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Assignment

1. Advantages of 2-D electrophoresis includes:

a. Visualized mapping analysis: One of the unique features of 2-DE is its 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 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 modifications usually change the protein MW and pI.

b. 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. 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 protein extract is digested directly for analysis.

c. Robustness: The use of immobilized isoelectric focusing (IEF) strips, ampholyte-based buffers, highly sensitive dyes, and gel imaging software made the reliability most likely from

human error process such as protein loss during extraction. The
becomes less variable when multiplexing electrophoresis
Differentially labeled samples run at the same time minimize
the possibility of artifacts resulted from technical errors

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

3 State the concept of 2-D electrophoresis and list 5 of its 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 depending on their isoelectric point
(pI). In the second dimension protein separation is performed
based on molecular weight using SDS Laemmli or Tris-Tricine buffer
• Applications of 2-D electrophoresis includes:
i Whole proteome analysis
ii Microscale protein purification
iii Bacterial pathogenesis
iv Cell differentiation
v Detection of biomarkers and disease markers

4 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 it's net charge
equals to zero. Proteins that are in a pH region below its 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 its pI. At this point
the migration ceases. As a result the proteins become focused

in 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 disadvantage as the ampholytes have some susceptibility to flow towards cathode and this gradient flow usually causes a reduction in the reproducibility.

NEPHGE: 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 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 speed, 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. It was found that 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.

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 pre-cast acrylamide gel matrix co-polymerized with a pH gradient on a plastic strip results in a stable pH value over the traditional ampholy

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.

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 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 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).