BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI
BITS PILANI - DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2011 – 2012
BIOT C344 PROTEOMICS
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 3hours **Date: 02.01.2012**
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 Desorption Electrospray Ionization (DESI) and Matrix Assisted Laser Desorption Ionization (MALDI) methods. How the Electrospray Ionization is different from DESI? Illustrate with suitable diagrams. [3+1=4.0]
2. What are mass analyzers and how the mass analyzer is coupled with the ionization source? Give Explain with respect to MALDI-TOF and Q-TOF. Provide schematic diagram for each. [1+3=4.0]
3. Briefly explain the basic principle of 2D PAGE, ligand blot assay and 2D-PAGE gel profiling of post translational modifications (PTM) of proteins with examples. [3.0]
4. Labeled amino acids play a major role in detection of proteins. Explain with suitable examples on any one method describing incorporation of labeled amino acids for protein identification. How the ICAT is different from the detection of labeled proteins as above? [2+1=3.0]
5. What are the role of phosphorylation and glycosylation in a cell? Give a brief account on identification of phosphorylated and glycosylated proteins in a cell. [1+2=3.0]
6. What is the principle of MudPIT? Give any two applications of MudPIT in identification of protein complexes. [1+3=4.0]
7. Why the proteins need to be fractionated prior to identification? Give different principles employed for protein fractionation and examples/methods for each. [1+2=3.0]
8. What are the different reporter genes used in proteomics and give a brief account on any one application? [2.0]
9. What are the basic differences between protein and protein complex identification? If you want to identify protein complexes what are the requirements with respect to your mass analyzer? [1.5+1.5=3.0]
10. Differentiate the X-ray diffraction and NMR and its applications in proteomics? [2.0]
11. What is the principle of Dual Color Labeling approach in microarray analysis? Explain with a suitable diagram. [1+2=3.0]
12. Write a short note on microfluidics and single cell proteomics. [2.0]
13. Write a short note on the following with examples: [2.0]
    a. Cancer biomarkers
    b. Biomarkers of neurological abnormalities
    c. Ovacheck
    d. Infectious diseases.
14. You have been exposed to different areas of proteomics, detection methods and applications in biotechnology, cancer genetics and biomedical applications. In case you have become a researcher in any of the above areas, how will you approach your problem with the help of genome data, proteins and DNA/Protein microarray? With your overall exposure to different areas in biology, what is the role of proteomics in biological discovery? Briefly discuss with respect to automation, data acquisition and informatics tools in proteomics. [2.0]

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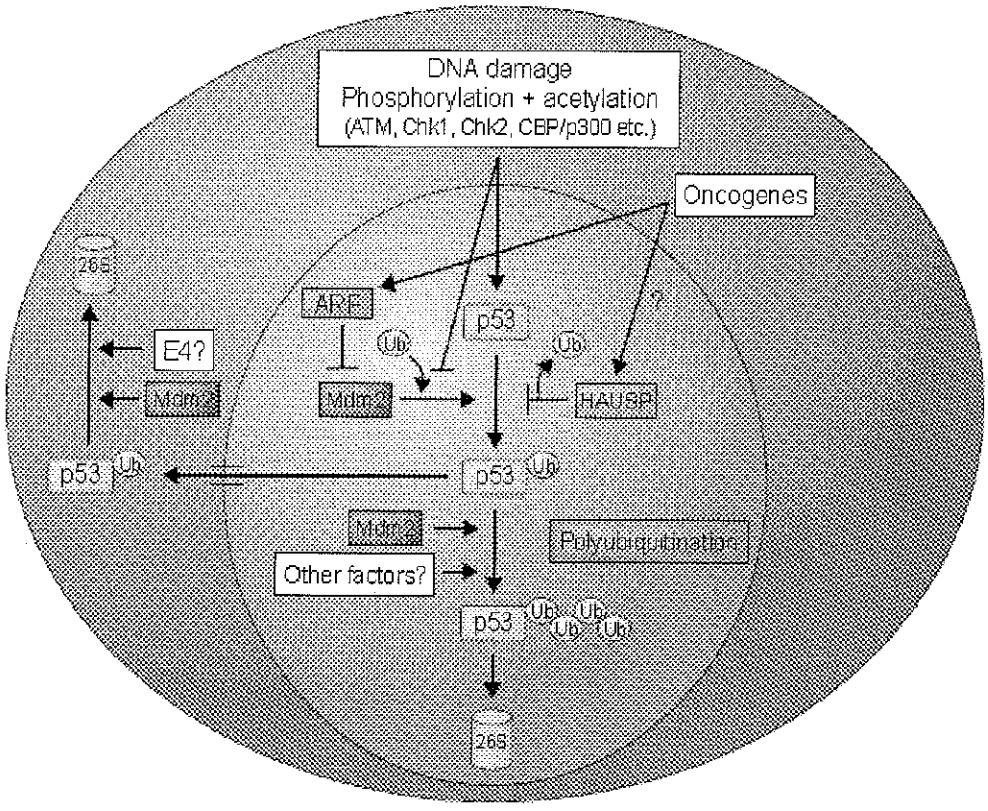
TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 13.11.2011**
Weightage: 20% **Max. Marks: 20**

