1. Indicate whether the following statements are true or false. If a statement is false, explain why. (15%, each score 3 points)

(1) CEN, a yeast centromere, is a sequence where the replication of yeast DNA is initiated.

(2) A plasmid had a unique *Eco*RI site. The plasmid after *Eco*RI cleavage, followed by DNA polymerase extension in the presence of dNTPs and DNA ligation lost the *Eco*RI site.

(3) In theory, there are 10^{390} kinds of proteins with 300 amino acids. (*note*, log 5=0.7, log 3= 0.477, log 2=0.3) .

(4) Allelic exclusion of immunoglobulin genes in B cells involves DNA rearrangement.

For the next question, choose either only 5 or 5' for your answer.

(5) Two mutations have the same phenotypic effect and map close together, complemetation test shows that the heterozygote has mutant phenotype. The two mutations are alleles of one gene.

(5') If a point mutation can not recombine with a deletion, the site of mutation must lie outside the deleted region.

2. Fill in the blanks in *part I* with the suitable answers (*in letter*) provided in *part II*. (22 %, each score 2 points)
part I.

1. Yeast two hybrid system is usually used _____

2. ____ yeast cells are induced to undergo sporulation by nitrogen source deprivation

3. _____ may be important in some human disease e.g. spinocerebellar ataxia and Wilm's tumor, where inheritance of these disorder depends on which parent contributes the mutant gene.

4. Embryonic stem (ES) cells from black mice were microinjected into blastocysts of white mice. If ES cells were dead for some reasons during the procedure of injection. The coat color of the mice offspring of the experiment will be ____. If ES cells survived during the procedure, the color of the offsprings will be ____.

5. Insertion site of transgene(in a plasmid vector) can be cloned by method of _____.

6. The component A of the commonly-used HAT selection medium stands for _____.

7. Gene transfer by ____ is subject to the endocytosis.

8. The drug G418 commonly used for selecting stable transfectants with ___ gene

9. *Cos* cells produce the viral ____, which triggers replication of viral DNA by binding to a DNA sequence called _____.

part II.

A. DEAE-dextran method
B. reporter gene
C. origin of replication
D. T antigen protein
E. electroporation
F. purine synthesis inhibitor
G. DNA methylation
H. aminopetrine
I. *neo*
J. plasmid rescue
K. imprinting
L. dihydrofolate reductase
M. black
N. thymidine kinase
O. white
P. chimeric of black and white
Q. gray
R. haploid
S. a type

T. diploid
V.α type
W. as a genetic assay for protein-protein interactions.
X.to clone new human genes by direct genetic complementation of yeast mutations.

3. Choose and answer only one question from the following questions ([1]-[3]) (8 %)

[1] An *E. coli* mutant is isolated that renders the cell simultaneously unable to utilize a large number of sugars, including lactose, xylose and arabinose. However, genetic analysis shows that each of the operons responsible for utilization of these sugars is free of mutation, neither the membrane transport systems for these sugars have mutation. What are the possible genotype of this mutant?

[2] *E.coli lac* operon consists with 3 structural genes:LacZYA. LacZ codes for the enzyme β-galactosidase, LacY for β--galactoside permease, LacA for β--galactoside transacetylase.
Allolactose is an isomer of lactose that is produced by β-galactosidase as an intermediate in the cleavage of lactose to galactose and glucose. It is allolactose, not lactose, that is natural inducer for the *lac* operon. Allolactose binds to the repressor and lowers its affinity for the *lac* operator, thereby opening the operon for transcription.
Can you suggest a reason why cells (*need to*) synthesize low levels of β--galactosidase and β--galactoside permease even when there is absolute no lactose in the medium?

