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BCH 412 ASSIGNMENT

1. Explain 3 advantages of 2 dimensional polyacrylamide gel electrophoresis.

i. VISUALIZED MAPPING ANALYSIS: Amongst other unique features, 2 dimensional electrophoresis is known for it's ability to resolve intact full length proteins (up to 5,000 proteins) in a single gell. This includes visualized detection of the physico-chemical properties such as MW and pI with possible quantification based on spot intensity. Proteins of interest could be characterized via peptide mass finger printing or when probed with antibodies.

ii. ROBUSTNESS: Over the years, multiple methodological improvements have contributed to increase the robustness of 2dimension electrophoresis workflow. The use of immobilized isoelectric focusing (IEF) strips, ampholytes based buffers, highly sensitive dyes and gel imaging software made the variability most likely from upstream processes such as protein lost during extraction. Moreover, 2-DE becomes less variable when multiplexing electrophoresis developed. Differentially labelled samples running at the same time minimized the possibility of artifacts resulted from technical errors.

iii. COMPATIBLE FORM FOR FURTHER ANALYSIS: 2-DE gel easily and efficiently couples with many other analysis and biochemical techniques. Thus it provides a compatible platform for subsequent analysis. Compatibility of ~~protein~~ 2DE includes bottom-up proteomics to identify proteins and characterize their amino acids sequences or alternatively proceeded by the top-down proteomics (shot gun) in which the crude protein extract is digested directly for analysis. Most

commonly, 2-DE fractionated proteins are subjected to either in-gel digestion to prepare tryptic peptides for mass spectrometric analysis or gels are validated for proteins of interest using western blotting.

2. Disadvantages of 2-DE
i Narrow dynamic range
ii Difficulty in separating hydrophobic and extremely acidic or basic proteins.

3. State the concept of 2DE and list 5 of it's applications.

The concept of 2-DE consists mainly of two steps of separation; first dimension and second dimension. In the first dimension, protein molecules are resolved based on their iso-electric point (pI). In the second dimension, protein separation is performed based on molecular weight using SDS Laemmli or Tris-Tricine buffers.

Applications of 2DE
i Whole proteome analysis
ii Cell differentiation
iii Microscale protein purification
iv Bacterial pathogenesis
v Detection of biomarkers and disease markers

4. Describe 3 techniques of iso-electric focusing in first dimension electrophoresis

i CONVENTIONAL IEF: The conventional method of IEF depends on the carrier ampholyte where proteins migrate in a solution media until reaching equilibrium state when it's net charge is equal to zero. Proteins that are in a pH level below it's isoelectric point (pI) will be positively charged and will migrate towards the cathode.

As it migrates through a gradient of increasing pH, however, the protein's overall charge will decrease until the protein reaches the pH region that corresponds to it's pI. At this point, the migration ceases. As a result, the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to it's pI.

ii IMMOBILIZED pH GRADIENT (IPG): IPG is an integrated part of polyacrylamide gel matrix fixed on a plastic strip. Co-polymerization of a set of non amphoteric buffers with different chemical properties is included. Ready-made IPG strips are available with different lengths and pI. A commercial pre-casted acrylamide gel matrix co-polymerized with a pH gradient on a plastic strip results in a stable pH value over the traditional ampholyte method. It has an ability to avoid cationic accumulation and to produce a better focused protein with less smearing

iii NON EQUILIBRIUM pH GEL ELECTROPHORESIS (NEPHGE)
This technique was developed to resolve proteins with basic to extremely high pI (7.0 to 11.0) that cannot be separated by traditional method. In contrast, IPG method allows the protein molecules to move at different rates across the gel based on the charge and the volt hours setting that determined speed pattern and reproduceability. A previous study comparing IPG and NEPHGE showed that protein loss was higher in IPG based method, especially for basic proteins. They found that the reproduceability of spots was slightly better in NEPHGE based method. The reproduceability of acidic proteins were similar in both methods.

5. In 2-D, what does 2nd dimension electrophoresis entail? State it's major differences from polyacrylamide gel electrophoresis.

This step separates proteins based on their molecular weight using a vertical electrophoretic device with either Laemmli buffer or Tris-Tricine buffer. Instead of loading protein sample within the wells, the first dimension rehydrated strip is carefully placed on top of the SDS-PAGE and sealed with agarose.

The major difference between 2nd dimension electrophoresis and polyacrylamide gel electrophoresis is that 2nd dimension electrophoresis separates protein based on their molecular weight while polyacrylamide gel electrophoresis separates protein based on their electrophoretic mobility (i.e their length, conformation and charge of their molecules.)