

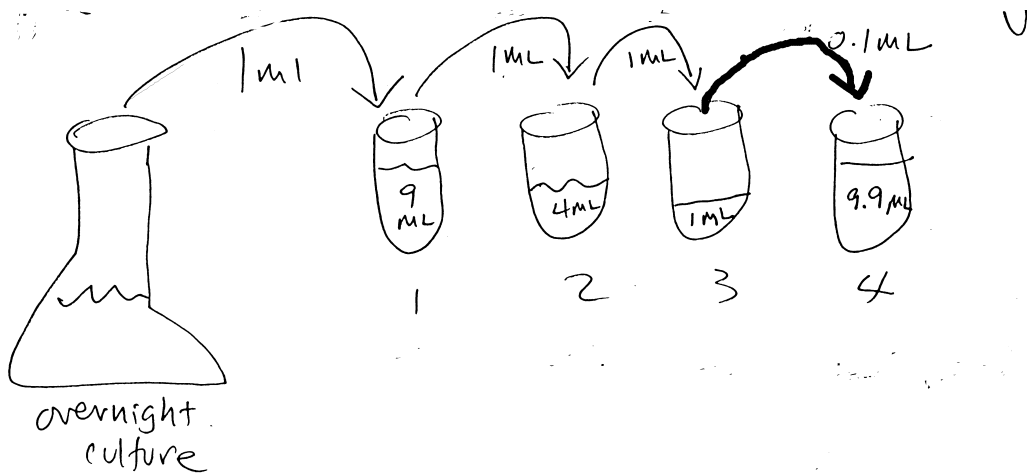
Biol 322 Fall 2012 Study Sheet for Quiz #2

Quiz #2 is scheduled for Thursday Nov 10th and will be worth 30-40 pts. This quiz will cover:

- *Mutagenesis Lab: Parts 1 & 2*
- *Bacterial Genetics: F' X F- Cross*
- *All lecture material through Tuesday Nov. 6*
- *It will not cover the Deaf by Design or complementation (we'll save this topic for the final quiz)*
- ***There will undoubtedly be a question (worth 10-12 pts) like practice problems 1, 2 & 10. You will not be allowed to use a calculator and partial credit will be stingy.***

You will be required to answer specific questions about the required reading assignments papers outside of class in a google.doc form (will be worth 10-15 pts. and due sometime after Nov 10)

☒ Problem 1 NO CALCULATOR 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.



Tube used for plating	Amt.per plate	Colony counts (duplicate results)
#2	0.25 ml	too many to count
#3	0.25 ml	161/173
#4	0.25 ml	4/1

a. Calculate the #cells/ml in the overnight culture.
problem continues on the next page

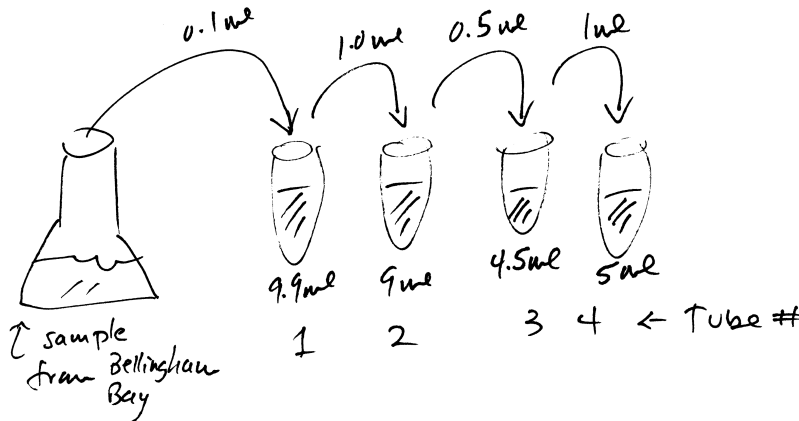
b. For various reasons, you want to make 10 ml of a 10^{-5} dilution of the original culture. Your lab partner doesn't want to do a serial dilution (because it seems like too much trouble) and

suggests that the dilution from the overnight culture could be done in a single step. How many μl of the overnight culture do you need to mix with 10 ml to make this dilution?

Problem 2 NO CALCULATOR 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.]

b. The percentage of cells that are mercury resistant. Show your work and circle your answer.