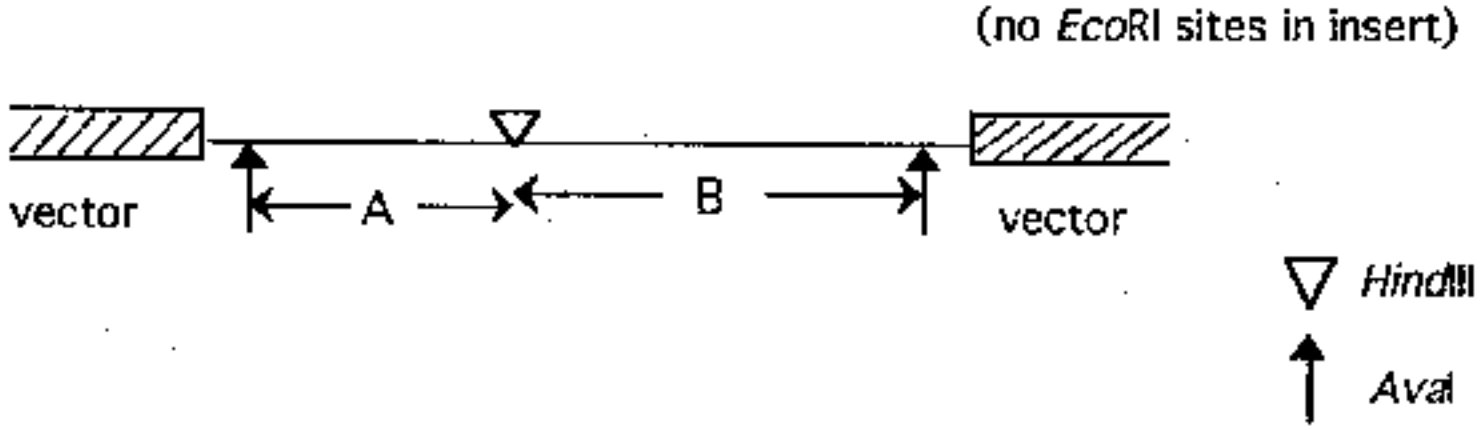
[3] You have the cDNA clones for two human genes, A and B, which you believe may be closely linked on a human chromosome, since these two genes are known to be close together in rats. Briefly describe the tools and procedures you would use to answer this question.

4. Choose only one and explain the term (used in gene cloning) (5%)

(1) enhancer trap strategy

(2) phage display strategy

5. (12 points) You have isolated a nearly full length kappa light chain immunoglobulin cDNA clone whose restriction map is shown below. The length of the clone insert is such that the clone must contain sequences which code for all of the light chain from about amino acid 5 to the CO_2 end (a.a. 216). That is, it does not code for the leader region. If fragment A is radiolabeled and used to probe Southern blots of **germline** DNA digested with *Eco*RI a single band of 5 kb is seen. When fragment B is used as a probe two bands of 4 kb and 2 kb hybridize. Note that there are no *Eco*RI sites in the cDNA insert of the plasmid. Only the label in fragment B can be protected from S1 digestion by hybridization using mRNA from the myeloma used to prepare the cDNA clone when the *Ava*I fragment (A + B) is 5' end-labeled with ^{32}P-ATP and then cut with *Hind*III followed by the isolation of singly end-labeled fragment A and singly end-labeled fragment B.



