## Biol 322 Fall 2012 Study Sheet for Quiz \#2

Quiz \#2 is scheduled for Thursday Nov $10^{\text {th }}$ 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
Colony counts plating

Amt.per plate (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 $\mu l$ 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 mercuryresistant 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?


C | L plate + mercury |
| :---: |
| Tube \# |
| volume plated |

| 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 |

[^0]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 soure
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. $\mathrm{lac}^{+}$ph ${ }^{+}$
b. lac $^{+} p h e^{-}$
c. $l a c^{-} p h e^{+}$
d. lac" pho
e. not enough information to draw conclusion about genotype
f. data are ambiguous for this strain

## Indicate genotype by letter (above)

$\qquad$ Strain $1 \quad$ Strain 2 $\qquad$ Strain 3 $\qquad$ Strain 4

$$
M M=\text { minimal media }
$$

$$
\begin{aligned}
& \text { = grawthof } \\
& \text { strain }
\end{aligned}
$$



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 <br> cells scored) |
| :--- | :--- | :--- |
| rpoB | lethal | $5 \times 10^{-4}$ |
| gyrA | resistance to nalidixic acid | $5 \times 10^{-6}$ |

From these results, the graduate student concludes that the rpo $B$ gene must be much larger than the $\operatorname{gyr} A$ gene. In other words, he concludes that the coding region of $r p o B$ gene must be 100 X 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 $g y r A$ 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 $l e u^{-}$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 $l e u^{-}$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 Saccaromyces cerevisae. 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 intracelluar 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 $a d e^{-}$mutants. The forward ( ade $^{+} \rightarrow$ ade $^{-}$) mutation frequency of this gene is about 5 $\mathrm{X} 10^{-5}$ 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 $a d e^{-}$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 $1 \times 10^{9}$.
- Assume a reversion frequency of $1 \times 10^{-10}$ 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 $=5 \mathrm{ml}$

TUESDAY, NOVEMBER 9, 2004

## Health Fitness

## The New Hork ©imes Germs, 1

## By MARY ROACH

I saw a television advertisement recently for a new product called an air sanitizer. A woman stood in her kitchen, spraying the empty space in front of her as though using Mace against an imaginary assailant. She appeared very determined. Where others are satisfied with antibacterial-laced spongare dish soaps, hand sanitizers and telephone es, dish soaps, hand sanitizers and teleph
wipes, here was a woman who sought to wipes, here was a won
sterilize the air itself.
As a casual student of microbiology, I find it hard to escape the absurdity here. This woman is, like any human being, home to hundreds of trillions of bacteria. Bacteria make up a solid third, by weight, of the contents of her intestines.
If you were to sneak into her bathroom while she was showering - and based on my general impression of this woman from the advertisement, I don't recommend this and secret away a teaspoon of the water at and secret away a teaspoon of the wate sou would find some 820 billion bacteria. Bacteria are unavoidably, inevitably - and, usually, utterly benignly - a part of our world. (Statistics courtesy of a University of Arizona microbiologist, Dr. Charles P. Gerba, a man who gave his son the middle name Escherichia, the E in E. coli.)
The fantasy of a germ-free home is not only absurd, but it is also largely pointless. Unless you share your home with someone very old, very young (under 6 months) or very ill, the few hundred bacteria on a

Mary Roach is the author of "Stiff: The Curious Lives of Human Cadavers."

2 Problem 8 A number of years ago a microbiologist named Bruce Ames came up with a simple way to test commercially produce products such as cosmestics and food additives for their potential to act as a mutagen. In this test his- (his = the amino acid histidine) strains of the bacteria Salmonella were treated with the compound in question and the frequencies of reverse mutations were compared in treated and untreated cultures. Briefly (1-2 sentences max) speculate as to why he chose to base his test on reverse mutations rather than forward mutations from his+ to his- given that the latter would be much more frequent.

Problem 9 One afternoon in the microbiology lab, you set up a cross between strain A and strain B (genotypes below). That same day, your freezer melts down and you lose all of your frozen stocks. From the mating mixture, you need to recover the recombinant strain (R) as well as both of the parental strains. The F-factor in strain B carries a gene that confers strep resistance. $\operatorname{str}^{\mathrm{R}}$ is dominant to $\operatorname{str}^{\mathrm{S}}$.

- On an extra sheet of paper, describe/diagram how you would go about isolating pure cultures of strains A, B and R. YOU MUST draw out a flow diagram to illustrate your answer (see board). Indicate what will/won't grow at each step.
- You have minimal media with any components you want to add. You also have MacConkey plates.
- Be very explicit about what media you are using at each step. Don't forget to add a carbon/energy source to your media and any other stuff the strain might need to grow.
- You also have a mk virus strain (called mk for male killer) that infects and lyses (kills) cells making pilli
- Make your strategy as simple and as little work as possible. You will lose points if your strategy is overly complicated.


## Strain A $\quad \mathbf{F}^{-} \quad$ str $^{S}$ mal $^{+}$lac $^{+}$thi ${ }^{-}$leu tet ${ }^{\mathbf{S}}$

$$
\text { Strain B } \quad \mathbf{F}^{\prime} \text { str }^{R} \quad \text { str }^{\mathrm{S}} \text { mal }^{-} \text {lac }^{+} \quad \text { thi }^{+} \text {leu' } \text { tet }^{R}
$$

## Strain $\mathbf{R}$

carbon/energy sources: $\quad$ lac $=$ lactose $\quad \mathrm{mal}=$ maltose
amino acids: leu $=$ leucine $\quad$ vitamins: thi $=$ thiamine
antibiotics: tet $=$ tetracycline $\quad$ str $=$ streptomycin

## Problem 10 MORE PRACTIVE 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.


[^0]:    tmtc $=$ too many to count

