Student Name : _____ 2013-10-03
Student ID : _____

Instructions:

Write neatly and clearly. Cross out with a single line any material you do not wish to have marked. Marks will be deducted for incorrect statements. Students must work independently and may not knowingly utilize resource materials or share resource materials with other students. Students may use pens, pencils, erasers and calculators only.

Electronic devices including cell phones, personal information managers and audio devices are prohibited.

Question	Mark	Total Marks
1		9
2		6
3		4
4		4
5		5
6		6
Total		**34**

(1) In the space provided, give short answers to the following questions: (10 marks)

(a) Explain the acronym MALDI. What does MALDI accomplish?

Matrix assisted laser desorption ionization is a method for producing gas-phase proteins that contain a small number of additional positive charges (protons).

(b) How are amino terminal blocking groups selectively removed during the chemical synthesis of peptides?

Anhydrous acid (TFA) will break the amine ester linkages to the N-terminal amine but will not break other ester or alcohol based linkages.

(c) What type of gel matrix would be optimal for separating polypeptides of 10 kDa?

18% PAGE

(d) What is Pulse Field Gel Electrophoresis and how does it separate nucleic acids?

Electrophoresis technique for separating very large molecules (eg. Genomic DNA). Using two orthoganol sets of electrodes, the electrical current is switched between the vertical and the horizontal direction. Separation is based upon reorientation time as opposed to filtration.

(2) You have subjected a highly purified polypeptide (below) containing containing 3 Arg/Lys residues and 3 Phe/Tyr residues to limited digestion using chymotrypsin. (6 marks)

$(X)_5$ Arg $(X)_{20}$ Phe $(X)_{20}$ Arg $(X)_{10}$ Tyr $(X)_{10}$ Lys $(X)_{30}$ Tyr $(X)_{10}$

(a) What products would you expect to generate?

1. $(X)_5$ Arg $(X)_{20}$ Phe
2. $(X)_{20}$ Arg $(X)_{10}$ Tyr $(X)_{10}$ Lys $(X)_{30}$ Tyr $(X)_{10}$
3. $(X)_5$ Arg $(X)_{20}$ Phe $(X)_{20}$ Arg $(X)_{10}$ Tyr
4. $(X)_{10}$ Lys $(X)_{30}$ Tyr $(X)_{10}$
5. $(X)_5$ Arg $(X)_{20}$ Phe $(X)_{20}$ Arg $(X)_{10}$ Tyr $(X)_{10}$ Lys $(X)_{30}$ Tyr
6. $(X)_{10}$
7. $(X)_{20}$ Arg $(X)_{10}$ Tyr
8. $(X)_{20}$ Arg $(X)_{10}$ Tyr $(X)_{10}$ Lys $(X)_{30}$ Tyr
9. $(X)_{10}$ Lys $(X)_{30}$ Tyr

(b) If you only observe five products in the limited proteolysis, what is the most likely explanation?

This would occur if one of the aromatic residues could not be cut due to lack of accessibility.

(3) You have recently isolated a novel enzyme that hydrolzyes cell-wall polysaccharides from a natural source. Not having any primary sequence information you treat an aliquots of the enzyme with N-ethyl maleimide and another with diethylpyrocarbamate. Only the aliquot treated with diethylpyrocarbamate retains hydrolytic activity. What can you conclude from these simple experiments?(4 marks)

Diethylpyrocarbamate modifies Histidine residues. As modification of His does not impair function we are unlikely to have a His residue that is participates in the hydrolytic mechanism. N-ethylmaleimide modifies Cys residue. As modifications of this amino acid impair hydrolytic function a Cys residues either participates in the hydrolytic mechanism or is located near to the active site.

(4) Can an unknown protein with a pI of 9.0 be visualized using native PAGE? Explain? (4 marks)

Yes.

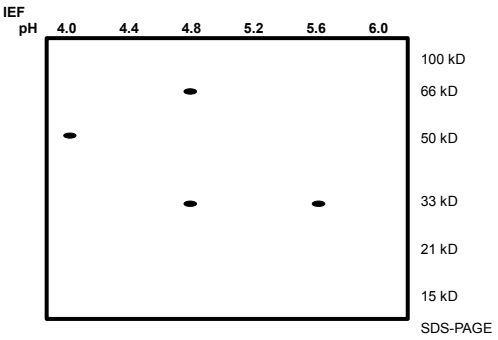
Using normal native PAGE buffers (pH 8.8), the net charge of the unknown protein is slightly positive and it will not enter the gel, unless the electrodes are switched (anode at top). In order to increase the net positive charge and allow the protein to move within the gel, low pH buffers are required.

(5) Box C/D RNA contain a conserved CUGAUGA sequence. You suspect one or more proteins cellular proteins specifically bind to the conserved sequence. Describe a strategy for purifying the cellular proteins that bind the conserved sequence of Box C/D RNAs. (5 marks)

The most efficient purification strategy is affinity chromatography. Covalently attaching the CUGAUGA sequence to an inert bead provides an affinity tag. Lysing cells and passing the soluble fraction through the affinity column will allow all cellular proteins that bind to bind.

To elute the bound sample, simply add excess CUGAUGA

(6) Consider the following 2D gel separation of an unknown protein mixture. The isoelectric focussing dimension was run in the pH range 4-6 followed by an SDS-PAGE separation in the vertical dimension. (6 marks)



a) What can be concluded about the number of proteins are present in the unknown protein mixture?

At minimum, there are 3 protein samples (at pI 4, 4.8 and 5.6) though there may be 4 (we do not know if the 2 bands at pI 4.8 are one protein or two AND we don't know if the bands can form oligomers).

b) Assume the identical unknown protein mixture is subjected to size exclusion chromatography and produces a single peak with an apparant molecular mass of 100 kD. What can we now conclude about the size and subunit composition of each protein in the mixture?

Knowing that each of these proteins must have a molecular mass of 100 kD from the SEC, and knowing their are a minimum of 3 proteins, the simplest interpretation is there are only three proteins with the following composition.

pI 4.0 band would correspond to a dimer (50 kD x 2 = 100 kD)

pI 5.6 band would correspond to a trimer (33 kD x 3 = 99 kD)

pI 4.8 band would correspond to a heterodimer. (66 kD + 33 kD = 99 kD)

(Note: Given the resolution and errors associated with SEC, 100 kD and 99 kD cannot be distinguished from one another)