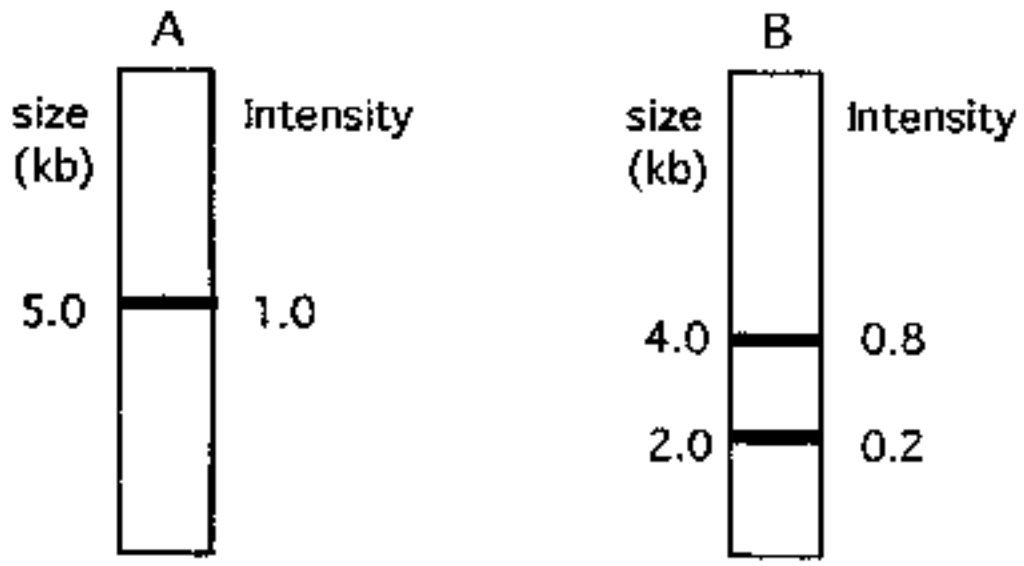
a. In what direction is this IgG gene transcribed relative to the map above?

b. Draw the likely positions of the V, J and C regions in the cDNA clone map.

c. Assume that the chromosome that has been rearranged to produce this IgG in the myeloma is the only light chain rearrangement that occurs, i.e., the homologous chromosome does not rearrange. Draw the patterns (two) to be expected when myeloma DNA is cut with *Eco*RI, Southern blotted, and hybridized with fragment A and fragment B. The patterns with germline DNA are shown below (the next page) along with the relative band intensities. Assume that the same amount of myeloma DNA is used as germline DNA gives the approximate relative intensity of each myeloma band in both of your patterns. Will any new bands likely appear in either of your blots? If so, how many and with what relative intensity?

(question 5 continued)

Germline Blots



6. (7 points) Select the number on the right column which represent the protein function of each proto-oncogene on the left.

proto-oncogene		protein function
(a) *ras*:	____	1. steroid receptor
(b) *fos*:	____	2. transcription factor
(c) *raf*:	____	3. growth factor
(d) *erbB*:	____	4. receptor for growth factor
(e) *sis*:	____	5. protein serine kinase
(f) *erbA*:	____	6. protein tyrosine kinase
(g) *src*:	____	7. GTPase

7. (10 points) Transposable elements have become powerful tools for gene cloning and gene expression. They can also be used as vehicles to deliver foreign DNA to organisms. The *Drosophila* P element has been adapted for this purpose and is now routinely used to put genes into flies.

(a) Describe the P element gene.

(b) Design two vectors containing the essential sequence of P element for investigating the expression of gene X in *Drosophila* embryos (both vectors are required). Also, show how do the vectors you designed work in this system.

8. (7 points) Describe how a cyclin machine regulate cell cycle progression.

9. (14 points) The rules of this question are: (1) each step is carried out by only one enzyme and (2) each enzyme can only be used once, even though some of them may function well in more than one step. (straight line represents DNA)

(I) 5'—NGCGCN'—3' (a)→ —NG CGCN'— (b)→ CGCN'—

(c)↓

CGCN'

(II) —NGCGCN'— + GCGC → (d) → GCGC → (e) → — GCGCN' —

(f)↓

NGCGCN'

(g)←

Write down the following number on your answer sheets, which can best carry out the enzymatic step on the above (a) through (g).

1. Rec A + ATP	2. Rec A	3. S1	4. S1 + EtBr
5. gyrase + ATP	6. topoisomerase I	7. helicase + ATP	8. DNase I + EtBr
9. *Hha*I	10. *Hha*I + EtBr	11. *Hha*I + ATP	12. T4 DNA ligase
13. exonuclease III	14. exonuclease V	15. E. coli DNA pol I + dNTP	