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



L plate + mercury

Tube #	volume plated	Duplicate colony counts	
2	0.25	50	60
3	0.25	4	1

L plate (rich)

	volume plated	Duplicate colony counts	
2	0.1	tmtc	tmtc
3	0.1	90	110
4	0.1	8	18

tmtc = too many to count

Problem 3 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:

lac (lactose): a carbon/energy source

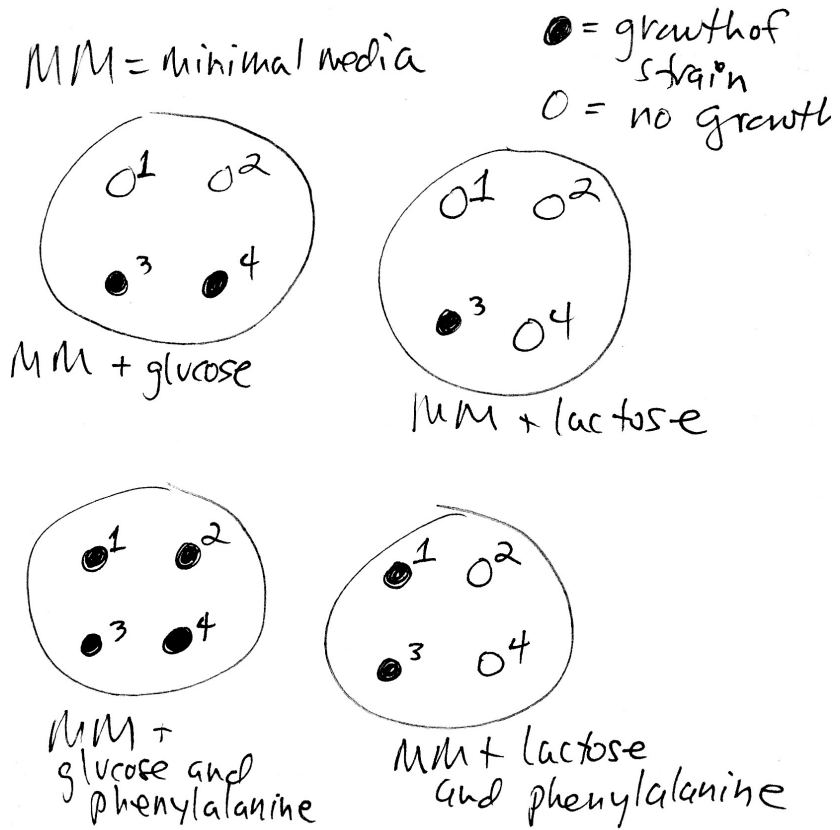
phe (phenylalanine: an amino acid)

Each strain is spotted on each of four different media. For each strain select one of the following genotypes:

- a. $lac^+ phe^+$ b. $lac^+ phe^-$ c. $lac^- phe^+$ d. $lac^- phe^-$
- e. not enough information to draw conclusion about genotype
- f. data are ambiguous for this strain

Indicate genotype by letter (above)

____ Strain 1 ____ Strain 2 ____ Strain 3 ____ Strain 4



✘ **Problem 4** A new genetics graduate student is studying mutation rates in *E. coli*. He does an experiment to determine the rate of EMS induced lethal mutations in the *rpoB* gene, which specifies RNA polymerase. The results are shown below. He also does an experiment to determine the mutation rate at another locus: *gyrA*. This gene specifies DNA gyrase, which has a critical role in DNA replication. This enzyme is the target of the antibiotic nalidixic acid.

Gene	Phenotype of mutant	Frequency (mutant cells/total cells scored)
<i>rpoB</i>	lethal	5×10^{-4}
<i>gyrA</i>	resistance to nalidixic acid	5×10^{-6}

From these results, the graduate student concludes that the *rpoB* gene must be much larger than the *gyrA* gene. In other words, he concludes that the coding region of *rpoB* gene must be 100X larger than the coding region of *gyrA* gene.

Part A What feature of the data supports his conclusion?

Part B The student discovers that the sequences of both the *rpoB* gene and the *gyrA* gene are in the GENBANK database and that the coding region of the *gyrA* gene is twice the size of the *rpoB* gene. He comes to you for help in determining why his original experiment was flawed. What do you tell him?

✘ **Problem 5** You have a *leu⁻* strain and are interested in measuring the rate of spontaneous reverse mutation. *leu* = the amino acid leucine.

a. You grow up an overnight culture of the *leu⁻* strain. To look for reverse mutations, what selective media would you use? No explanation necessary.

b. Is this sentence true or false or N? *The vast majority of new mutations in the leu gene will go undetected in your assay.*

Circle: True or False or N (not enough info).

One sentence explanation. NO credit if no explanation.

✘ **Problem 6** A friend of yours is working with the yeast *Saccharomyces cerevisiae*. Yeast cultures can be grown up and handled like bacteria cultures. In yeast, mutants in the *ade 2* gene cannot biosynthesize adenine and are pink when grown on rich media because of the intracellular accumulation of a red pigment. Wild-type *ade 2⁺* colonies are white.

a. Your friend grows up a wild-type (haploid) culture of yeast and sets up a screen to find spontaneous *ade⁻* mutants. The forward (*ade⁺* → *ade⁻*) mutation frequency of this gene is about 5×10^{-5} mutant cells/total.

