**Dr. Reichler's Bio 325** class time:_____ Print Name:_____KEY_____
**Exam #2** October 29, 2007

Read each question carefully and don't hesitate to ask if a question seems unclear. If possible, answer each question in the space provided, but if needed, continue on the back. If you use a drawing as part of your answer, be sure to also include a written explanation. These questions have specific answers, although for some, more than one answer is possible. To receive full credit you must clearly and fully answer the question being asked. This exam is worth 103 points with the points for each question noted in parentheses.

1. A) Would the amino acid sequence of a nuclear-localized protein be different when it is in the cytoplasm versus when it is in the nucleus? Why or why not? (4pts)
*No, nuclear localization signals don't get modified in any way when proteins are transported to the nucleus.*

B) Would your answer be different if the protein was localized **in** the nuclear membrane? Why or why not? (4pts)
*No, membrane bound proteins retain their signal peptides, so the protein sequence will not be changed.*

2. How can mRNA degradation lead to the specific localization of a protein? (6pts)
*If the degradation is not everywhere, so the mRNA is protected in certain regions, then the protein will only be translated in that area.*

3. How could inserting some DNA into a eukaryotic cell, but not disrupting any part of the gene, inhibit the production of a protein? (6pts)
*Any one of: If the DNA inserted was a sequence that would form a double-stranded RNA that matches the mRNA for the protein, then this will form a siRNA that will degrade the mRNA thereby reducing the amount of protein. OR The inserted DNA could code for an insulator that would decrease transcription levels thereby decreasing protein levels. OR The inserted DNA could disrupt a transcription factor needed that would reduce mRNA and therefore protein levels.*

4. In the paper looking at the tight regulation of alternatively spliced isoforms, what techniques would allow the visualization of the different versions of the mRNAs for a certain gene? Include the procedures used, what order the procedures would be done in, and how each procedure would allow the visualization of the different sized mRNAs? (8pts)
*In the following order: Reverse transcription- to make cDNA from the RNA. PCR- to amplify the mRNA we wish to see. Gel- to visualize the different sized bands.*

5. In eukaryotes, give **two** reasons that the presence of transcription factors that recognize sequences within a gene's promoter would not be sufficient for that gene to be transcribed? Explain why each reason is inhibiting transcription even in the presence of the appropriate transcription factors. (8pts)
*Any two of: The formation of G-quadruplexes, four-stranded DNA, inhibits binding of transcription factors. If the DNA is tightly packaged, the presence of the transcription factors will not matter. Methylation of the DNA will inhibit the binding of transcription factors.*

6. Would you expect the promoters of different homeobox genes to be similar or different? Why? (6pts)
*Different- each homeobox gene is expressed in different tissues, so they need different promoters to be activated differently.*

7. What part of a gene would a transposon be inserted that would alter the localization of the gene's mRNA? (6pts)
*The 3'-UTR. This is where information about mRNA localization is.*

8. Could RFLP (Restriction Fragment Length Polymorphism) analysis help identify whether a B-cell was totipotent? Why or why not? (8pts)
*Yes, as B-cells differentiate they rearrange their DNA. If the size of the region that codes for the antibody is smaller, then the B-cell is differentiated and no longer totipotent.*

9. Reporter genes can be used to determine when a gene is being expressed. Why would you **not** be able to determine the precise time of transcriptional activation using a reporter gene? (6pts)
*The reporter protein is visualized, and some time passes between transcription and protein production.*

10. Why are four reactions necessary for proper sequencing using dideoxy nucleotides? (6pts)
*The sequencing reaction stops when each ddNTP is incorporated. For the complete sequence four different ddNTPs are needed, and each reaction is done separately.*

11. Why does PCR (polymerase chain reaction) require both heating and cooling? (6pts)
*The heating separates the DNA strands, and the cooling allows the primers to bind and DNA polymerase to elongate the DNA.*

12. When inserting a gene into a bacterial plasmid, sometimes two different restriction enzymes are used to cut the two ends of the gene of interest. Why is it **not** necessary to treat the plasmid with phosphatase when using two different restriction enzymes? (6pts)
*The plasmid will not have matching sticky ends so it will not be able to religate to itself.*

13. The deletion of what gene in bacteria would result in the lac operon being continually expressed? Why? (6pts)
*The repressor. The repressor inhibits expression of the lac operon. Without it, transcription will be constitutive.*

14. To make fruit flies (*Drosophila*) with legs instead of antenna, would you <u>need</u> to modify many of the fly's genes? Why or why not? (6pts)
*No, homeobox genes tell the different parts of the embryo what they will become. So the change of one or a few homeobox genes will be enough to change the fate of these cells.*

15. Would binding of microRNA to the mRNA for the protein 'itsover' <u>necessarily</u> lead to a rapid decline in the amount of 'itsover' protein present? Why or why not? (8pts)
*No, even though the miRNA will inhibit translation, the stability of the 'itsover' protein may be independent of its translation.*

**Bonus:** How could a L1 type retrotransposon missing one of its ORFs (open reading frames) still be able to replicate? (3pts)
*It could use the proteins that it needs to move, like reverse transcriptase or RNA-binding protein, that were produced by another transposon or virus.*