Questions: 1－3

An experimenter generates a library of plasmids containing 10-15 kilobase (kb) inserts from the genome of a bacterium by partially digesting the bacterial genomic DNA with *Eco*RI and cloning the resulting fragments into the *Eco*RI site of a plasmid vector. The experimenter must then identify the plasmids containing the *purB* gene. To do this, 5 of the plasmids from the library were digested with *Eco*RI and the digests were separated by gel electrophoresis (Figure 1). In a second experiment, the same 5 plasmids were analyzed by PCR using primers derived from sequences internal to *purB* and electrophoresis was performed on the PCR products (Figure 2). Both gels were stained with ethidium bromide to visualize the DNA.

Figure 1. Electrophoresis of digests

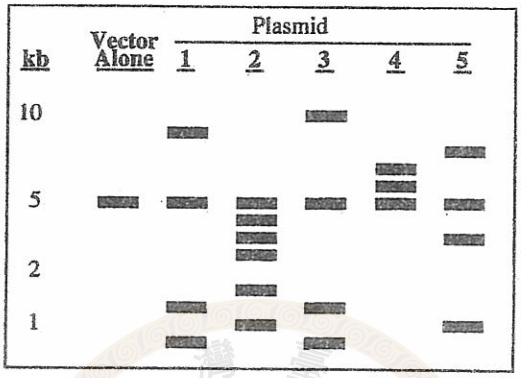
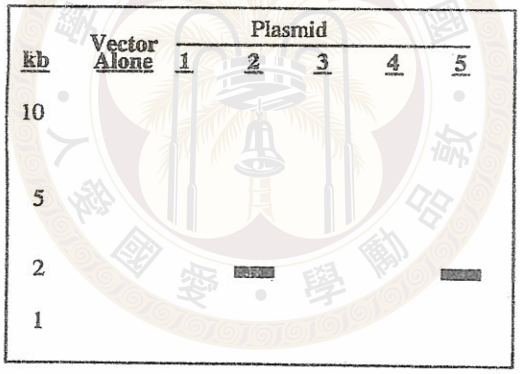


Figure 2. Electrophoresis of PCR products



1. The inserts in which of the following pairs of plasmids may overlap? (2%)
   (A) 3 with 4 only
   (B) 3 with 5 only
   (C) 1 with 2 and 3 with 4 only
   (D) 1 with 3 and 2 with 5 only
   (E) All of the inserts may overlap.

2. Which of the following methods would NOT be a useful alternative to using PCR to determine which plasmids contain *purB*? (2%)
   (A) Testing for complementation of a *purB* auxotroph
   (B) Sequencing the inserts
   (C) Hybridizing the plasmids with a probe complementary to *purB*
   (D) Mapping each plasmid with several restriction enzymes
   (E) Footprinting with DNase

3. The part of *purB* complementary to the *purB*. primers is contained in which of the following plasmids? (2%)
   (A) 2 only
   (B) 5 only
   (C) 2 and 5 only
   (D) 1, 3, and 4 only
   (E) 1, 2, 3, 4, and 5

見背面

Questions: 4－6

When normal human fibroblasts are cultured in medium containing calf serum, they divide with an average generation time of approximately 22 hours (M = 1 hr, G_1 = 10 hr, S = 6 hr, G_2 = 5 hr). To determine the effects of serum deprivation on cell cycle distribution, cells were incubated for 48 hours in medium with or without serum. At the end of this incubation, cells were harvested and stained with propidium iodide, which binds to DNA and fluoresces when exposed to ultraviolet light. The stained cells were analyzed for DNA content (fluorescence) in a flow cytometer. The results with serum are shown in Figure 1a. If deprived of serum, the cells stop proliferating and enter a quiescent state (Figure 1b).

In a second experiment, cells were deprived of serum for 48 hours and then treated either with serum alone or serum plus cycloheximide (CHX), an inhibitor of protein synthesis. At various times after treatment, RNA was isolated from the cells. Equal amounts of total cellular RNA from each sample were analyzed by gel electrophoresis and Northern blotting to detect the level of c-*fos* mRNA. The c-*fos* protein is involved in regulating cell proliferation. The results of this experiment are shown in Figure 2. ("–" indicates serum alone and "+" indicates serum plus CHX.)

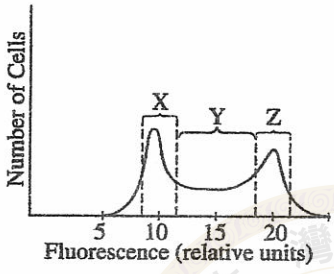


Figure 1a. With serum

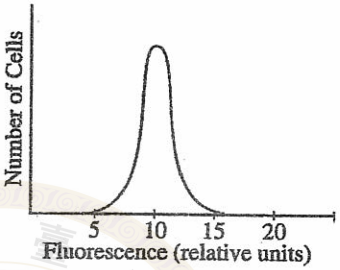


Figure 1b. Without serum

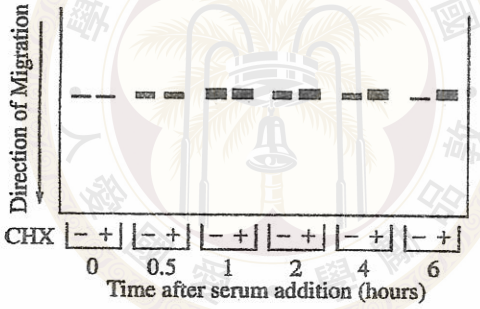


Figure 2. c-*fos* mRNA

4. In Figure 1a, the cells in the region labeled Y are in what stage of the cell cycle? (2%)
   (A) G_1
   (B) S
   (C) G_2
   (D) M
   (E) G_0

