ITSI-Life Science NEWSFLASH

Highlighting Recent Technologies and Approaches used in Biomedical Research.

Proteomics on Formalin-Fixed Paraffin-Embedded Tissues

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Formalin-Fixed Paraffin Embedded Tissues (FFPE) are commonly used in healthcare to preserve tissues from a wide variety and different stages of a disease. However formalin cross links the protein and poses a great obstacle for downstream proteomics application. The development in this area is critical for identification of new biomarkers and for validation of biomarkers that change at different stages of a disease like cancer. A number of protocols have been recently published dealing with the isolation of proteins from FFPE samples that are compatible for proteomics.

The paper recently published by Tanca *et al*[1] demonstrates for the first time the applicability of 2D-DIGE to FFPE tissues. The 2D-DIGE technology marketed by GE Healthcare, is a variant of the classical 2D-PAGE that allows multiplexed analysis of 2 to 3 protein samples on a single gel[3]. It involves the use of fluorescent dyes typically Cy3 and Cy5, to label "test" (Cy3) and "reference" (Cy5) protein samples respectively. A third dye (typically Cy2) is also used to allow the inclusion of a universal internal standard, consisting of equal aliquots of all samples in the experimental batch. The Cy2 labeled sample is included in all gels. After mixing the labeled samples and performing 1st and 2nd dimension electrophoresis, the gel is scanned at three wavelengths to capture the Cy2, Cy3 and Cy5 signals. The images obtained (three per gel) are merged, and differentially pseudo-colored to obtain a single image with three different colors (Figure 1). After automated spot detection and statistical analysis with a DIGE enabled software, spots representing differentially expressed proteins, are picked, tryptic in-gel digested using robotic workstations, and sequenced by LC/MS/MS or MALDI-TOF MS. The use of fluorescent dyes increases sensitivity, and the use of a universal internal standard allows gel-to-gel comparisons, and eliminates significant gel-to-gel variations. 2D-DIGE can be performed using a "minimal labeling" or "saturated labeling" protocol. Saturated labeling protocol is used when the protein amount is limiting e.g. for analysis of samples acquired by laser microdissection. The power and versatility of 2D-DIGE has been widely demonstrated in many recent studies[4-6].

Tanca *et al*[1] modified existing protein extraction methods to obtain reproducible two-dimensional difference in gel electrophoresis (2D-DIGE) for comparative proteomics of FFPE tissue. Currently 2D-DIGE is considered as the most refined technology for performing comparative proteomics[2]. Tanca *et al*[1] compared fresh-frozen and FFPE tissues from sheep liver and skeletal muscle tissues for reproducibility among FFPE tissues and understand the difference between protein isolation and representation comparing fresh-frozen vs. FFPE tissues. The authors labeled proteins from the FFPE and fresh-frozen tissue and pooled sample with three different cyanine dyes prior to 2D-Electrophoresis.

Their 2D-DIGE based comparison of fresh-frozen and FFPE tissues yielded interesting observations. For example, high and medium MW proteins seems to be poorly represented in FFPE because of formalin induced cross linking. Basic proteins and proteins rich in lysines are also under represented owing to their reactivity with formalin. Authors also observe a unique acidic shift which suggests the possibility of formalin modifying basic residues of all the proteins in the tissue. Authors observe excellent reproducibility among FFPE samples with satisfactory resolution. They however express concerns about the under representation of high and medium molecular weight proteins, acidic shift, and high visible background and hence comparisons should only be made between FFPE tissues and not with fresh tissues. This paper opens the way for performing reproducible and differential proteomics investigations using 2D-DIGE technology on widely available FFPE tissues.

ITSI - biosciences (www.itsibio.com) offers 2D-DIGE as a service. All biological and clinical samples can be analyzed. The typical turnaround time is 2 weeks. As little as 30ug of protein can be analyzed. The deliverable includes raw and analyzed data, fold-difference of proteins that show statistically significant difference in expression between the test and reference samples and gene ontology classification of the identified proteins.

