※ 考生請注意：本試題不可使用計算機。 請於答案卷(卡)作答，於本試題紙上作答者，不予計分。

1. Please explain why sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) can be used to determine the molecular weight of unknown proteins. (10%)

2. When we are generating monoclonal antibodies, we need to use hypoxanthine-aminopterin-thymidine (HAT) medium to select hybridoma cells. Please explain the mechanism why HAT medium can be used to select hybridoma. (10%)

3. Please draw a diagram and explain the pattern of normal human serum protein electrophoresis. (5%)

4. Describe the enzymes involved in lipoprotein metabolism. What are the locations of these enzymes? What are the substrate, cofactor (if any) and product of their respective actions? (8 points)

5. Describe Cori cycle and its physiological role.(5 points)

6. 說明 apo E 和 Alzheimer disease 的關係。(6 points)

7. 說明維生素 D 的生理功能，並說明何種血漿檢驗最能反映維生素 D 在人體內的濃度以及其檢測方法。(6 points)

8. 請簡述下列名詞及其相關分子機制：
   (1) lac operon (8 分)
   (2) microRNA (biogenesis and function) (8 分)
   (3) Protein translation (initiation, elongation, and termination) (9 分)

9. An abrupt temperature downshift may induce a cold-shock response in both prokaryotes and eukaryotes. During times of temperature decrease from 37℃ to 10℃, CspA, the major cold-shock protein of *Escherichia coli* is expressed up to 10% or more of the total cellular protein. Please read the following ABSTRACT regarding one study on CspA and then answer the questions.

ABSTRACT

When the gene for CspA, the major cold shock protein of Escherichia coli, was disrupted by a novel positive/negative selection method, the deltacspA cells did not show any discernible growth defect at either 37 or 10 degrees C. By two-dimensional gel electrophoresis, total protein synthesis was analyzed after temperature downshift in the deltacspA strain. The production of the CspA homologs CspB and CspG increased, and the duration of their expression was prolonged, suggesting that both CspB and CspG compensate for the function of CspA in the absence of CspA during cold shock adaptation. Interestingly, the production of the 159-base 5'-untranslated region (5'-UTR) of cspA from the

(背面仍有題目,請繼續作答)

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chromosomal cspA::cat gene, detected by primer extension, failed to be repressed after cold shock. When an independent system to produce CspA was added to the deltacspA strain, the 5'-UTR production for the cspA::cat gene was significantly reduced compared to that of the deltacspA strain. By examining the expression of translationally fused cspA and cspB genes to lacZ in the deltacspA strain, it was found that cspA is more strongly regulated by CspA than cspB is. We showed that the increased expression of the 5'-UTR of the cspA mRNA in the deltacspA strain occurred mainly at the level of transcription and, to a certain extent, at the level of mRNA stabilization. The mRNA stabilization in the deltacspA strain was observed for other mRNAs, supporting the notion that CspA functions as an mRNA chaperone to destabilize secondary structures in mRNAs.

Questions

(1) What did the authors find when the CspA knockout *E. coli* cells were cultured in a low temperature? (5%)

(2) What did the CspA protein act on its own 159-base 5'-UTR? (5%)

(3) Please describe one kind of possible techniques to produce CspA. (5%)

(4) Please describe one kind of possible methods to clone CspA gene in fusion with lacZ. (5%)

(5) What is the major finding of this study? (5%)