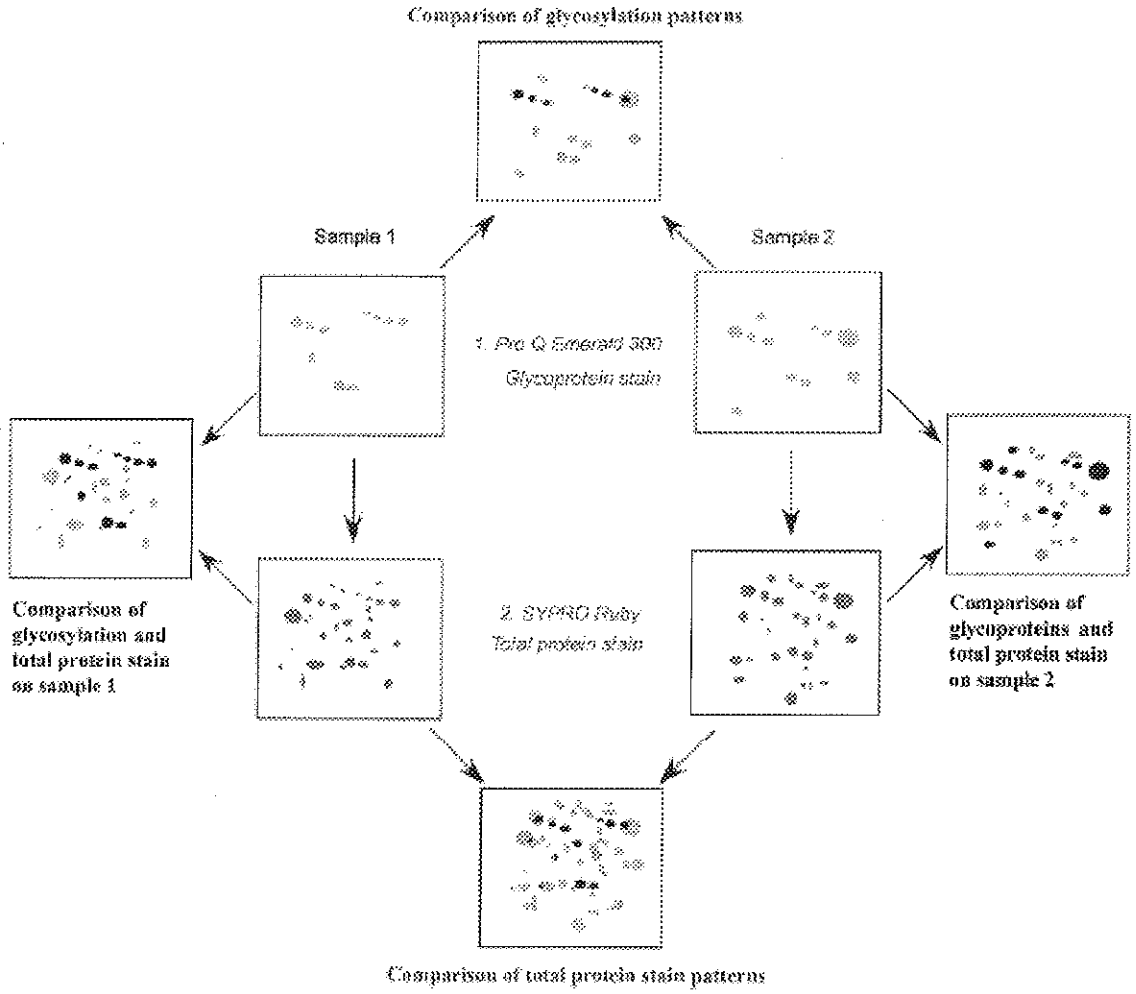
Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. Protein phosphorylation during the process of p53 stabilization is given below. Several mechanisms for regulating p53 protein levels are depicted here. The p53 is ubiquitinated within the nucleus by Mdm2 and possibly other factors which leads to its degradation by the 26S proteosome either in or out of the nucleus. DNA damage-induced upstream factors can block this cascade of events by targeting Mdm2 and p53. ARF and HAUSP represent antagonists of Mdm2 in this process. The mechanism of oncogenic activation of HAUSP and the role of other factors including E4 ligases in Mdm2-mediated ubiquitination of p53 are yet to be elucidated. Based on your understanding with protein phosphorylation answer the following: [6.0]



