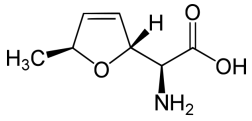
Be sure to describe how you would make stock solutions, dilutions, and how you would set up each growth curve sample. Be sure to show the concentration of antibiotic (in molarity) in each well of the plate. Assuming these antibiotics inhibit both growth rate and maximal growth, how would you interpret your results (in other words, how would you get an answer from your results)?

Additional information:

- The formula weights of the sodium salts of these antibiotics are 522.6 for mupirocin and 179.2 for furanomycin. Standard stock solutions are 20mM (11.4mg/ml) muprocin and 50mM (8.96mg/ml) furanomycin; these antibiotics are soluble at these concentrations in water. Neither can be autoclaved.
- *P. fluorescens* has been reported to be resistant to mupirocin to >10mM (Minimum Inhibitory Concentration, MIC; the concentration required to stop growth completely). *E. coli* is sensitive to 200µM mupirocin (MIC) and 2µM furanomycin (MIC).



Mupirocin (a.k.a. pseudomonic acid, Bactroban)



Furanomycin

Experiment 2:

In your preliminary experiment (above), your hypothesis is proven wrong; *P. fluorescens* is apparently sensitive to furanomycin. It grew poorly, only ~20% of normal growth at 5µM furanomycin. **Describe how you would more carefully test the sensitivity of *P. fluorescens* to furanomycin.** Make it a convincing experiment, with appropriate controls and replicates; it's going to to be included in a published paper! There's no need to repeat anything you've already described in Experiment 1, such as stock solution preparations, how each well is mixed, serial dilutions, &c; just show what would be in each well. **Then be sure to show how you would present and interpret your results in tables and/or graphs.**

DON'T PANIC! Start with a basic, simple experiment, then add the controls, comparisons, replicates, and any other complexity you need to make the results convincing. What is the question? What do you want to measure? What controls do you need to compare this measurement to in order to understand what it means?

(NOTE : This scenario is realistic. Mupirocin works by inhibiting isoleucyl-tRNA synthetase (it actually mimics the Ile-tRNA junction), so the organisms can't charge isoleucine onto tRNA for protein synthesis, and so protein synthesis stops. Furanomycin works by actually being mistaken for isoleucine and is charged onto tRNAs, then is incorporated into proteins in place of isoleucine. Protein synthesis continues, but generates defective proteins. So although they are both isoleucine analogs, their structures and mechanisms of action are quite different, and therefore their mechanisms of resistance are distinct.)