**Sample Exam Questions Zabulionis Unit FALL/12**

It is in your best interest to try the questions first before looking at the answers; this gives you a chance to see how your thinking works for these types of questions.

**1.** Ampicillin plates are prepared to a final "working concentration" of 100 μg/mL. If the stock solution of amp is 10 mg/mL, how much antibiotic stock solution would be mixed with how much medium to achieve the working concentration in 1 litre final volume? (Show your work.) (3 marks)

**2a.** If an overnight culture was diluted to $10^{-7}$ and then 278 colonies were counted after plating 100μL, what is the estimated number of cells per mL in this overnight culture? (Show your work for part marks.) (4 marks)

**2b.** What assumption is made in determining the number of cells per mL in this overnight culture? (1 mark)

**3.** Some mutants of *E. coli* have twice as many pores (adhesion zones) on the outer surface of the cell. Would you expect such strains to have more, fewer, or the same number of transformants as normal strains using the calcium chloride/ice/heat method used in class? Why? (4 marks)

**4.** Given the data below, propose a biological model (it can be in 2 parts) to explain these results:

Growth (number of transformants) on LB + ampicillin plates after transformation of *E. coli* JM101 cells with pGREEN plasmid that codes for ampicillin resistance and increasing concentration of $CaCl_2$:

| $CaCl_2$ Concentration: | Growth (number of transformants) |
|---|---|
| 0 mM | 0 |
| 25 mM | 24 |
| 50 mM | 40 |
| 100 mM | 150 |
| 200 mM | 30 |

(6 marks)

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## ANSWERS:

**1.** use $C_1V_1 = C_2V_2$ $C_1 = 10$ mg/mL $= 10{,}000$ μL/mL
$V_1 = ?$
$C_2 = 100$ μg/mL
$V_2 = 1$ L $= 1{,}000$ mL

$$V_1 = \frac{100\ \mu g/mL \times 1{,}000\ mL}{10{,}000\ \mu L/mL} = 10\ mL.$$

Therefore, 10 mL of ampicillin stock solution will be added to 990 mL of medium.

**2a.** 278 cells/100μL = 2780 cells/mL = $2.78 \times 10^3$ cells/mL.
cells were diluted $10^{-7}$ ; therefore multiply by $10^7$

$$2.78 \times 10^3 \text{ cells/ mL} \times 10^7 = 2.78 \times 10^{10} \text{ cells/ mL}$$

**2b.** Each colony represents one living cell in the overnight culture.

**3.** Mutants with twice as many pores present a larger number of entry points for plasmid molecules than normal cells since it is assumed that plasmids enter the cells through the adhesion zones (pores). If the rate of transformation is dependent on cell competence (i.e. ability to take up a plasmid molecule) and not establishment of the plasmid, it would be expected that the mutants would have more transformants than normal cells. If the LPS (on the outer membrane which prevent the plasmid from getting close to the outer membrane) is the limiting factor for transformation; once a plasmid molecule gets by the LPS it has twice the probability of contacting the outer membrane at an adhesion zone (pore); consequently, mutants could have twice the number of transformants as normal cells.
Another explanation: if the limiting factor is getting the plasmid through the adhesion zone and into the cell, the mutants would have more transformants. If only 1 out of the approximate 400 pores in a normal cell allow a plasmid through the adhesion zone, having 800 pores (mutant cells) would have two pores that allow a plasmid through which would double the number of transformants provided that there are not so many plasmid molecules that each pore has a plasmid at the adhesion zone (i.e. it only takes one plasmid molecule to get in the cell to form a transformant).

OR:

Mutants will produce the same number of transformants as the normal cells; although there are more entry points for the plasmid with the mutants, the number of transformants is limited by the plasmid being "established" by the cell. The limiting factor to form a transformant is the cell's ability to recognize the plasmid resistance gene, transcribe and translate it, transport the resistance protein to the site where it can work on the antibiotic; and have the plasmid replicated so copies are available for daughter cells, i.e. the plasmid gets into the cell relatively easily, the limiting factor for the number of transformants is only some cells have the ability to express and replicate the plasmid (establishment).

**4.** At 0 mM $CaCl_2$ there are no transformants indicating that calcium chloride is required for transformation possibly to neutralize the negative charges on the pGREEN plasmid DNA, the membranes, and the attachment points of LPS to the membrane proteins. The optimal calcium chloride concentration is 100mM; this concentration may neutralize the maximum amount of negative charges allowing the most pGREEN molecules to get tight to the adhesion zone of JM101 cells (adhesion zones are the entry point of the plasmid into the cell).

At 200 mM $CaCl_2$, the plasmid molecules could still get into the cell but the calcium chloride is toxic to the many cells by causing disruption of important pathways, e.g. maybe cells cannot take up nutrients.

OR

At 200 mM $CaCl_2$, the concentration of calcium chloride is so high that the osmotic pressure outside the cell is greater than inside causing many cells to lose water desiccating the cells and thus killing the weaker ones or rendering them dormant.