Biol 322 Fall 2010 Answers to some Study Sheet 2 Questions

Quiz #2 is scheduled for Thursday Nov and will be worth ~ 20 points
Quiz covers
- Forward and Reverse Genetics Lecture
- Bacterial genetics I – assessing genotypes and dilution problems
- It will not cover the Deaf by Design or the articles on antibiotic resistance

Problem 2

Read the abstract below edited from a recent Science paper.

Haploid Genetic Screens in Human Cells Identify Host Factors Used by Pathogens
Jan E. Carette,1 Carla P. Guimaraes,1 Malini Varadarajan,1 Annie S. Park,1 Irene Wuethrich,7 Alzbeta Godarova,1 Maciej Kotecki,2 Brent H. Cochran,2 Eric Spooner,1 Hidde L. Ploegh,1,3 Thijn R. Brummelkamp1,*
Loss-of-function genetic screens in model organisms have elucidated numerous biological processes, but the diploid genome of mammalian cells has precluded large-scale gene disruption. We used random insertional mutagenesis to generate null alleles in a human cell line haploid for all chromosomes except chromosome 8. Using this approach, we identified host factors essential for infection with influenza by screening mutant lines for resistance to H1N1 influenza virus. This approach has both conceptual and practical parallels with genetic approaches in haploid yeast and other model organisms.

a. Is this experiment an example of forward or reverse genetics? Defend your answer in one sentence. forward

b. What is insertional mutagenesis?
As the name indicates a chunk of foreign DNA is inserted in a gene sequence causing, typically, a severe loss of function
Give one specific example from a lab exercise or reading assignment this quarter.
T-DNA mutation in aha3 gene
transposon mutagenesis in Sleepless stud
Problem 3  You want to determine the concentration of cells in an overnight culture of *E. coli* using the viable cell count method. You do the following set of serial dilutions and plate duplicates from tubes #2, #3 & #4.

a. Calculate the #cells/ml in the overnight culture. Use cell count from tube 3.

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

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

b. For various reasons, you want to make 10 ml of a \( 10^{-5} \) dilution of the original culture. Your lab partner doesn't want to do a serial dilution (because it seems like too much trouble) and suggests that the dilution from the overnight culture could be done in a single step. How many \( \mu l \) of the overnight culture do you need to mix with 10 ml to make this dilution?

\[
10 \text{ ml} / 10^{-5} = 10,000 \text{ ml} / 10^{-5} = 0.1 \text{ ul} \rightarrow \text{not even a P2 would do an accurate job of measuring this small amount}
\]

Problem 4  Over the years Georgia Pacific has dumped considerable quantities of mercury into Bellingham Bay. You are interested in determining if mercury-resistant bacteria can be isolated from the microflora of the bay, so you collect a “slurry” of bacteria from a tide pool near Boulevard Park. From the data shown below, determine the:

a. Viable cell count in the slurry. [Show your work and circle your answer. Use scientific notation. I will not count zeros or decimal points.] Use cell count from tube #3. Viable cell count/ml = \( 1 \times 10^7 \)

b. The percentage of cells that are mercury resistant. Show your work and circle your answer. Use cell count from tube #2. MercuryR cell/ml = \( 2.2 \times 10^5 \)

\[
\% \text{ resistant} = \frac{2.2 \times 10^5}{1 \times 10^7} \times 100 = 2.2\%
\]

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 5  You have four strains of *E. coli* creatively named Strain 1, strain 2 and so on. You want to establish the genotype of each strain with respect to two gene loci:

Indicate genotype by letter (above)

\[ \begin{array}{cccc}
_b & \_Strain 1 & \_d & \_Strain 2 \\
\_a & \_Strain 3 & \_c & \_Strain 4 \\
\end{array} \]