Name - Sani Ojonugwa Aishat
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Assignment.

1. Advantages of 2-D electrophoresis Includes:-

i Robustness

During the last few years, several methodological improvements have Contributed to Increase the robustness of 2-DE Workflows. The use of Immobilized Isoelectric focusing (IEF) strips, amphilyte based buffers, highly sensitive dyes, and gel imaging Software made the variability most likely from upstream process such as protein loss during extraction. Moreover, 2-DE becomes less Variable when multiplexing electrophoresis developed. Differentially labelled Samples run at the the Same time minized the possibility of artifacts resulted from technical errors. Finally, the recent Improvements of the gel image analysis minimized the former

high percentage of spot Identification failure estimated to reach 60% which Considered one of the major Contributors to variability seen with 2-DE.

ii Visualized mapping analysis

One of the unique features of 2-DE is it's ability to resolve intact full-length proteins (up to 5000 protein) in a single gel. This includes visualized detection of the physico-chemical properties such as MW and pI with possible quantification based on the spot intensity. proteins of interest could be characterized via peptide mass finger printing or when probed with antibodies. Moreover, 2-DE allows Seperation and Identification of PTMs and protein isoforms. In several cases, PTMs Could be recognized by horizontal or vertical shifting of a protein spot as these modifications usually change the protein MW and pI.

iii Compatible platform 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. For example, stained gels can be followed by spot excision, destaining, protein extraction, digestion and analysis of the tryptic peptides by mass spectrometry. Although Coomassie blue could be reversibly destained and compatible with mass spectrometry, silver staining, it is not compatible because of the ~~using~~ usage of formaldehyde or glutaraldehyde during the fixing and sensitization step that results in lysine residue cross-linking within the protein chain interfering MS analysis and this will hinder trypsin digestion. Various ~~identi~~ ~~fication~~ modifications in the silver nitrate stain approach were performed to overcome this drawback compatibility

2. Disadvantages of 2-D electrophoresis
(i) Difficulty in seperating hydrophobic and extremely acidic or basic proteins.
(ii) Narrow dynamic range of 2-DE.

3. State the concept of 2-D electrophoresis and list 5 of its applications

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

Application of 2-D electrophoresis Include
(i) whole proteome analysis
(ii) Cell differentiation
(iii) Detection of biomarkers and disease markers
(iv) Bacterial Pathogenesis
(v) microscale protein purification

4. Describe 3 techniques of isoelectric 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 the ~~eq~~ equilibrium state when its net charge equals to zero. proteins that are in a pH region below its ~~et~~ isoelectric point (pI) will be positively charged and will migrate through a gradient of increasing pH However the protein's overall charge will decrease until the protein reaches the pH region that corresponds to its 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 its pI. Although this conventional method is easy to prepare and does not require much casting

equipments, it has a main ~~adva~~ disadvantage as the ampholytes have some susceptibility to flow towards cathode and this gradient flow usually causes a reduction in the reproducibility.

iii Immobilized pH gradient (IPG)

Immobilized pH gradient strip (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. Usually, short length IPG strips are used for fast screening while longer one for maximal and comprehensive analysis. A commercial precasted acrylamide gel matrix co-polymerized with a pH gradient on a plastic strip result in a stable pH value

the traditional ampholyte method. It has an ability to avoid cationic method. It has acr accumulation and to produce a better-focused protein with less smearing. There are many other advantages of using IPG strips over ampholytes such as reduced cathodic drift, higher mechanical strength as the strips are casted on a mechanical strength as the strips are casted on a plastic backing that minimizing gel breakage, and higher protein loading capacity due to the sample loading method.

iii. Non-equilibrium pH gel electrophoresis (NEPHGE)

Non-equilibrium pH gel electrophoresis (NEPHGE) technique was developed to resolve proteins with basic to extremely high pI (7.0 to 11.0) that cannot be seperated by the traditional method. In constrast, IPG method allows the protein

molecules to move at different rate across the gel based on their charge and the volt hours setting that determine speed pattern and reproducibility. A previous study comparing IPG and NEPHGE techniques showed that protein loss was higher in IPG-based methods, especially for basic proteins. they found the based method. About half of detected basic protein spots were not reproducible by IPG-based 2-DE, whereas NEPHGE-based method showed excellent reproducibility in the based gel zone. the reproducibility of acidic proteins was similar in both methods.

5 In 2-D, what does second dimension electrophoresis entail? state its major difference from polyacrylamide gel electrophoresis.

this steps separate proteins based on their molecular weight using a vertica

electrophoretic device with either laemmli buffer or Tris-Tricine buffer. Instead of loading protein sample within the wells, the first dimension-rehydrated strip carefully placed on the top of the SDS-PAGE and sealed with agarose.

The major difference between second dimension electrophoresis and polyacrylamide gel electrophoresis is that second dimension electrophoresis separates proteins based on their molecular weight while polyacrylamide gel electrophoresis separates proteins based on their electrophoretic mobility (i.e their length, conformation and charge of the molecule).