FALODE DORCAS
16/0C108/005
BCH 4/12

1 Explain 3 advantages of two-dimensional electrophoresis (2-DE)

a) 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, ampholytes-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 same time minimized the possibility of ~~[illegible]~~ artifacts resulted from technical error. Finally, the recent development 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

b) Visualized mapping analysis

One of the unique features of 2-DE is its ability to resolve intact full-length proteins (up to 5000 proteins) 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 separation 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 modification usually change the proteins MW and pI.

c) Compatible platforms 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 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 thus will hinder trypsin digestion. Various modifications in the silver nitrate stain approach were performed to overcome this drawback.
Compatibility of 2-DE includes bottom-up proteomics to identify proteins and characterize their amino acid sequences or alternatively preceded by the top-down proteomics (shotgun) in which the crude proteins extract is digested directly for analysis. In another powerful combination, antibody-based analysis could be coupled before or after 2-DE. For instance, immuno-affinity purification can be used to pre-fractionate a protein of interest prior to running 2-DE such as phosphorylated or ribonucleoproteins.
Most commonly, 2-DE fractionated proteins

are subjected to either in-gel digestion to prepare tryptic peptides for mass spectrometric analysis or gel are validated for protein of interest using Western blotting.

2. Highlight 2 disadvantages of two-dimensional electrophoresis

Two disadvantages of 2-D electrophoresis are:
a) Difficulty in separating hydrophobic and extremely acidic or basic proteins.

b) 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 mainly of two steps of separation; 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.

Applications 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 equilibrium state when its net charge equals to zero. Proteins that are in a pH region below its isoelectric point (pH) 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 its pH. At this point, the migration Ceases. As the result, the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient Corresponding to its pH.

Although this Conventional method is easy to prepare and does not require much Costing Equipments, it has a main disadvantage as the ampholytes have some susceptibility to flow towards Cathode and this gradient flow Usually causes a reduction in the reproducibility

II 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 of non-amphoteric buffers with different chemical properties is include. Ready-made IPG strips are available with different lengths and pI. Usually, short length IPG strips are Used for fast Screening while longer are for

maximal and comprehensive analysis. 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.

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 plastic backing that minimizing gel breakage, and higher protein loading capacity due to the sample loading method.

1 Non-equilibrium pH gel electrophoresis (NEPHGE)

Non-equilibrium pH gel electrophoresis (NEPHGE) technique was developed to resolve proteins with basic to extremely high (7.0 to 11.0) that cannot be separated by the traditional method. In contrast IPG method allows the protein molecules to move at different rate across the gel based on the charge and the Volt hours setting that determine spread pattern and reproducibility.

A previous study comparing IPG and NEPHGE techniques showed that protein loss was higher in IPG-based method, especially for basic proteins. They found the reproducibility of spots was slightly better in NEPHGE-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 basic gel zone. The reproducibility of acidic proteins was similar in both methods.

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

This step separates proteins based on their molecular weight using a vertical electrophoresis 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 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).