BITS PILANI – DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
SECOND SEMESTER 2010 – 2011
BIOT C344 PROTEOMICS
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 3hours | **Date: 02.06.2011**
Weightage: 40% | **Max. Marks: 40**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. Briefly discuss on the principle and operation of Electrospray Ionization (ESI) with a suitable diagram and differentiate with Matrix Assisted Laser Desorption Ionization (MALDI). Illustrate with suitable diagrams. [3.0]
2. What are mass analyzers and list the different types available for mass analysis? Explain Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) with a suitable diagram. [3.0]
3. Write the principle of the 2D PAGE. Explain in detail on: [6.0]
   a. Sample preparation
   b. First Dimension Systems
   c. Second dimension Systems
   d. Protein visualization methods
   e. Detection of specific groups of proteins
   f. Image analysis
4. Write a short note on functional proteomics and comparative proteomics. How isotope-coded affinity tags (ICAT) are used in quantitative proteomics for protein identification? Explain with a suitable diagram. [4.0]
5. What are glycoproteins and mentions its role in cellular functions in eukaryotes. Briefly explain on proteome-wide identification of glycoproteins and methods involved. [3.0]
6. Write a short note on multidimensional protein identification (MudPIT) technology in proteomics. How MudPIT will be used for the analysis of yeast whole cell lysates? Compare the probable results from MudPIT with 2D PAGE analysis. [4.0]
7. What is tandem affinity purification (TAP)- TAG approach in proteomics? How TAP-TAG is different from Immobilized Metal Affinity Chromatography (IMAC)? Explain the two methods with suitable diagrams for each. [3.0]
8. Briefly explain the following on Calmodulin (Cmd1): [3.0]
   a. What is calmodulin (Cmd1)?
   b. Mention the predicted protein partners of calmodulin (Cmd1). Mention at least any five functions, and protein partners involved in each category.
   c. How the Cmd1 can be indentified with respect to its specific functions in the cell? Explain with a suitable method for Cmd1 localization.
9. Explain Fluorescence Resonance Enegy Transfer with a suitable diagram with specific proteins involved. Why Green Fluorescent protein is not useful in FRET? What the principles by which FRET is measured? Mention any five cellular functions where FRET can be used? [4.0]
10. What is the role of phosphotransferase system in bacteria? Briefly explain on the components and functions of the phosphotransferase system (PTS), and methods for protein analysis and identification. [2.0]

PTO

11. Write a short note on Protein microarrays and compare with DNA microarrays. Why protein microarrays are required and mention the limitations of mRNA based array techniques in biological sample analysis? Mention advantages and disadvantages of each application in proteome and genome analysis. [3.0]
12. Briefly discuss on the following: [2.0]
    a. Differentiate between the genetic analyses and proteomic analysis in clinical diagnosis.
    b. Biomarkers, characteristics and weaknesses of current cancer biomarkers
    c. Surface enhanced Laser Desorption ionization time-of-flight mass spectrometry (SELDI-TOF/MS).
    d. Differences between SELDI and Matrix Assisted Laser Desorption Isonization time-of-flight mass spectrometry (MALDI-TOF/MS).

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SECOND SEMESTER 2010 – 2011
BIOT C344 PROTEOMICS TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 10.4.2011**
Weightage: 20% **Max. Marks: 20**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

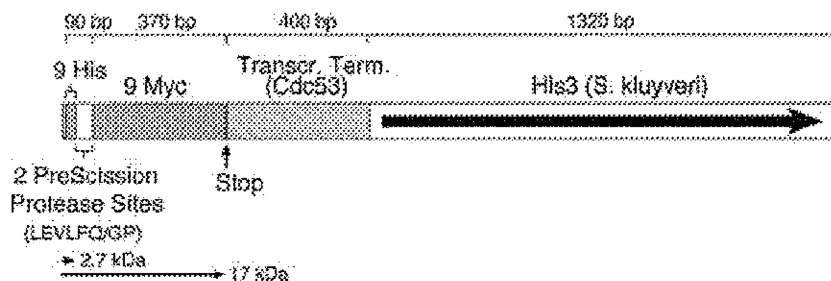
1. A combined multidimensional chromatography-mass spectrometry approach known as MudPIT" enables rapid identification of proteins that interact with a tagged bait while bypassing some of the problems associated with analysis of polypeptides excised from SDS-polyacrylamide gels. However, the reproducibility, success rate, and applicability of MudPIT to the rapid characterization of dozens of proteins have not been reported. Based on your understanding with MudPIT answer the following: [8.0]

   a. Is it possible to get a reproducible data on bona fide partners for budding yeast Gcn5p by using MudPIT? Justify with your answer with suitable explanation.

