Guide Questions for Topic: Using Recombinant DNA to Manipulate Gene Expression

1. The terms "expression plasmid" and "expression construct" refers to _____.

2. When engineering an expression construct, researchers often insert a cDNA for a their gene of interest next to a viral promoter. Why is this the case? In other words, what advantage does this provide in experiments?

3. What do the terms "transfection" and "infection" have in common with each other in the context of using recombinant DNA molecules for experiments? How do those terms differ from each other?

4. If you wanted to acquire an expression construct encoding the transcription factor that you are assigned for the take home assignments, what would be the simplest first step in trying to do so?

5. As noted in the Video Lecture, transfection of cells with a p53 expression construct causes to undergo apoptosis. Based on your understanding of p53 regulation from class, which of the following expression construct would you predict to counteract the apoptotic effect of p53 transfection if it was co-transfected alongside p53 (i.e., if cells were transfected with BOTH the p53 expression construct and your selection below)?

6. What does the term "transient transfection" refer to?

7. What does the term "stably-transfected cells" refer to?

8. During transfections, only a tiny fraction of cells become stably transfected. How, then, do researcher isolate populations of stably-transfected cells from transiently-transfected and non-transfected cells?

9. What does the term "point mutation" refer to?

10. What does the term "truncated protein" refer to?

11. What does the term "site-directed mutagenesis" refer to?

12. If you wanted to transfect a truncated version of p53 into cells, but only have access to an expression construct encoding normal p53, how might you go about acquiring a construct that encodes the truncated p53 you want?

13. A mutant form of the kinase ERK in which lysine-72 is mutated to arginine (aka, ERK-K72R) functions as a dominant-negative protein when over expressed in cells. Based on this information, what can you tell me about ERK-K72R?

14. What does the term "GFP-fusion protein" protein refer to?

15. If you transfected cells with an expression construct encoding a histone H3-GFP fusion protein and then examined the cells by fluorescence microscopy, what would you expect to observe?

16. What does the term "transgenic mouse" refer to?

17. In 2012, Senturk and Manfredi published a study in the journal **Genes & Cancer** showing that transgenic mice in which MDM2 is over expressed develop spontaneous tumors at an accelerated rate. Based on your growing understanding of p53 and its regulation, are you surprised by this data? Explain.

18. If you transfect cells with an siRNA that is complementary to mRNA encoding the protein ERK, what effect would it have on ERK protein?

19. Which of the following siRNAs would inhibit apoptosis induced by accumulation of DNA damage if transfected into cells? (HINT: Think about the working model of p53 regulation that we have discussed.)