1. (5 pts) You are a fifth year graduate student whose Ph.D. thesis concerns the cloning and analysis of a novel transcription factor that regulates drug metabolism. After struggling for a few years, experiments are going just great and you are nearly ready to publish a major paper and start postdoctoral study. Your advisor has just returned from a scientific meeting and has some very disturbing news. She heard a rumor that Dr. Evil has a) also cloned your gene b) has similar results to yours and c) has a manuscript under review at a leading journal. You have a brief time window to avoid being scooped (i.e., time is of the essence). The final experiments for your paper require the use of a liver cell line that stably expresses the promoter of your gene driving an appropriate reporter. Outline the approach you would use to generate this cell line. Be sure to mention how you would get the DNA into the cells, what type of selection you would use (positive or negative), which antibiotic or other selection would be preferred and what reporter would work best in these experiments. The reporter should be sensitive and have a large dynamic range. There is more than one way to accomplish your goal - full credit depends on how well justified your chosen approach is.
2. (5 points) Highlight some of the important issues to consider when selecting a reporter gene to use for gene expression assays.

3. (5 points) What is the fundamental mechanism shared among most transfection methods that allows cells to take up DNA from the surroundings?

4. (5 points) Retroviral gene transfer is a useful and efficient technique for getting cloned genes into cells or embryos. Would this be the method of choice for introducing cloned genes into early mammalian embryos? If this is not the method of choice, what would be? Justify your answer.
5. (6 points) Transgenic mice are important experimental models to evaluate gene function. What are three important things we would like to know about any founder animal before undergoing a large scale breeding project? Describe how would you test to ensure that these criteria are met?

6. (4 points) What are some problems and pitfalls in targeted gene disruption experiments in mice?
7. (5 points) Discuss the features of the sterol regulatory element binding proteins that distinguish them from other closely related transcriptional regulatory proteins and describe how these features specifically influence cholesterol regulation in the cell.

8. (4 points) Compare and contrast conventional, confocal and two photon stimulated fluorescence microscopy. Be specific.
9. (3 points) What is a chromosomal translocation? Describe two types of chromosomal translocations that can lead to the formation of cancers.

10. (3 points) With a diagram, explain the physics and principles of laser tweezers.
11. (10 points) You are a molecular biologist who really loves eating shrimp after work as much as you enjoy working in the lab. To combine your two loves, you have decided to create a biotech company (www.jumboshrimp.com) based on producing the largest shrimp anywhere. You took an invertebrate biology course at UCI and learned that shrimp and related animals express an, as yet, unidentified growth inhibitory gene when cultured at high density. You really need to a) clone this gene, b) figure out where this gene is expressed to begin to understand how it works and c) knock it out so that your shrimp will grow to large size in aquaculture. How would you go about accomplishing these goals? How would you prove to the patent office that your gene is the decisive factor in regulating shrimp growth in culture? Assume that all of the standard sorts of methods we discussed in class will work in shrimp (except targeted gene disruption). Assume that shrimp heterozygous for disruption of the growth inhibitory gene will be large enough to be detected but not big enough for your company.
12. (10 points) In the example above, you have identified a putative gene that can regulate the growth of shrimp when they are cultured at high density. You also know where and when the gene is expressed. The next step is to understand the factors that regulate expression of this gene. Describe the approach you would take to characterize the promoter of this gene and identify elements required for correct temporal and spatial expression in both cultured cells and transgenic animals. What reporter gene constructs would you make and why? Assume that transgenesis in shrimp is possible.
13. (10 points) The experiments you described above have provided you with an important gene and information on what regions of its promoter are required for temporal and spatial expression. Unfortunately, although the shrimp grow to large size when the gene is knocked out using the approach you described in question 11, their fecundity is impaired. One thousand fold fewer embryos are produced per mating, despite the increased size of the animals. Oops. Fortunately, the consultant you have hired (your former Ph.D. advisor) has a bright idea. Why not just identify the promoter element responsible for regulating expression of the gene in response to high density culture and then modulate its activity rather than knocking the gene out and getting 1000 fold less shrimp? You described how to do this in your answer to question 12, right? As it turns out, you identify a single promoter element that is required for the activation of the gene when the shrimp are cultured at high density. The same element is required for correct spatial expression during development. The sequence of the element is the following: AGGTCATCAGATGACCT. What can you deduce about the protein(s) from the sequence of the binding site? Describe how you could identify a protein(s) that binds to this site. How would you identify cDNAs encoding the protein that binds to this element?
14. (5 points) OK, you are really making progress now. Genes closely related to your shrimp growth inhibitory gene have just appeared in the EST databases from *Drosophila* and human. The *Drosophila* gene maps to a region on chromosome 2 where a previously uncloned gene required for germ cell formation is located. Loss-of-function mutations at this locus produce embryos lacking most of their germ cells. Cool. This suggests a possible model for why the shrimp deficient in this activity are less fecund. Which of the known embryonic signaling pathways is most likely to be involved? How would you go about determining where your gene fits into the pathway?
15. (5 points) It gets better. The human EST is from a testis cDNA library. Considering that the shrimp produce fewer embryos and that flies produce fewer germ cells, you hypothesize that the protein is required for the formation of sperm. Wow, perhaps one could use this gene as a target to develop new male contraceptives. Describe an experimental approach that would demonstrate that sperm are produced when the gene is on but not produced when the gene is off?