

八十四學年度 生命科學 所 甲 組碩士班研究生入學考試

科目 細胞與分子生物學 科號 0003 共 5 頁第 1 頁 \*請在試卷【答案卷】內作答

1. Restriction enzyme *Bam*HI recognizes DNA sequence GGATCC and cuts between the two G's to generate ends with 4 nucleotides GATC-overhang. *Sau*3A recognizes sequence GATC and cuts in front of G, also generating ends of GATC-overhang. You are making an *E. coli* genomic library. You digest total genomic DNA with *Sau*3A into completion, and ligate the *Sau*3A fragments into the *Bam*HI site in your recombinant plasmid pBR322. (1) What is the chance to regenerate one *Bam*HI site in your recombinant plasmids? (2) What is the chance to regenerate two *Bam*HI sites in your recombinant plasmids? (3) What is the average size of your insert DNA? (20%)
  
2. True or not true, if not true, why not? (15%)
  - (1). The role of a gene in a metabolic pathway is inhibitory because the pathway progression is enhanced when the gene is knocked out.
  - (2). Antisense RNA is transcribed by reverse transcriptase.
  - (3). Co-transfection of various kinds of DNA may be used in transient expression assay.
  - (4). Specificity of sequence recognition in transcription factors is determined by both DNA and transactivation domains.
  - (5). pYAC is a shuttle vector because it contains both bacterial and yeast origin of replication.
  
3. Short assay (15%)
  - (1). A phenotype is lost and mutation of YFG gene is found. When the intact YFG gene is successfully introduced into yeast, the phenotype is, however, not restored, why?

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科目 細胞與分子生物學 科號 0903 共 5 頁第 2 頁 \*請在試卷【答案卷】內作答

- (2). Professor Liu did a DNA transfection experiment in cells and found that the expression of the endogenous thymidine kinase gene was turned off while another endogenous gene YCL was turned on. He forgot which DNA of the following: (a) Ac, (b) Ty, (c) P element, (d) Tn10, (e) SV40 viral DNA was used in transfection. Which one is the mostly likely? Why? (hint: Ac, Ty, P element and Tn are transposable DNA of maize, yeast, *Drosophila* and bacteria, respectively).
- (3). Mutation in either repressor gene or operator gene of *E. coli lac* operon may result in constitutive expression of the operon. Please design a genetic method to distinguish those two mutants.
4. Please read each statement below carefully. Indicate whether the statement is correct or incorrect. If incorrect make the necessary corrections to make it correct. (10%)
- (1). After one replication of a duplex DNA molecule, some of the daughter DNA molecules contain no parental material.
- (2). An inhibitor that blocked all RNA synthesis would not immediately affect DNA synthesis.
- (3). Of all the known DNA polymerase, only the RNA-dependent DNA polymerase (reverse transcriptase) of RNA tumor viruses does not require a primer.
- (4). Because membranes are essentially impermeable to charged metabolites, the  $K'_{eq}$  for diffusion of charged metabolites out of the cell is much less than 1.

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- (5). If a cloned and labeled genomic restriction fragment is used to probe a Southern transfer of a digest of genomic DNA made with the same restriction enzyme, then the number of labeled bands always will be equal to the number of times the cloned sequence is repeated in the genome.

5. Please fill in the blanks. (20%)

- (1). (a), which consist of long (b) chains linked to a protein core, are found mainly outside the cell as part of the extracellular matrix.
- (2). The  $\text{Na}^+\text{-K}^+$  ATPase is required to (c) and (d).
- (3). If the cell is synthesizing DNA, it needs to import  $\sim 10^6$  (e) molecules from the cytosol every 3 minutes in order to package newly made DNA into chromatin. (f) direct nuclear proteins to nucleus.
- (4). (g) are found in all eukaryotic cells. They contain oxidative enzymes such as catalase and urate oxidase at high concentrations.
- (5). Enzyme-linked receptors are transmembrane proteins with their (h) domain on the outer surface of the plasma membrane.
- (6). Intracellular movements in eukaryotic cells are generated by motor proteins, which bind to either an (i) or a (j) and use the energy derived from repeated cycles of ATP hydrolysis to move steadily along it.

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6. Please name and describe the two well-characterized types of coated vesicles. (5%)
7. Please discuss the current model of how  $G_s$  protein couples receptor activation to adenylyl cyclase activation. (5%)
8. You have cloned a mouse cDNA. The clone was generated from fragment produced by cleavage with the restriction endonuclease *EcoRI* (2.2 kb). Your analysis of the cloned cDNA indicates positions for a few other restriction sites is in Fig. 1a (size markers are indicated as kb). In a second experiment, you used two primers at the positions indicated in Fig. 1b to perform polymerase chain reactions with the mouse genomic DNA as a template. The products are separated with electrophoresis and shown in Fig. 1c. (10%)
  - (1). Please show the positions on the gene for the restriction enzyme sites of *EcoRI*, *BglII*, *BstEII*, *HindIII* and *HpaII*.
  - (2). Please explain the PCR results.

