Bi 430/530 Winter 2008 Name:_____
Problem set 2, 50 points
Due by 11:30AM on Friday, March 10

In working through these questions you may use any source (books, internet, co-workers, classmates, etc.), but answers must be in your own words.

1. (15 points) A new hereditary disease that affects liver function in maqaque monkeys has been described, and it is caused by the loss of function of an enzyme normally expressed in liver cells. You know the sequence of the gene (both wild type and mutant versions), and you decide to try to correct the problem by growing the defective liver cells in culture, transforming them to restore the wild type version of the gene, and then moving the engineered cells back to the monkey with the defective liver. Describe a protocol that will allow you to engineer the mutant cells to contain a wild type version of this liver enzyme (there are several possible answers to this question).

2. (10 points) A) Your lab has cloned two genes ("W" and "X") from a newly identified virus, and you think that their protein products may interact with each other to form a functional complex. Design an experiment to determine whether these proteins interact within the context of the eukaryotic cell. B) You believe that one of the proteins interacts with human proteins in the cell following infection. Indicate how you would modify the above experiment to identify human proteins that can interact with the protein product of gene W.

3. (10 points) Selectable markers are crucial for generating transgenic cells and organisms. Explain why. Counter-selection is a useful method for manipulating transgenes following transformation. Give one example of a use for counter selection.

4. (15 points) 3. A bacterium isolated from a hyper-saline environment has been brought into the lab, and you want to see whether it contains a specific gene that you think is associated with salt tolerance in bacteria. You'd like to clone the gene that confers this salt tolerance. Describe a series of steps that will allow you to do that, starting with a genomic DNA library. Assume in your calculations that the bacterial genome is 6 Megabases in length.
   A. What cloning vector will you use, and what practical size of DNA can you clone in this vector?
   B. How many clones will you need for 99.9% confidence that you will isolate the gene of interest?
   C. How will you screen the library for the gene of interest?