Name:

Quiz 2 PL S/MCB/MIC 340 10/3/2002 (20 points)

- 1. Describe the three main properties of plasmid vectors used for cloning genes. Diagram a typical plasmid vector. (10 points)
 - (1) Origin of replication.
 - (2) Antibiotic resistance gene(s).
 - (3) One or more unique restriction sites for cloning.



RES = Restriction enzyme site

- 2. <u>Outline</u> the steps involved in isolating a <u>genomic</u> clone of a brain-specific gene that encodes a very abundant and easily obtainable protein of an unknown function. (10 points)
 - (1) Isolate mRNA from brain.
 - (2) Make double-stranded cDNA using reverse transcriptase.
 - (3) Clone the cDNA into plasmid or phage vector to generate a cDNA library.
 - (4) Transform (or infect bacteria if using a phage library) bacteria and induce expression of the cDNA clones in bacteria.
 - (5) Imprint/blot/lift/adhere bacterial colonies onto filter membranes, lyse bacteria and denature DNA.
 - (6) Use an antibody raised against the brain protein to identify the colonies expressing the specific cDNA coding for the protein.
 - (7) Cut out the cDNA from the plasmid within the specific clone identified above and use it as a radioactive probe to identify the gene coding for the protein from a genomic library.

3. **Bonus question:** You have attempted to disrupt a mouse gene essential for skeletal development by targeted gene insertion. A heterozygous mutant mouse obtained through this procedure produces a litter that segregates for mice that are severely deformed. This information indicates that you have been successful in disrupting the gene of interest. Do you expect the mutant gene to be larger or smaller than the normal copy? Explain your answer. (5 points)

It depends on the type of vector used to generate the mutation and the size of the Neo^r gene. If the vector contained a large deletion in the gene of interest in addition to the Neo^r gene, then the size of the mutated gene would be probably smaller. However, if the targeting vector had a simple Neo^r gene insertion into the gene of interest and no other deletions had been made, then the size of the mutated gene will be larger—that is, its size will be equivalent to the size of the gene itself plus the Neo^r gene.