

# Unit 4 - Week-3: Two-dimensional gel electrophoresis (2-DE)

## Course outline

### How to access the portal ?

### Week-1: Basics of proteins and proteomics

### Week-2: Gel-based proteomics

### Week-3: Two-dimensional gel electrophoresis (2-DE)

L11. 2-DE: Second dimension, staining & destaining

L12. 2-DE: Gel analysis

L13. 2-DE Applications

L14. 2-DE Applications (contd.) & Challenges

L15. Lab session - Protein/peptide pre-fractionation using OFFGEL FRACTIONATOR & data analysis

Download Videos

Weekly Feedback

Quiz : Week-3 Assignment

Assignment-3 Solutions

### Week-4: Difference in gel electrophoresis (DIGE) & Systems Biology

### Week-5: Basics of mass spectrometry

### Week-6: Basics of mass spectrometry and sample preparation

### Week-7: Quantitative Proteomics

### Week-8: Advancement in Proteomics

### Text Transcripts

## Week-3 Assignment

The due date for submitting this assignment has passed. As per our records you have not submitted this assignment.

**Due on 2019-09-18, 23:59 IST.**

### Week-3 Assignment

1) After isoelectric focussing in 2-Dimensional Electrophoresis, the IPG strips are equilibrated. Herein the equilibration is a conditioning process that involves which of the following? **1 point**

- Coating of proteins with SDS and breaking of disulphide bonds with Dithiothreitol
- Alkylation of Sulphydryl groups of Cysteine residues
- Both a and b
- None of the above

No, the answer is incorrect. Score: 0

Accepted Answers: Both a and b

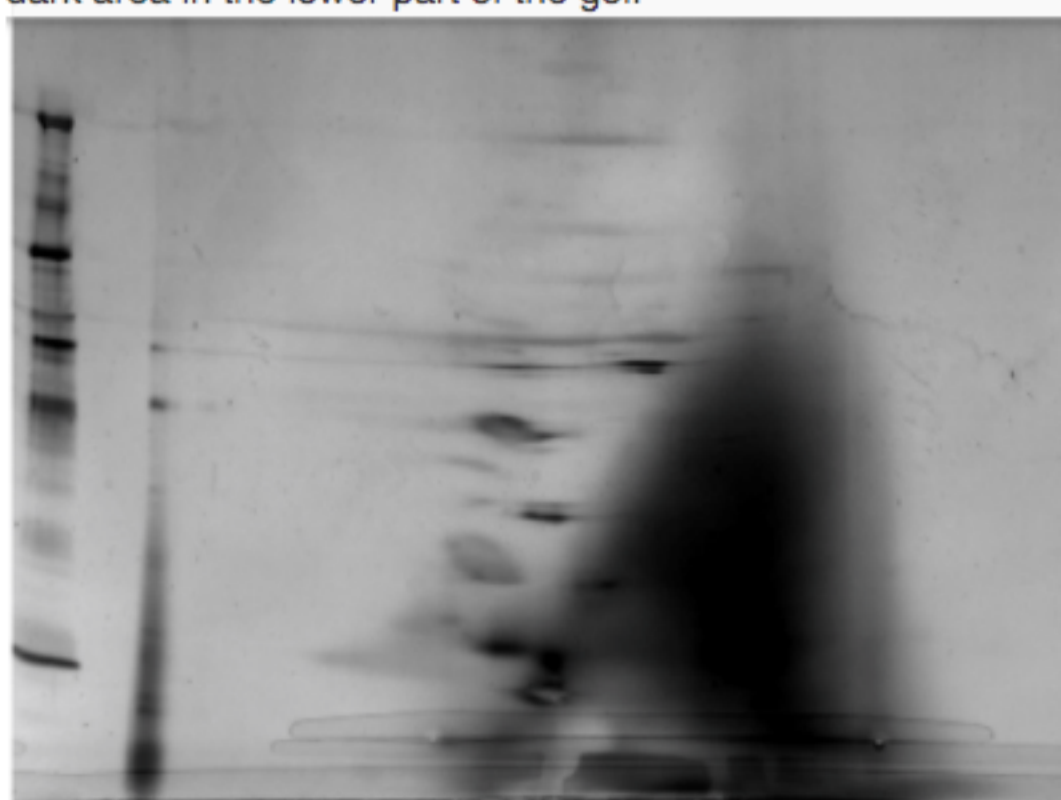
2) Bradford assay is often used to quantify the protein concentration in a protein sample. Which of the following statements is NOT correct about the Bradford assay? **1 point**

- The absorbance shift of Coomassie brilliant blue dye forms the basis of the assay
- Under acidic conditions, red form of the dye is converted into its blue form
- Absorbance is always measured at 280 nm.
- Bradford assay is based on Beer Lambert law

No, the answer is incorrect. Score: 0

Accepted Answers: Absorbance is always measured at 280 nm.

3) Abhilash did a proteomic experiment using 2DE. After running and staining the gel, he got the image as shown below with a big dark area in the lower part of the gel. **1 point**



What possible mistakes he might have done during the experiment?