Assuming that she can screen about 1000 small colonies per plate of rich media, how many plates must she set up to have find at least one *ade⁻* mutant?

b. With your help, she finds one *ade⁻* mutant. Her next experiment is to measure the frequency of spontaneous reversions of this mutation. She grows up an overnight culture in rich media and

then sets up 10 plates spread with 0.1 ml of undiluted culture. The plates are minimal medium plus adenine. After an overnight incubation, what did her plates look like?

- The density of the overnight cells culture was 1×10^9 .
- Assume a reversion frequency of 1×10^{-10} revertant cells/total

7. Your mom has read a New York Times article entitled “Germs, germs everywhere..” She asks you if the 820 billion number stated in the article is an exaggeration. Based on your experience in the lab, fully assess the validity of the statement. **2-3 sentences**

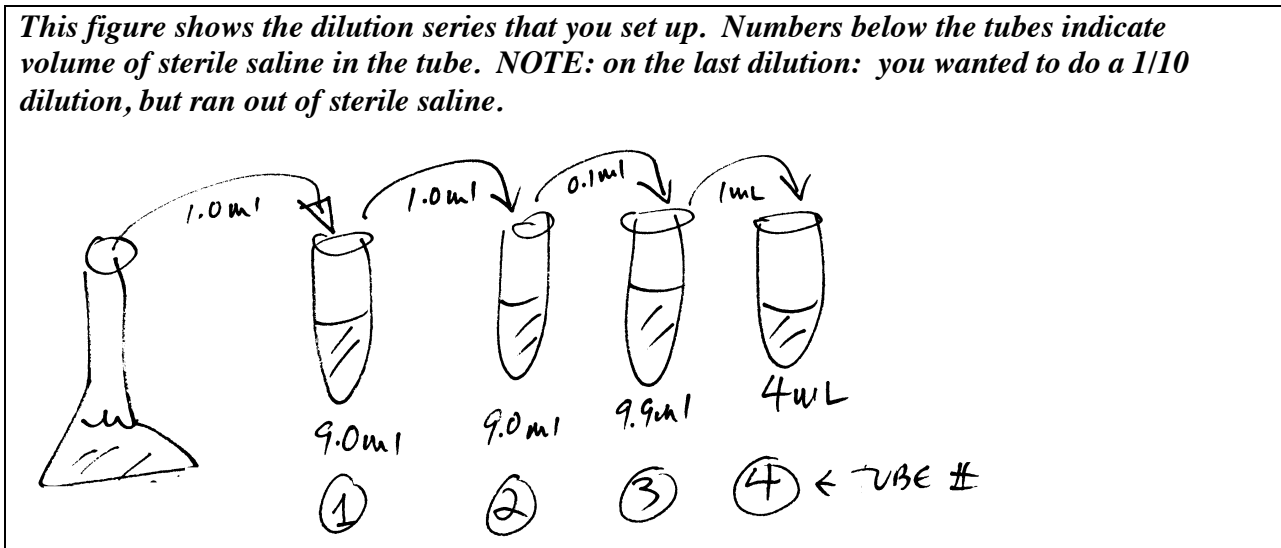
NOTE: one teaspoon = 5 ml



Problem 10 MORE PRACTICE PROBLEMS: NO CALCULATOR

One arena for intense selection for antibiotic resistance bacterial strains is found in animal husbandry in which antibacterial agents are used for growth promotion. Chickens receiving antibiotics, such as virginiamycin, in their feed gain more body weight than animals that do not receive antibiotics. You go to a commercial chicken feedlot to determine the frequency of virginiamycin-resistant bacteria in this environment. You return to the lab with a “slurry” of bacteria and perform the following analysis .

This figure shows the dilution series that you set up. Numbers below the tubes indicate volume of sterile saline in the tube. NOTE: on the last dilution: you wanted to do a 1/10 dilution, but ran out of sterile saline.



Volume plated on:

Tube #	L plate (rich)	L plate + virginiamycin	Duplicate colony counts	
1		0.2	535	515
2		0.2	47	53
3		0.2	0	1
3	0.1		108	92
4	0.1		19	27

tmtc = too many to count

From the data shown above, determine the:

a. Total viable cell count in the slurry. [Show your work and circle your answer. Use scientific notation. I will not count zeros. You must show your work to get credit for this question. Ditto for part b.]

b. The % of cells that are virginiamycin resistant.