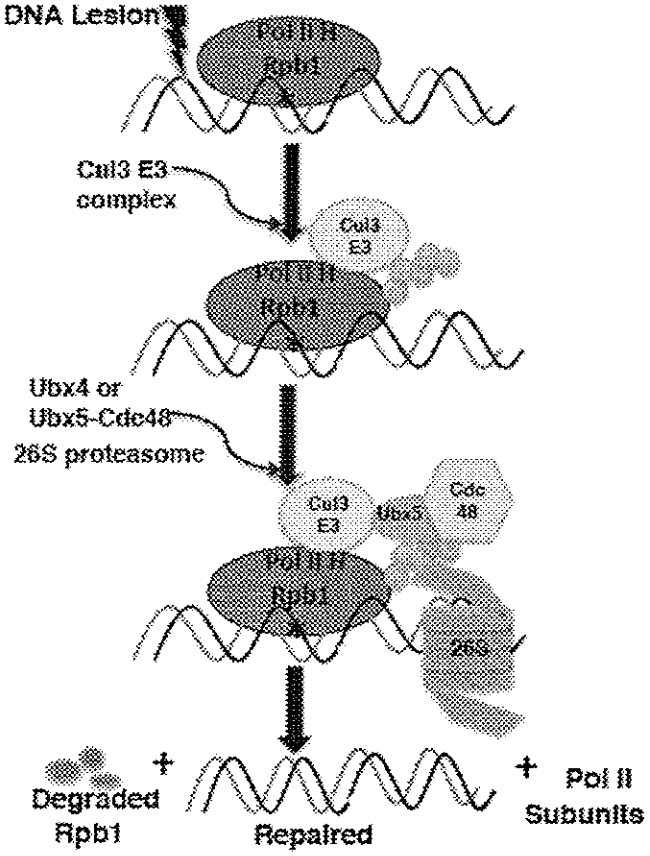
a. How will you purify the phosphoproteins and phosphopeptides and name the proteins which undergo phosphorylation?
b. How will you detect the phophoproteins and elaborate the techniques?
c. What are your conclusions on the post-translational modifications of proteins in this cascade?