5. Cells growing in the presence of serum were labeled for 3 hours with ^{3}H-thymidine and then analyzed by flow cytometry. Which of the following regions defined in Figure 1a will contain radioactive cells? (2%)
   (A) X only
   (B) Y only
   (C) Z only
   (D) Y and Z only
   (E) X, Y, and Z

6. Based on the results shown in Figure 2, the differences in the amounts of c-*fos* mRNA in the presence *versus* the absence of cycloheximide at 2, 4, and 6 hours is best explained by which of the following? (2.5%)
   (A) c-*fos* mRNA is degraded by an unstable nuclease.
   (B) The c-*fos* promoter is regulated by an unstable transcriptional activator.
   (C) The c-*fos* protein activates its own promoter.
   (D) Splicing of c-*fos* pre-mRNA requires an unstable splicing factor.
   (E) c-*fos* mRNA is degraded by cycloheximide-induced nuclease.

接次頁

7. Which of the following coenzymes is *NOT* a prosthetic group, i.e. covalently linked to an enzyme? (2.5%)
(1) Lipoate, (2) Biotin, (3) Thiamine pyrophosphate (TPP), (4) Nicotinamide adenine dinucleotide (NAD^+)
(A) 1, 3, 4
(B) 2, 3
(C) 3, 4
(D) 1, 2, 3, 4
(E) 2, 3, 4

8. Which of the following monosaccharide is a component of the carbohydrate moiety of proteoglycans and glycoproteins? (2.5%)
(1) Glucose, (2) Galactose, (3) Ribose, (4) Xylose, (5) Mannose
(A) 1, 3, 4
(B) 1, 2, 4, 5
(C) 2, 5
(D) 1, 2, 3, 4
(E) 2, 3, 4

9. Which of the following coenzymes is/are required for citric acid cycle? (2.5%)
(1) Coenzyme A, (2) Biotin, (3) Flavin adenine dinucleotide (FAD), (4) Nicotinamide adenine dinucleotide (NAD^+)
(A) 1, 3, 4
(B) 2, 3
(C) 2, 4
(D) 1, 2, 3, 4
(E) 2, 3, 4

10. Which of the following statements is/are *CORRECT*? (2.5%)
(1) Human cells can only metabolize D-isoforms of monosaccharides.
(2) D-glucose and D-mannose are epimers of each other.
(3) The most abundant form of D-glucose in aqueous solution is β-D-glucopyranose.
(A) 1
(B) 1, 3
(C) 2, 3
(D) 1, 2
(E) 1, 2, 3

11. Antibodies are gamma-globulin proteins secreted by B lymphocytes to identify and neutralize pathogens such as bacteria and viruses. Antibodies are also very useful experimental tools for biologists. What kind of experimental assays require antibodies as a reagent? (2.5%)
(1) Immunoblotting, (2) Immunofluorescence, (3) Enzyme-linked immunosorbent assay (ELISA), (4) Immunoprecipitation, (5) Immunoelectron microscopy.
(A) 1, 2
(B) 1, 2, 3
(C) 2, 3, 4, 5
(D) 1, 2, 3, 4
(E) 1, 2, 3, 4, 5

12. What is the protein secondary structure? Please describe the most common secondary structures in proteins and how to characterize them experimentally? (6%)

13. Proteomics has been used in cancer research, briefly describe how to design the approach? (6.5%)

14. Please describe at least two molecular mechanisms of glutamate functions on cells. (12.5%)

15. Briefly describe the differences between prokaryotic and eukaryotic translation in (A) mRNA processing for translation, (B) translational initiation. (6.5%)

見背面

16. Describe the following terms. (6%)
    (A) Eukaryotic DNA-dependent RNA polymerase I
    (B) Telomerase
    (C) *Cis*-elements in transcriptional regulation

17. What is acyl-CoA:cholesterol acyl transferase? Please explain the functional role of this enzyme. (5.5%)

18. How dietary triacylglycerols are digested, absorbed and transported to adipose tissue and liver? (7%)

19. What are the three major sources of high-energy phosphates which take part in energy conservation or energy capture? (4.5%)

20. How is pyruvate moved from muscle cells to liver cells in human body? (2%)

21. What are the three ways used by animals to convert α-amino nitrogen to varied end products? (3%)

22. What are the three key enzymes involved in amino acid biosynthesis in human? (3%)

23. The Michaelis-Menton equation for competitive inhibition is shown below. Based on this equation, please write down the apparent Vmax ____(A)____ (2%) and apparent Km ____(B)____ (2%) for competitive inhibition. When this equation is transformed into the so-called "double-reciprocal plot" or "Lineweaver-Burk plot", the slope is ____(C)____ (1%) and the Y-intercept is ____(D)____ (1%). (total 6%)

    Michaelis-Menton equation for competitive inhibition: $$Vo = \frac{Vmax\,[S]}{\alpha Km + [S]}$$

24. Briefly explain why K_m can be used to approximate the dissociation constant of the ES complex when $k_2 << k_{-1}$. (3%)

25. Please describe the essential features of a plasmid that can be used to overexpress recombinant protein in *E. coli*. (3.5%)

試題隨卷繳回