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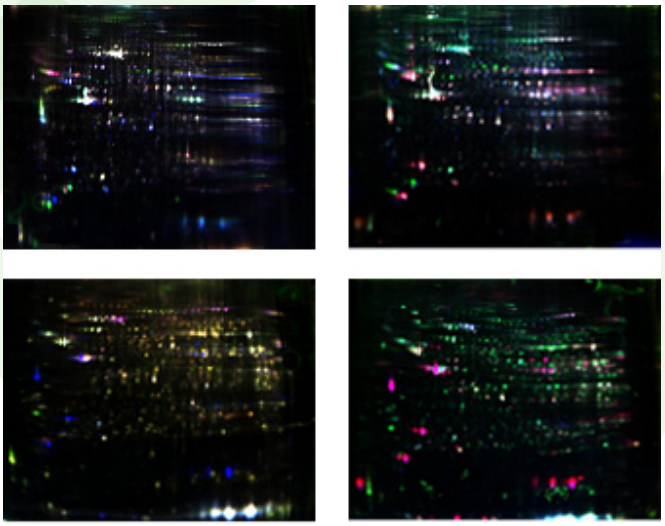
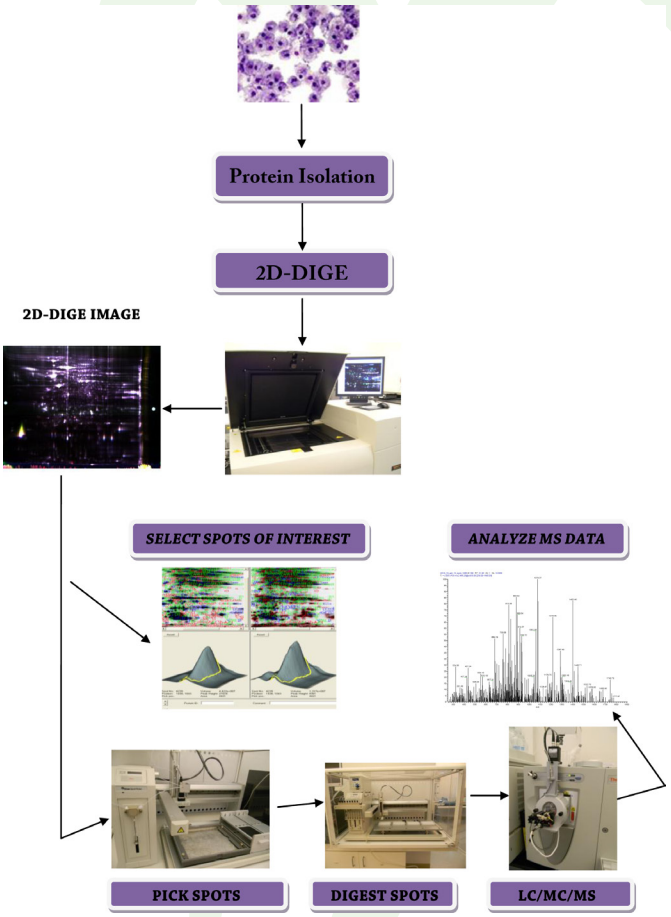
Figure 1: Illustration of the ITSI—Biosciences standard 2D-DIGE workflow process. Protein samples separately labeled with cyanine fluorescent dyes (Protein 1, Cy3; Protein 2, Cy5; Protein 3, Cy2) are subjected to 1st and 2nd dimension electrophoresis. The gel is scanned at 3 wavelengths and candidate spots are identified and selected using a DIGE-enabled software, which also provides data on statistical significance of expression differences. Proteins of interest are picked and digested with a robot, sequenced using nano-LC/MS/MS technology and annotated. Annotation includes Gene Ontology classification and pathway mapping.





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