Biol 322 Fall 2012 Answers to some Quiz 2 Study Sheet Questions

Problem 1

a. Use cell count from tube 3.

Overall dilution for tube 3 = $[10^{-1}]$ X $[2 \times 10^{-1}]$ X $[5 \times 10^{-1}] = 1 \times 10^{-2}$

Viable cell count/ml = $167 \times 4 \times 10^{2} = 6.7 \times 10^{4}$

b. 10 ml/10^5 = 10,000 ml/10^5 = 0.1 ul → not even a P2 would do an accurate job of measuring this small amount

Problem 2

a. Use cell count from tube #3. Viable cell count/ml = $1X10^7$

b. Use cell count from tube#2. MercuryRcell/ml = 2.2×10^5
% resistant = $2.2 \times 10^5/1X10^7 \times 100 = 2.2$

c. ***Why do you do a serial dilution rather than one single dilution?***

Serial dilutions are a more accurate way of producing a very dilute suspension of cells. Also, you typically need a series of dilutions to ensure that one of the platings give you a countable number of colonies in the 30-300 range.

Problem 3

Indicate genotype by letter (above)
_b___ Strain 1 __d__ Strain 2 _a___ Strain 3 __c__ Strain 4

Problem 4

Part a: The rpoB mutation rate is 100X that of gyrA

Part b: He is looking for very different kinds of mutations in the two genes -- loss-of-function in rpoB and a type of gain-of-function (altered function) in gyrA. Mutations causing a LoF occur with much greater frequency than mutations causing GoF

Problem 5

a. Minimal media without leucine.
b. True: most new mutations will not reverse or suppress the original mutation

Problem 6

Part a. 5×10^{-5} = 1 mutant per 20,000 cells
She should set up a minimum of 20 plates, but of course she may find no mutants in 20 plates:
For your personal enrichment: for each colony that she looks at, there is a 0.99995 (19,999/20,000) probability that it will be wildtype. If she looks at exactly 20,000 colonies, the probability that they will all be wildtype = 37% [0.99995 E 20,000]. So she may need to look at more than 20,000.
Part b. She will see a continuous lawn of E. coli because both the wild-type and mutant cells will grow. How should she have set up the experiment?

Problem 9

- Use a selection over a screen if possible:
- *Work up a clear flow chart…*

Strain A by itself: *treat culture with mk virus and select on MM + maltose (as sole carbon source) + thiamine + leucine (strain can't make the latter two compounds)*
Strain B by itself: *select on plates containing tetracycline and streptomycin (either rich media such as nutrient agar or mimimal media with lactose or glucose plus leucine)*
Recombinant: *select on minimal media with streptomycin and maltose as the sole carbon source (+ thiamine and leucine)*

Problem 10

a. Use plates from tube 3: 108+92/2 = 100 cells
100 cells/0.1ml = 1 X 10E3 cell/ml in tube 3
Viable cell count in the slurry = [1 X 10E3]/[1 X10E-4] =1 X10E7 cells/ml

b. Use plates from tube 2: 47+53/2 = 50 cells
50 cell/0.2 ml = 2.5 X10E2 resistant cells/ml in tube 2
Resistant cells per ml in slurry = [2.5 X10E2]/[1 X10E-2] = 2.5 X10E4
% resistant = 2.5 X10E4/1 X10E7 (resistant cells/total cells) X100 = 2.5 X10E-1 = 0.25%