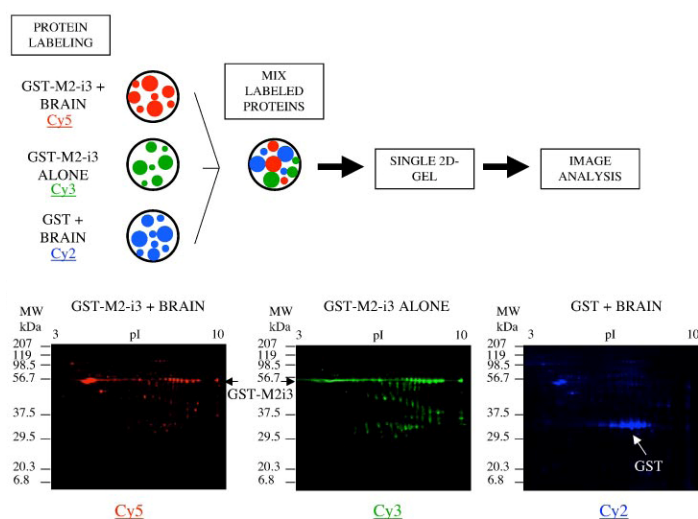


2-Dimensional Electrophoresis to Isolate Protein Binding Partners

In previous editions of this Application Note we have touted the benefits of the DIGE methodology¹. In this Application Note, we cite a successful work by a research group that utilized DIGE and identified a protein of interest within The LSUHSC Proteomics Core Facility. The details of this work were published in JBC 2006².

As a reminder, the DIGE system utilizes three covalently attached fluorochromes or dyes to the primary amines of proteins. These dyes are spectroscopically unique in their excitation and emission spectra, but are size and charge matched so their influence on migration through a 2D gel is the same. This allows for up to three different samples to be labeled with a unique dye and co-loaded onto the same gel. The resultant image set can then be combined into a single image, or “multiplexed” to analyze difference among the protein species.

Utilizing 2D-DIGE, Lanier *et al.* investigated which proteins in rat brain cells interact specifically with the 3rd intracellular loop of the M3-muscarinic receptor. The group prepared a fusion construct of the i3 loop region carrying a glutathione-S-transferase tag (GST-M2-i3). This construct and control were expressed and attached to glutathione-Sepharose beads for the binding experiment. A rat brain lysate was prepared and incubated with the GST fusion protein and controls to yield the following samples: (1) GST-M2-i3 + brain, (2) GST-M2-i3 alone, and (3) GST + brain. The resultant protein materials were labeled with the DIGE dyes, Cy5, Cy3 and Cy2, respectively, (the scheme to the right). The images are generated by utilizing the unique fluorescence for each of the dyes.



When 3 images are overlaid, or multiplexed, the proteins that are unique to that protein subset become clear. In the example to the left, samples unique to the GST-M2-i3 + brain are displayed in red (spot c and d only appear in the Cy5 channel), while protein spots that are in the GST-M2-i3 and GST + brain show in purple shade (spot b; Cy5-red and Cy2-blue together). Spots that are common to the purification of the fusion constructs would be in GST-M2-i3 and GST-M2-i3 + brain; these appeared in yellow to orange shade (spot a; Cy5-red and Cy3-green together). **Spot c** was identified as rat protein SET utilizing MALDI mass spec and database search.

Validation The authors confirmed the identification and quantification using Western Blotting. Also, *in vitro* binding experiments with subsequent cellular physiological experiments were performed to verify the results.

References:

1. LSUHSC Proteomics Core Facility Applications Newsletters, Jan 2007 and Jul 2009
2. Simon V, Guidry J, Gettys TW, Tobin AB, Lanier SM. The proto-oncogene SET interacts with muscarinic receptors and attenuates receptor signaling. J Biol Chem. 2006 Dec 29; 281(52):40310-20.