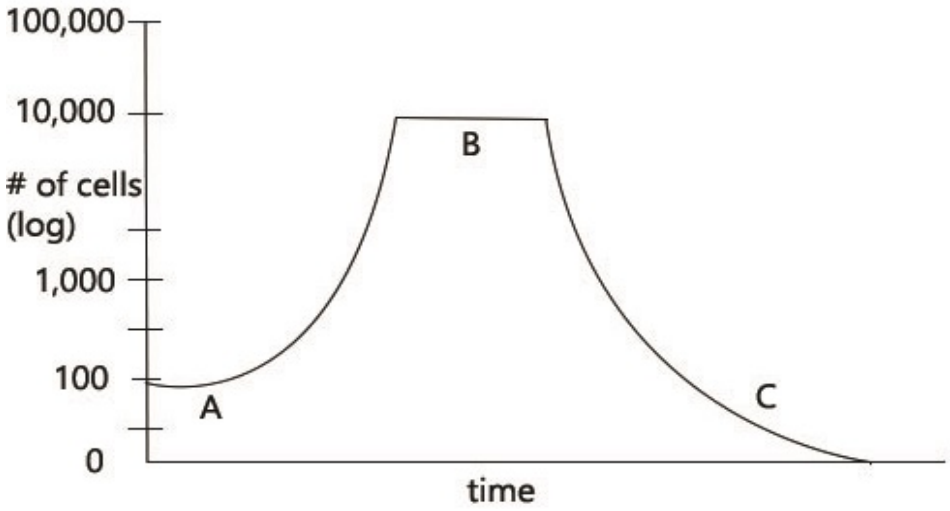
Bacterial Transformation Post lab Questions:

1. This graph represents typical bacteria growth and death on any culture plate. This trend occurs in both Luria Broth/ agarose and Luria broth/ Agarose/ Ampicillin/Arabinose plates.



Describe what is happening in the three phases (A,B, and C) and give a name to each phase. Why is there a decline as time goes on? What is happening/ Think in terms of the bacteria's resource(s) that may be depleting or accumulating as time goes on.

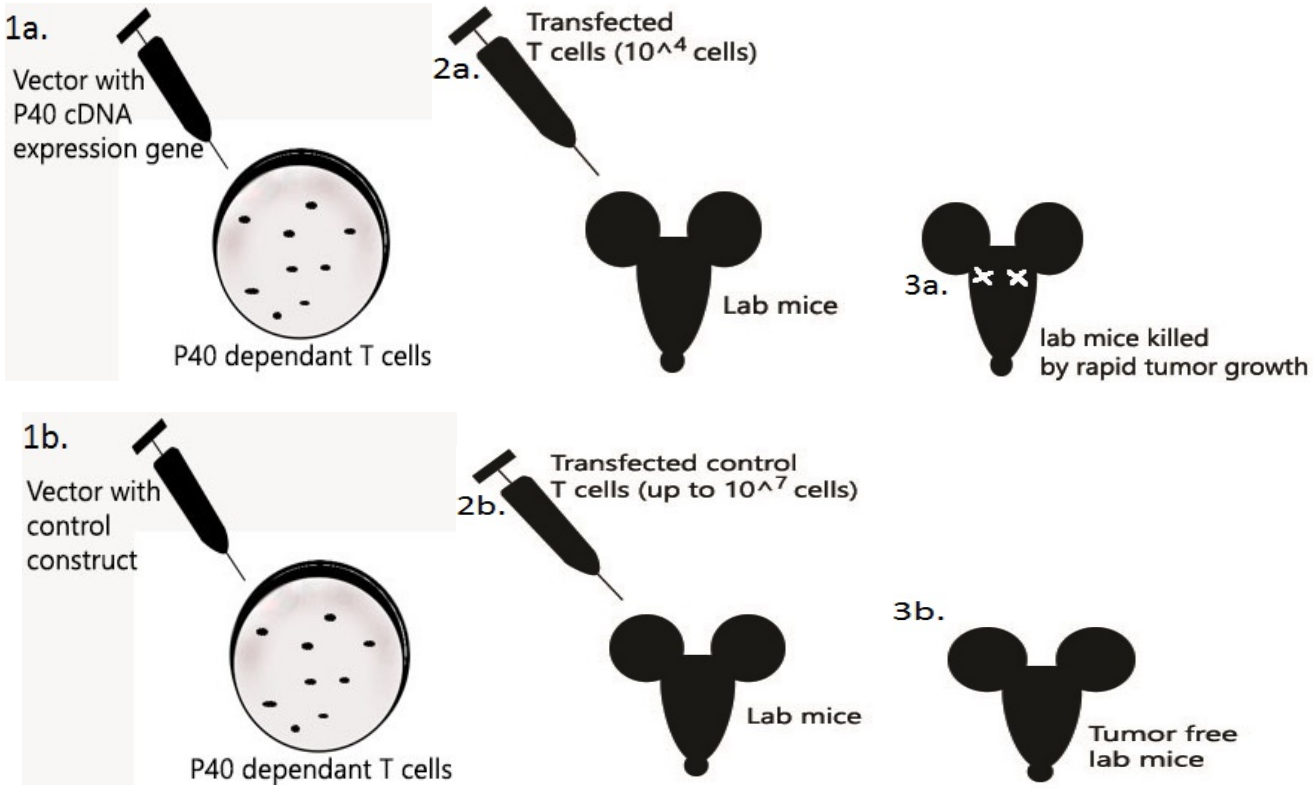
2. Why won't spilling a bucket of the plasmid pGLO on your hands give you “glowstick fingers”?