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Fig. 1a

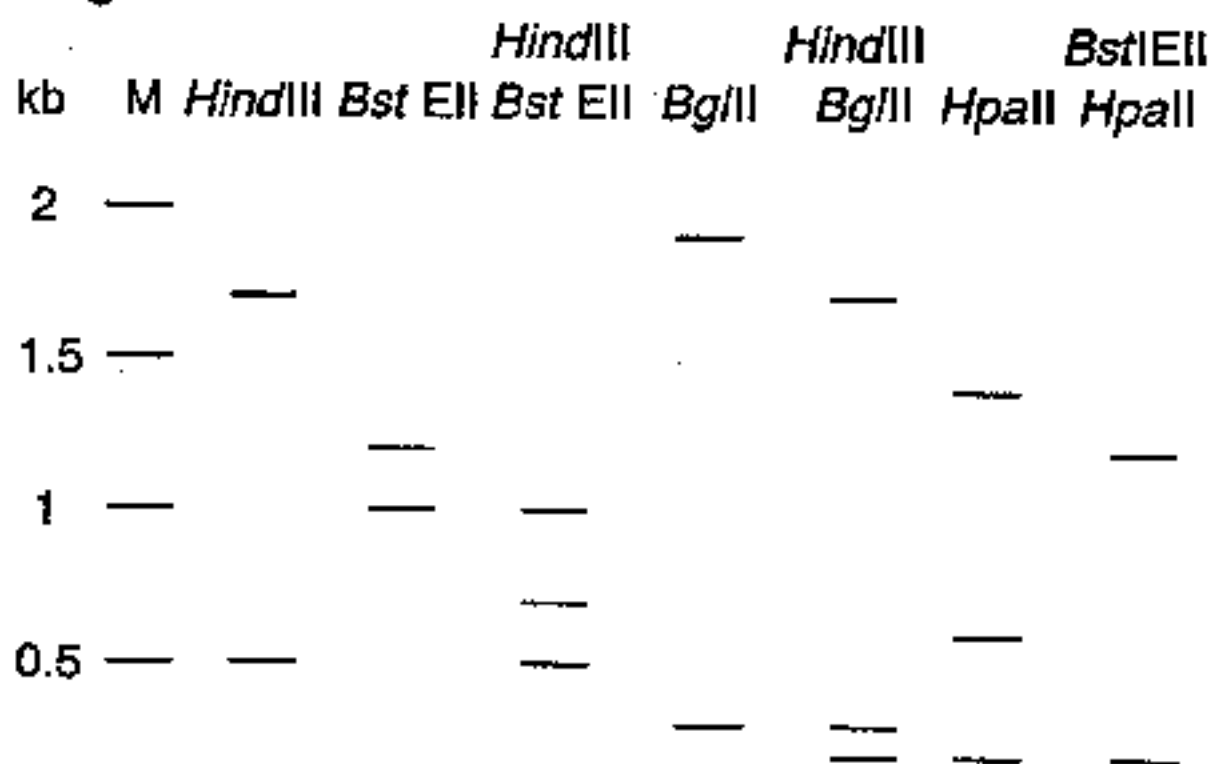


Fig. 1b

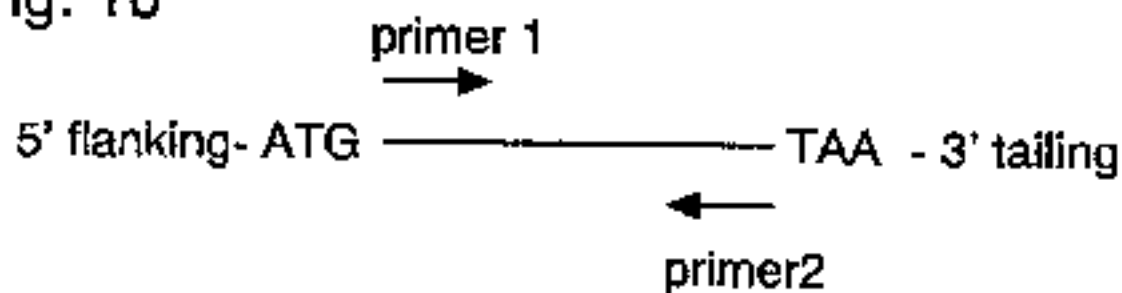


Fig. 1c

