Name:

Quiz 3 PL S/MCB/MIC 340 11/14/2002 (30 points)

- 1) (10 pts.) Diagram a typical gene-targeting vector for creating gene knock-outs in mice using homologous recombination. Briefly describe at least three important components of the vector and their individual uses.
- targeted gene insertion requires
 - the desired gene.
 - neor, a gene that encodes an enzyme that inactivates the antibiotic neomycin (and its relatives).
 - tk, a gene that encodes thymidine kinase, an enzyme that phosphorylates the nucleoside analog gancyclovir. DNA polymerase fails to discriminate against the resulting nucleotide and inserts this nonfunctional nucleotide into freshly-replicating DNA.



2) (15 pts.) Briefly describe at least two distinct approaches to cloning genes from eukaryotes that we have discussed in this class. In your answer, consider the available information that dictates the particular approach.

If have seq. info, genomic library screening. Screen using a DNA probe.

If have no seq. info but have protein product, cDNA expression library screening. Screen using an antibody raised to the protein of interest.

3) (5 pts.) Draw diagrams illustrating how the restriction endonuclease *Bam*HI (a 6 base cutter – G*GATCC - that leaves a 5' overhang) digests a double-stranded DNA molecule. Use the following conventions to diagram DNA molecules: OH=hydroxyl, P=phosphate, A=adenine, G=guanine, C=cytosine, T=thymine. Show the polarity at all free ends of DNA molecules.

See midterm exam.

4) (Bonus question: 5 pts.) Why is the yeast *Sacchromyces cerevisiae*, a single-celled fungus, a good system for molecular biology and utility in biotechnology? Provide at least two advantages and your reasoning!

Grows fast. Genetically well characterized (have many mutations). Genome seq. known. Etc.