3. Why is the recovery step at 37 degrees Celsius? (HINT: the conversion from Celsius to Fahrenheit is C *9/5 +32=F). What else is at that temperature that makes bacteria happy and divide quickly?

The following paragraph is from a scientific journal published in 1991. Read it examine the diagram below to answer questions 4-6.

Summary:

To test the transforming potential of deregulated P40/Interleukin 9 expression, we transfected a mouse P40-dependent T cell line with P40 cDNA, and examined the tumorigenicity of the resulting transfectants. When the cells, which grew autonomously in vitro, were injected intraperitoneally or subcutaneously into syngeneic mice, a very high tumor incidence was observed with as few as 10^4 cells per inoculum. Animals died as a result of widespread dissemination of lymphomatous tissue to abdominal and thoracic organs. The same P40-dependent cell line transfected with a control construct did not form tumors even after injection of 10^7 cells. These results indicate that uncontrolled expression of P40 can support T cell proliferation in vivo, and may be a transforming event involved in the development of certain T cell tumors



Source:

Uyttenhove, C. "Autonomous Growth and Tumorigenicity Induced by P40/interleukin 9 CDNA Transfection of a Mouse P40-dependent T Cell Line." Journal of Experimental Medicine 173.2 (1991): 519-22. Web.

4. Based on the paragraphs above, what do you think Transfection is? How is it different from Bacterial Transformation?

5. Why do you think the researchers infected mice with cells that could become tumors? Why didn't they use bacteria to test tumor growth instead? Think about the difference in complexity of the two different types of cells being discussed here and provide at least 3 different reasons.

6. (Optional) Do you think that the experiment performed above was ethical? Is there a benefit to our understanding of tumor growth that makes the sacrifice worth it? If not, then what do you propose as an alternative method to better treat people with cancer in similar situations? If so, then would you personally be able to perform the experiment and inject mice knowing that they will die? Why?

7. How will the transformation of bacteria affect the inheritance and hereditary transfer of genes into the next generation of bacteria? What would happen to that inheritance when the transformed bacteria are placed into an environment without Amp? (think back to the background images in your lab manual discussing the “cancer cure” bacteria)

Questions 8 and 9 are based on a 2002 published, scientific ecology paper on Genetically modified Organisms

8. The ability and practicality of genetically modifying organisms does not stop at bacteria. "The Ecology of Genetically Modified Mosquitoes" was published in Science by Thomas W. Scott. It details the feasibility of "*Mosquito Population replacement-- that is, the release into natural mosquito populations of genetically modified mosquitoes (GMM) rendered refractory to pathogen-- to reduce or eliminate disease transmission*". It is one matter to genetically modify an organism in a controlled environment, but a much more complicated one to measure the effects of its release. This application of GMOs is meant specifically to reduce or eliminate the risk of Plasmodium Malaria transmission by mosquitos.

"The premise of the GMM approach is to reduce the number of competent mosquito vectors, thereby decreasing human infection But we need to know the extent to which vector populations must be reduced in order to elicit the required public health outcomes. Most population models assume random mating, which is almost certainly not the case. The most plausible natural circumstance is assortative mating- the tendency for certain phenotypes to mate with one another-yet this phenomena has been little studied in mosquitoes"

8a. What does the term "Competent mosquito vectors mean"? Do they simply mean dead or alive mosquitoes?

8b. What do researchers expect the release of GMM to do to the population of wild-type mosquitoes? How can the inheritance of transgenes in the mosquito vector population accomplish this goal when competing against Mendelian inheritance (normal non GMM inheritance)?

9.- *"Now that mosquito transformation is well established in the laboratory, we need to study the effects of genetic modification on the ability of these mosquitoes (relative to wild type mosquitoes) to survive, reproduce, and carry out critical physiological processes. Ecologists need to study gene flow, with emphasis on mosquito mating patterns and reproductive behavior, mosquito population size and structure, mechanisms of population regulation, genetic exchange between neighboring populations, and fitness and phenotypic effects of colonization and mass rearing. Regulation of population size could be beneficial or detrimental to the spread and stability of transgenes, depending on the circumstances. If mosquitoes mate assortatively and their populations are restructured into reproductively separate subdivisions, different mechanisms of population regulation could lead to an unpredicted advantage for one population over the other."*

9a. Why would GMMs have more complications upon release than a plate of transformed bacteria you made in lab? Explain two factors (within the species itself or environmental) that could affect the fitness of GMMs or other more complex multicellular organisms, but not bacteria.

9b. Among the many environmental factors, how could insecticides, used as population control affect the success of transgene inheritance? (think about the initial population difference between wild types and released GMMs)

9c. How could pesticides ensure the spread of transgene mosquitoes? (How was the success of