Unit 2, Module 3
Transformation Questions Expanded Name_____
Version A

First (overall) question:
What were you trying to accomplish by treating the *E. coli* with the pAMP?

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1. Why must sterile technique be used even after bacteria are introduced to the tube?

   (Would it be a disadvantage for the tube to contain various unknown bacteria and fungi in addition to the *E. coli* that are the subject of the experiment?)

2. What makes the $CaCl_2$ solution quite different from the normal conditions enjoyed by *E. coli* inside the large intestine? Be specific about at least three differences.

   (*E. coli* is a bacterium that normally lives inside the large intestine, that last meter or more of the human digestive tract. In this lab you have placed the *E. coli* into a tube containing only 50mM Calcium Chloride solution placed in an ice bath on the lab bench. Describe at least three ways the test tube environment is different from the large intestine environment normal to *E. coli*.)

3. In what ways could $CaCl_2$ interact with the cell membrane of the cells? Give at least two specific examples of possible interactions.

   (Calcium ion, Ca^{+2}, is a divalent cation. The cell membrane is constructed of phospholipid bilayer plus proteins. Describe two features of the membrane structure that Ca^{+2} could attract to. If it were attracted to those two or more locations, how might each interaction affect the attractions, arrangements, and/or resulting structures that are normal in the cell membrane?)

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1. What negatively charged functional groups are plentiful on DNA? (The next question gives a big enough hint for the answer.)

2. If Ca^{+2} interacts with the negatively charged phosphate groups on DNA, what change(s) might take place in the overall arrangement of the plasmid in the presence of plentiful Ca^{+2} ions?
   (What is the normal arrangement of DNA? Do you think the presence of negatively charged phosphate groups all along the "backbone" of each DNA single strand tends to keep the DNA in a "stretched out" form or in a "wadded together" form? Could the presence of Ca^{+2} have any effect on the overall form of the DNA? Say how.)

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Why did you make the transfer of control cells in step 6 before doing anything with the transformed cells in step 7?

(On page 7 you made predictions about how many cells would grow on the plates that you have just inoculated. If you have not already done so, make the predictions. Since there is a chance of contamination any time cells are transferred, why is it better to have a chance of contaminating transformed cells with non transformed cells than the other way around?)

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(A lawn is millions of tiny colonies, many, **many** more than tens or hundreds of colonies.)

Page 11.

Each colony grew from how many cell(s) at that location on the plate before incubation? (What would happen if a single bacterium was put on a nutrient plate? How would the plate look next day? What if two were put 3 millimeters apart on the plate? How would it look?)

Extra questions:

Sample question and answer:
Question: Which plate(s) demonstrate(s) that you labeled the plates differently from one another? Explain your answer.

Answer: The labels on the plates show that they were all labeled differently. For example, in comparing the two LBA plates, one plate was labeled "Luria broth agar plus plasmid," and the other LBA plate was labeled "Luria broth agar minus plasmid."

A. Which plate(s) demonstrate(s) that the *E.coli* cells were not killed by the $CaCl_2$ you suspended the cells in? Explain your answer.

B. Which plate(s) demonstrate(s) that the *E. coli* cells were not killed by the ice-cold or the heat-shock treatment you gave the cells? Explain your answer.

C. Which plate(s) demonstrate(s) that the *E. coli* cells were not killed by the Luria broth you added to the cell solution? Explain your answer.

D. Which plate(s) demonstrate(s) that the *E. coli* cells were not killed by the pAMP plasmid you added to the cell solution? Explain your answer.

E. Which plate(s) demonstrate(s) that the antibiotic Ampicillin kills the cells that did not receive the Ampicillin-resistance gene? Explain your answer.

F. Which plate(s) demonstrate(s) that not every cell in the "+plasmid" tube was transformed? Explain your answer.