- Did not perform steps to remove nucleic acid and lipids
- IPG buffers/SDS complexes got stained, because carrier ampholytes were not washed out from the SDS gel
- He might have used 24 cm IPG strip that is why the gel is bad
- Absence of SDS while making gel for second dimension

No, the answer is incorrect. Score: 0

Accepted Answers: Did not perform steps to remove nucleic acid and lipids  
IPG buffers/SDS complexes got stained, because carrier ampholytes were not washed out from the SDS gel

4) While performing 2-Dimensional electrophoresis, Vipin added luke-warm agarose solution over the IPG strip. What is the purpose of adding agarose solution? **1 point**

- Proteins focussed on IPG strips cannot migrate in the absence of agarose gel
- Adding it helps the SDS-PAGE to run faster
- It will prevent the gel from drying up
- All of the above

No, the answer is incorrect. Score: 0

Accepted Answers: It will prevent the gel from drying up

5) Shalini performed some experiment on human plasma sample in order to identify the protein biomarkers for malaria diagnosis. She started her experiment from depletion of the sample to remove the abundant proteins such as serum albumin. After depletion and quantification, she processed her sample for Mass spectrometry. Why she preferred to deplete the sample before moving forward? **1 point**

- Because the removal of abundant proteins could significantly reduce the complexity of plasma proteins
- As mass spectrometer cannot detect high abundant proteins, so depletion was done
- If these abundant proteins are not removed, their tryptic peptides dominate the less abundant ones which might have higher significance
- The depletion step is not at all required for processing these types of samples. She wasted her time and sample for doing depletion

No, the answer is incorrect. Score: 0

Accepted Answers: Because the removal of abundant proteins could significantly reduce the complexity of plasma proteins  
If these abundant proteins are not removed, their tryptic peptides dominate the less abundant ones which might have higher significance

6) Which of the following statement(s) tells the difference between 1D-SDS-PAGE and 2DE? **1 point**

- Isoelectric focussing is done for 2DE and not for 1D-SDS-PAGE
- Discontinuous gels are used in 2DE, whereas for 1D-SDS-PAGE continuous gels are used
- Unlike 1D-SDS-PAGE, 2DE is performed for the analysis of pure protein sample
- SDS is not used in case of 2DE

No, the answer is incorrect. Score: 0

Accepted Answers: Isoelectric focussing is done for 2DE and not for 1D-SDS-PAGE

7) Which of the following software is NOT used for 2D gel analysis? **1 point**

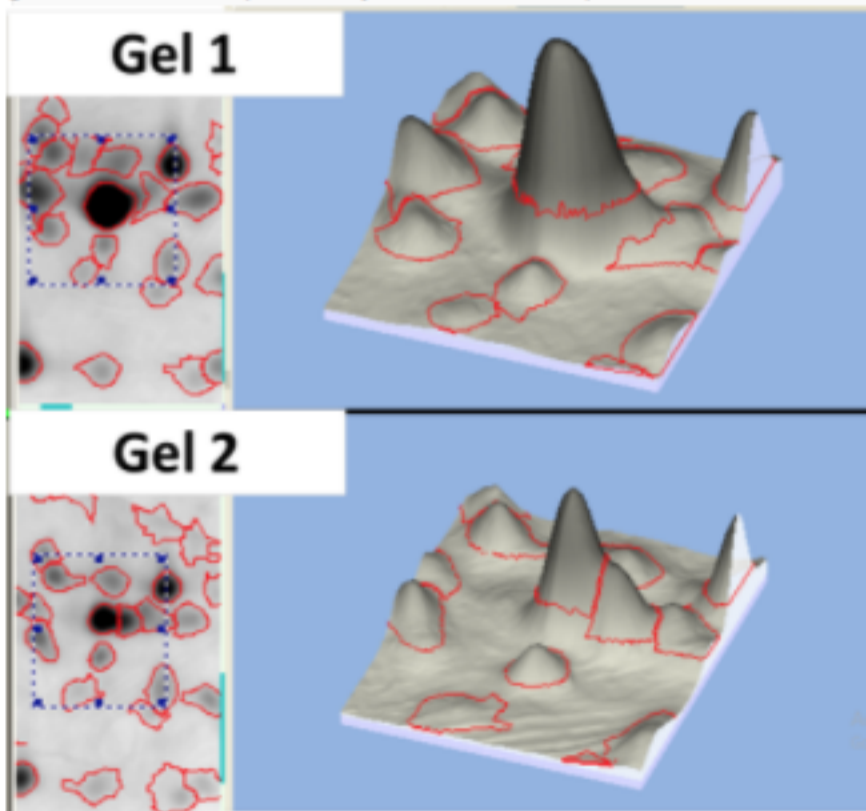
- PDQuest
- Dymension
- Maxquant
- Delta 2D

No, the answer is incorrect. Score: 0

Accepted Answers: Maxquant

8) Information for Q 8 and 9 **1 point**

Given below is a picture obtained while doing 2D gel analysis in Image master platinum software. The figure shows the data from two samples A and B with the gels 1 and 2 respectively. Answer the questions 8 and 9 based on the figure.



What can be inferred from the figure provided?

- Detected spots are encircled with red line both on right and left side
- Right panel shows the 3D view of the selected spots
- Peaks represents the relative intensity of proteins
- All of the above

No, the answer is incorrect. Score: 0

Accepted Answers: All of the above

9) Which of the following is correct about the analysed gels shown above? **1 point**

- Sample A and B are showing the differential expression for the proteins
- Sample A and B are the technical replicates
- Sample A has higher peak intensity for all the selected protein spots
- 2D gel obtained for sample B is of bad quality that is why showing less peak intensity

No, the answer is incorrect. Score: 0

Accepted Answers: Sample A and B are showing the differential expression for the proteins

10) While analyzing 2D gels in Image master Platinum, there are three parameters for spot detection settings. "Smooth" is one of those parameters. What does smooth parameter setting actually do? **1 point**

- It tells about the number of times ImageMaster smooths the image before detecting spots
- This parameter works to mark the distance between the two spots on the gel
- It works to detect all real spots and split as many overlapping spots as possible
- None of the above

No, the answer is incorrect. Score: 0

Accepted Answers: It tells about the number of times ImageMaster smooths the image before detecting spots  
It works to detect all real spots and split as many overlapping spots as possible