Final Exam

You can use any source of information to help answer these questions. You can ask for general help or information from anyone to help answer these answers, but you are not allowed to work out the specific answers with other students or anyone else.

“I have neither given nor received unauthorized aid on this test.”

Date: ______________________________________

Signed: ____________________________________________ Name: ____________________________________________

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 (mupirocin), you suspect that it might also be resistant to the other (furanomycin). **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 each well of the plate. Assuming these antibiotics inhibit both growth rate and maximal growth, how would you plot your results? What would your results look like if your hypothesis is correct? What if it is not correct?

Additional information:

- Both mupirocin and furanomycin are soluble (as sodium salts, which is how you buy them) to at least 10mg/ml in water. The formulas weights of these salts are 522.6 for mupirocin and 179.2 for furanomycin. Neither can be autoclaved.
- *P. fluorescens* has been reported to be resistant to mupirocin to >6000 µg/ml (Minimum Inhibitory Concentration, MIC; the concentration required to stop growth completely). *E. coli* is sensitive to 128µg/ml mupirocin (MIC), and 4µg/ml furanomycin (MIC).

![Mupirocin](image1.png)  ![Furanomycin](image2.png)

*Mupirocin* (a.k.a. pseudomonac 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 ~25% of normal growth at 1µg/ml (~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 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, 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.)