   b. Can we use tagged polypeptides to identify new protein interactions? If so how many different kinds of tagged proteins can be expressed in a cell? For example, proteins involved in transcription and progression through mitosis with a new tandem affinity purification (TAP) tag. Explain.

   c. Is it feasible for an investigator working with a single ion trap instrument in a conventional molecular/cellular biology laboratory to carry out proteomic characterization of a pathway, organelle, or process (*i.e.* "pathway proteomics") by systematic application of TAP-MudPIT?

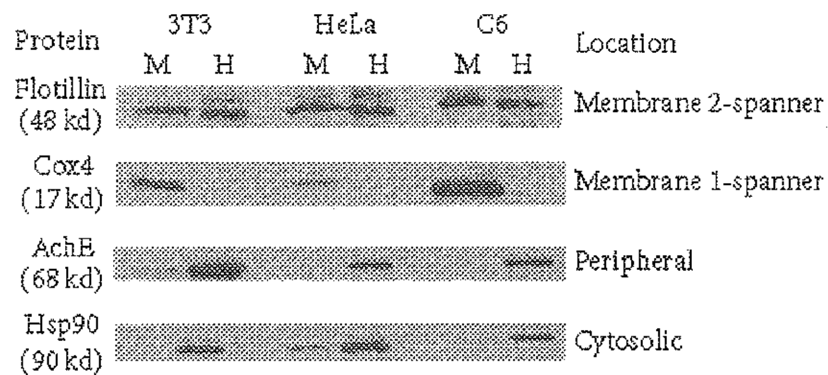
   d. Chromosomal integration gene cassette is given below. If expressed in a mutant *S. cerevisiae* strains, how will you identify whether it is expressed or not and explain with a suitable method to purify the protein with a schematic diagram.



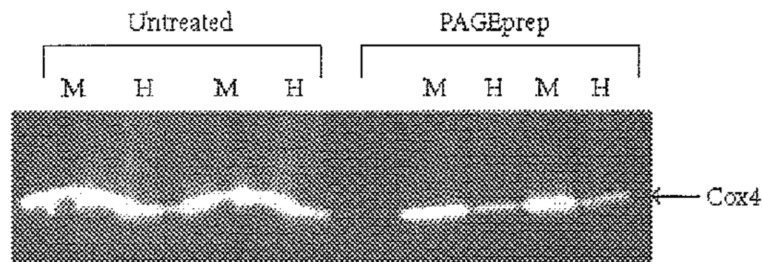
2. Differentiate Multidimensional Protein Identification Technology (MudPIT) with 2D and 1D PAGE based Protein identifications (principle, advantages and disadvantages). [3.0]

3. Based on the sequences from several genomes, transmembrane proteins have been predicted to comprise approximately 30% of eukaryotic proteomes. Membrane proteins are the most elusive and the most sought after proteins in drug discovery. They play a key role in signal transduction, cell adhesion, and ion transport and are important pharmacological targets. Yet, because of their hydrophobic and basic nature, and frequently large size, their isolation is not easy. Traditional methods for membrane isolation are often cumbersome and protein yields are poor. Although proteomics technologies have made rapid progress in the characterization and investigation of soluble proteins in recent years, MPs have lagged behind. The major challenge of membrane proteome study is the low solubility due to the complex structure and hydrophobic nature of the membrane proteins and their low abundance. Therefore, new strategies for the identification and characterization of these special kinds of proteins are of great interest in modern proteomic research. [6.0]

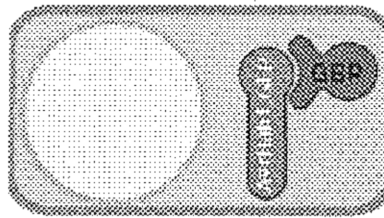
   a. With the help of the following diagram what information you derive out in terms of protein expression and localization. Briefly explain.



