Micro 201 Dove and Ravel Problem Set
April 24th 2018
Answers should be typed and sent no later than 5 pm on May 1st to:
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1. Provide three examples of second messengers in bacterial cells. Describe how these small molecules exert their regulatory effects.

(Your answer to question 1 should be no more than a page, single line spacing.)

2. You are studying a genetically tractable bacterium that contains an ortholog of the RNA-binding protein Hfq.

A. Describe at least two distinct ways in which you would attempt to identify small regulatory RNAs from this organism. Discuss the potential benefits and limitations of the approaches you describe.

B. Describe how you would identify the regulatory targets (both direct and indirect) of any small RNA you might identify. Include important controls.

C. One of the small RNAs you identify appears to influence the expression of ten different genes. Design an experiment to test whether any of the corresponding transcripts are direct regulatory targets of the small RNA. Include important controls.

D. You find that one small RNA in particular influences the expression of a large number of genes (several hundred). Describe two possible ways through which this RNA might exert its effects. Provide examples to illustrate your answer.

(Your answers to question 2 should total no more than 2 pages, single line spacing.)

3. You are studying a genetically tractable bacterium that contains an ortholog of RppH (RNA pyrophosphohydrolase) from E. coli. You find that despite repeated attempts you are unable to delete the corresponding gene from your bacterium.

A. How would you rigorously test whether or not the RppH ortholog is essential in your bacterium?

B. You find that the ortholog of RppH is indeed essential in your organism. How might you test whether the essential function of this protein is to remove pyrophosphate from the 5' end of transcripts in your organism?

(Your answers to question 3 should total no more than 2 pages.)

4. Madeupfor classia is a recently-discovered bacterial species that can cause respiratory infections in immunocompromised patients. Antibiotic resistant strains have already been observed, and your lab just got a grant to study this bug.

A. You are excited about the unfolded protein response and first want to identify disaggregases in M. classia. How would you find disaggregases? Detail the technique(s) used to find candidate
proteins AND to validate that they have disaggregate activity. Include the strains required, necessary controls, and expected results.

B. In a follow up to the study that we read for class, Yuan et al (“A bacterial global regulator forms a prion,” Science, Jan 2017) identified a prion-forming transcription terminator in bacteria. They suggest that such prions could increase fitness under specific conditions by increasing epigenetic diversity. *Madeupfor classia* is predicted to encode a protein with a prion-forming domain, so, naturally, you are interested in whether prion-forming proteins could contribute to phenotypic drug resistance in *Madeupfor classia*. Describe an experiment to test this hypothesis. Include strains, controls, and expected results.

(Your answers to question 4 should total no more than a page.)