

LSUHSC Proteomics Core Facility

Applications Newsletter

January 3rd, 2007

2-Dimensional Fluorescence Difference Gel Electrophoresis (2-D DIGE)

2-D Gel Electrophoresis Optimized for the Expression Proteome

2-D DIGE is a method to label protein samples with fluorescence dyes before 2-D gel electrophoresis. The labeled samples are mixed and run on the same gel. These fluorescence labels are size and charge matched and designed to allow the same proteins labeled with different tags to migrate in the gel identically. Each label absorbs and emits at unique wavelengths. During image normalization by software (like DeCyder), the images are overlaid and protein spots are compared quantitatively. Hence, this technique minimizes gel variations normally observed among different gels. This technique enables more accurate analysis of differences in protein abundance between samples. With subsequent biological (preferred) or experimental replicates, these differences can be verified unambiguously.

To date, three labels are available via Amersham Biosciences (GE Healthcare). Routinely, two samples to be compared are labeled with Cy3 and Cy5 (see the workflow chart at the right). Cy2 can be used to label a 3rd sample, or the internal standard pooled from a series of samples when multiple gels are required to complete the biological variation study. CyDyes, with the advantage of sensitive fluorescence detection, requires as little as 125 pg per protein, giving a linear response to protein concentration of up to 4 orders of magnitude.

Currently, this Facility utilizes minimal labeling in which ~2-5% of lysine residues of proteins are labeled. At this percentage, there is no deleterious effect in tryptic digestion for sequential protein identification by mass spectrometry. The automatic Amersham 2-D DIGE Ettan system uses large format gels that give the highest resolving power. We suggest that a minimum of 200 µg total protein extract per sample be used. However, further fractionation is recommended to reduce the sample complexity.

Dr. Steve Lanier's group of LSUSHC Pharmacology recently published a paper entitled "The Proto-oncogene SET Interacts with Muscarinic Receptors and Attenuates Receptor Signaling", Simon *et al.*, JBC, v 281, p40310 (2006). In their 'Interactome' study, they utilized 2-D DIGE in combination with protein identification by mass spectrometry in Proteomics Core Facility to analyze GST bound proteins from rat brain cytosolic fraction.

*References and further information about 2-D DIGE can be found in GE Healthcare Amersham Biosciences website: <http://www.amershambiosciences.com/>