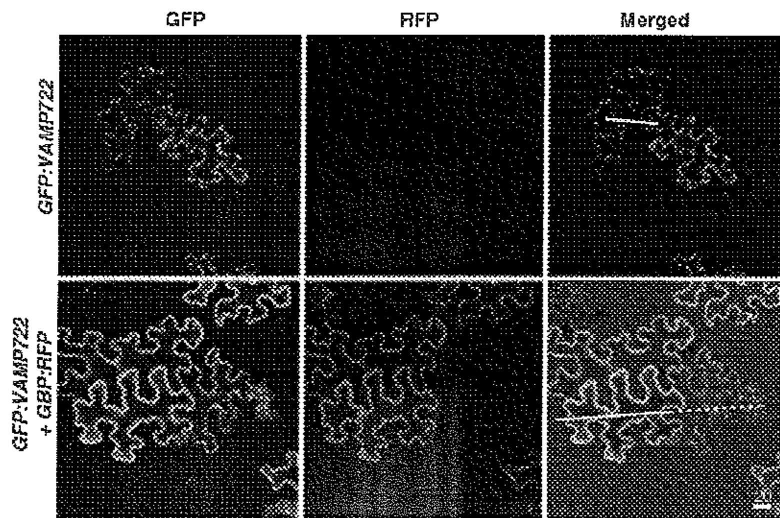
   b. What are the different methods which can be specifically used to purify membrane proteins and list each one with advantages and disadvantages including the one given as an example? How the PAGEprep is advantageous over the other methods? Justify with your answer.



4. A key challenge in cell biology is to directly link protein localization to function. The green fluorescent protein (GFP)-binding protein, GBP, is a 13-kDa soluble protein derived from a Llama heavy chain antibody that binds with high affinity to GFP as well as to some GFP variants such as yellow fluorescent protein (YFP). A GBP fusion to the red fluorescent protein (RFP), a molecule termed a chromobody can be used to trace in vivo the localization of various protein antigens (chromobody technology). The *Agrobacterium tumefaciens*-mediated transient expression assays (agro-infiltration) and virus expression vectors (agroinfection) was used to to express functional GBP:RFP fusion (chromobody) in the model plant *Nicotiana benthamiana*. Refer to the interactions as below: [3.0]



The Confocal imaging of green fluorescent protein-binding protein:red fluorescent protein (GBP:RFP) (bright) and GFP:VAMP722 (grey) 2 days after agroinfiltration of *Nicotiana benthamiana* leaf tissue is provided. What is your inference in the findings? Briefly explain.



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II SEMESTER 2010 – 2011
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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 20.2.2011**
Weightage: 25% **Max. Marks: 25**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. Explain in detail any two ionization methods used in mass spectrometry with suitable diagrams. [4.0]

2. What is the principle involved in the ion-trap mass spectrometer and mention how the ions are trapped for the MS/MS analysis? Briefly mention the significance of ion-trap mass spectrometer in analyzing the complex mixture of proteins. [3.0]

3. Differentiate between the Time-of-Flight Mass spectrometer and the Quadrupole Time-of-Flight Mass spectrometer. Draw suitable diagrams if required. [4.0]

4. What is the principle of Fourier Transform Ion Cyclotron Resonance mass spectrometry? Explain with a schematic representation. [3.0]

5. Why pre-gel fractionation is preferred in 2D-PAGE analysis? Explain with at least any two methods. [3.0]

6. Write a short note on sample preparation and chemical components of a standard 2D PAGE buffer and mention each of their functions. [2.0]

7. Differentiate between conventional IEF, NEPHGE and Immobilized pH gradients. [3.0]

8. Explain with any two examples for the detection of specific groups of proteins through ligand blot overlay technique. [3.0]

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 14.3.2011**
Weightage: 8% **Max. Marks: 8**

Name:

ID No:

Note: Answer to the the point

1. Write the principle of Isotope-Coded Affinity tag strategy used in proteomics. [2.0]

2. How the ^{15}N incorporation and labeled amino acids are used in identification of proteins? [2.0]

3. What is the role of phosphorylation and glycosylation in protein functions? How it is identified? [2.0]

4. Write a short note on proteome-wide identification of glycoproteins. [2.0]