2. Two different protein samples are run on two 2-D gels as shown below and the gels are stained for glycosylation using Pro-Q Emerald 300 dye and imaged. Gels are then stained for total protein using SYPRO Ruby dye and imaged again. Differences in glycosylation and protein expression are identified by evaluation of pseudo-colored differential display maps. [6.0]



a. With the help of the above diagram what information you derive out in terms of protein expression patterns of the sample 1 and sample 2 and identification. Briefly explain.
b. What are the different methods which can be specifically used to purify glycoproteins and explain the one which is most suitable with advantages and disadvantages including the one given as an example?
c. How will you proceed for the detection of N-linked and O-linked glycoproteins?

3. The DNA damage response in cells is given below. The Rpb1 subunit of RNA Pol II holoenzyme (H) irreversibly stalled at sites of DNA lesions is ubiquitinated by the Cul3–RING ligase complex. UbRpb1 can independently recruit proteasome and Ubx5-Cdc48 complexes. UbRpb1 is extracted from its binding partners in an unfolding reaction dependent on Ubx4 or Ubx5-Cdc48 and is threaded into the 26S proteasome. It is observed that there are numerous proteins accumulated on proteasomes in *cdc48-3* mutants. Based on your understanding with protein identification by MudPIT answer the following: [5.0]



a. What are the protein complexes you will be identifying based on MudPIT and mention brief outline on the procedure you follow for protein complexes identification?
b. How will you purify the protein complexes for MudPIT analysis and reason out why you need to instead of total cell lysate?
c. What is your understanding on the above cascade in cellular pathway and explain mechanisms?

4. Briefly explain with suitable diagrams. [3.0]
   a. Isolation methods for subcellular organelles
   b. Charged proteins
   c. Protein isolation methods based on isoelectric point

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 25.9.2011**
Weightage: 25% **Max. Marks: 25**

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

1. What is peptide mapping for protein identification? Mention the importance of in silico protein sequence data and theorical tryptic peptides and the peptide mass library. Give schematic representations. [4.0]

2. What is difference between MS and tandem MS or MS/MS. Explain. [3.0]

3. Explain the following peptide ionization for protein identification with suitable diagrams and mention advantages of each methods. (a) MALDI and (b) ESI [6.0]

4. What is the difference between Fourier Transform Ion Cyclotron Resonance mass spectrometry and Quadrupole Time-of-Flight Mass spectrometer (QqTOF)? Explain with a schematic representation. [6.0]

5. Differentiate between 1D PAGE and 2D PAGE for protein analysis. [4.0]

6. Briefly explain on different protein detection methods for 2D PAGE gels. [2.0]

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QUIZ-II (CLOSED BOOK)

Duration: 20 min. **Date: 19.12.2011** **Max. Marks: 7**

Name:

ID No:

Note: Answer to the point

1. Write any four points on Green Fluorescent Protein (GFP) as a protein localization tool in proteomics research. [1.0]

2. What is Cmd1, and mention any two roles from the interaction map. [1.0]

3. How the non covalent interactions of the protein complexes are maintained during characterization of functional protein complexes using mass spectrometry. [1.0]

4. What is collisional cooling in TOF analysis of macromolecular complexes. [1.0]

5. What is degradosome, and mention its functions? [1.0]

6. What is the composition of RNA polymerase protein complex, mention the subunit composition and interaction partners with RNA polymerase? [1.0]

7. How many different proteins are present in the *E. coli* ribosome complex and how these are present as an intact single protein assembly as ribosome? [1.0]

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 10.10.2011** **Max. Marks: 8**

Name:

ID No:

Note: Answer to the point

1. Write any one method to identify proteins on a ligand blot overlay or affinity blotting on a nitrocellulose membrane. [1.0]

2. Write any four image analysis software packages for 2D Protein gel. [1.0]

3. Why the pre-gel fractionation is required in protein separation by gel electrophoresis. Name any two methods. [1.5]

4. Write the principle of Isotope-coded affinity tag method for protein identification. [1.5]

5. Write the difference between in situ and in vivo metabolic labeling. Give example. [1.5]

6. Briefly write on a method of protein identification involving cells growing in culture medium with natural isotopic abundance of amino acids and the other with one or more isotopically substituted aminoacids . [1.5]