Molecular Biology
MIDTERM- sample

1. Write all answers on answer sheets provided. Put your name on each page and label the pages according to the following format: #1 of two; #2 of two etc.

PART I [Questions #1-30 (3 point each)]

1. True or False: In DNA hybridization experiments, DNA fragments are separated by electrophoresis and then must be denatured with NaOH in order for hybridization to take place.
2. Describe briefly the difference between a polyclonal antiserum and monoclonal antibody.
3. Using the figure at right, which structure (LEFT or RIGHT) is found in RNA? During RNA synthesis, to which carbon, will a phosphodiester bond be made by the incoming nucleotide?
4. Why is it that GC-rich regions of DNA require greater energy to promote strand separation as compared to AT-rich regions?
5. Describe the noncovalent interactions required to maintain the double strandedness of DNA.
6. If the temperature for hybridization is below the Tm, explain why you will or will not see a signal on a Southern.
7. In which direction is DNA synthesized?
8. What is the function of a ribosome and of what is it composed?
9. The function of DNA ligase is to catalyze the formation of a ______ bond.
10. What is GenBank and how does one get access to it?
11. An____ has three parts: a phosphate, a pentose, and an organic base.
12. How is Agrobacterium tumefaciens used to make a transgenic plant?
13. What is needed to set up a reaction for PCR (polymerase chain reaction)?
14. What ensures the fidelity (error-free process) of protein synthesis?
15. Give an example of a reporter gene and explain why it is useful to study of gene expression.
16. Describe three types of post-transcriptional modifications to RNA.
17. Describe how an alpha helix structure is formed in a protein.
18. What role does the 16S rRNA play in bacterial protein synthesis?
19. How are amino acids delivered to the ribosome during the process of translation?
20. Which end of the protein is synthesized first during translation?
21. The function of the promoter region of a gene is ________________________.
22. If you wanted to simultaneously assess transcript profiles for 1000 genes, would you use microarray (gene chip) analysis, Northern analysis, or RT-PCR? Why?
23. For a 3 KB cDNA, assuming that 1 amino acid has a mass of 100 daltons, what is the maximum mass of the gene product? Why is the size probably smaller?
24. What advantage in creation of genetic maps do molecular markers have over traditional phenotypic genetic markers?
25. What is the basis for the specific DNA banding pattern resulting from cleavage with a particular restriction enzyme such as EcoRI?
26. When a cDNA is isolated, and its sequence compared to that of the corresponding genomic DNA, they are not always the same; the cDNA sequence may appear to have multiple gaps as compared to the genomic DNA counterpart. Why?
27. What is a transgenic organism?
28. What is spliced out from a primary transcript that contains two introns and three exons?
29. If you wanted to screen for genes expressed in roots, and later use these clones for expression of the encoded proteins, what type of library would you prepare?
30. Multiple polypeptides are encoded by a (an) ________mRNA.
PART II (10 points) Choose one of the following:

Problem 1:
From an *E. coli* cell extract, you assay enzyme activity for beta-galactosidase. You divide the extract into two samples, one of which you treat with SDS (sodium dodecyl sulfate). Both samples are further divided into 2 samples each which are alternatively assayed for enzyme activity and subjected to Western Analysis (immunological testing with beta-galactosidase antibody).

These are the results:

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Antigenic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract only</td>
<td>YES</td>
</tr>
<tr>
<td>Extract +SDS</td>
<td>NO</td>
</tr>
</tbody>
</table>

Give a molecular/biochemical explanation of these results.

Problem 2:
You have cloned a new bacterial gene encoding enzyme X, sequenced the DNA, and deduced the promoter and coding region as shown above. You prepare a gene construct with the deletion above; the deletion does not remove any of the promoter nor does it alter the open reading frame. When both the control and deletion genes are expressed in *E. coli* and assayed for enzyme X, you find that only the control shows enzyme activity. WHY? How can you test your hypothesis?
Problem 3:

1. You screen two libraries- cDNA; genomic
2. Clones are isolated having homology to PSY- 10 clones from each library
3. These are subcloned into pBluescript.
4. Protein expression is induced with IPTG and proteins separated by SDS-PAGE.

Results:

* Genomic clones: 0/10 gave expression
* cDNA clones: 2/10 gave expression

Question:

* Why zero genomic clones
* Why only 2 cDNA clones