Systems Microbiology 1.084J/20.106J PROBLEM SET #3 – Due Monday Oct. 23rd

Problem 3.1

a. Describe the stringent response, and at what level and what components of cellular activity it regulates.
b. Most biosynthetic operons need only be under negative control for effective regulation, while most catabolic operons need to be under both negative and positive control. Why might this be?
c. Why are promoters from *E. coli* under positive control not close matches to the promoter consensus sequence for *E. coli*?
d. The attenuation control of some of the pyrimidine biosynthetic pathway genes in *E. coli* actually involves coupled transcription and translation. Can you propose a mechanism whereby the cell could somehow make use of translation to help it measure the level of pyrimidine nucleotides?
e. How could quorum sensing be considered a regulatory mechanism for conserving cell resources?

Problem 3.2

a. How does homologous recombination differ from site-specific recombination?
b. What does an F^+ cell need to do before it can transfer chromosomal genes?
c. What do you think are the most useful transposons for isolating a variety of bacterial mutants? Why are such transposons so useful for this purpose?
d. Describe why the discovery and use of transformation and the use of the F plasmid, were important milestones in the history of genetics. What insights did they contribute?
e. Describe three different ways that foreign DNA can enter the cell. How can homologous recombination favor the integration of more than a single or a few genes into the chromosome?

Problem 3.4

a. Explain why in generalized transduction one always refers to a transducing particle but in specialized transduction one refers to a transducing virus or phage.
b. What are the essential characteristics of a cloning vector? What characteristics of plasmids make them especially useful as vectors for molecular cloning? What characteristic(s) of the F plasmid makes it less useful for use *in vitro*?
c. What are the general properties of insertion elements? Class II transposons?

Problem 3.5

Five Hfr strains A through E are derived from a single F^+ strain of E. coli. The following table shows the time of entry (in minutes) of the first 5 genetic markers observed in interrupted mating experiments. Please answer a-c, below, based on the data.

STRAIN A		**STRAIN B**		**STRAIN C**		**STRAIN D**		**STRAIN E**	
mal^+	1	ade^+	3	pro^+	3	pro^+	10	his^+	7
str^s	11	his^+	28	met^+	29	gal^+	16	gal^+	17
ser^+	16	gal^+	32	xyl^+	32	his^+	26	pro^+	23
ade^+	36	pro^+	44	mal^+	37	ade^+	41	met^+	49
his^+	51	met^+	70	str^s	47	ser^+	61	xyl^+	52

a) Draw a map of the F^+ plasmid, showing the positions of all the genes relative to the origin, and their distance apart in minutes.
b) Show the insertion point and orientation of each Hfr strain.
c) In using each of these strains, which gene would you chose to use as a marker, to get the greatest number of "exconjugants" (eg. recipient cells that have successfully received an F plasmid)?