Panorama™ Ab Microarray – Cell Signaling: A Valuable Tool for Studying the Differential Expression of Proteins in Cells

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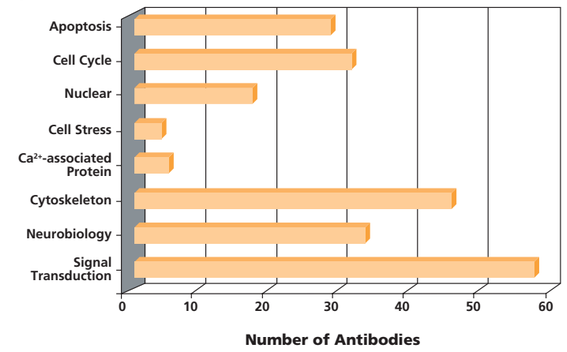
ABSTRACT

Antibody arrays are a promising new tool for mass analysis of protein level changes in cells responding to different stimuli. Here we describe a novel antibody array system called Panorama™ Ab Microarray – Cell Signaling that contains 224 antibodies spotted on FAST™ nitrocellulose-coated slides that can detect protein levels as low as a few nanograms per ml. The antibodies spotted are specific for proteins important in various areas of cell signaling such as phosphorylation, cell-cycle, apoptosis, nuclear signaling and cytoskeleton proteins. Furthermore, for some of the protein targets, the Panorama Ab Microarray can detect phosphorylated and non-phosphorylated forms of the target protein. We found that treatment of the slides post-spotting is important for the array performance (ratio of signal to background) and its stability. Panorama Ab Microarray was used to analyze changes in protein expression in F9 embryonic carcinoma (EC) cells stimulated to differentiate by all-trans-retinoic acid. We found that the level of several proteins, among them cell cycle regulators and kinases, was either up- or down-regulated. For more than ten protein targets, the results obtained by the Panorama Ab Microarray were confirmed by immunoblotting.

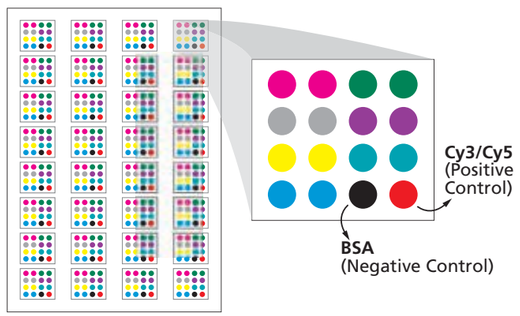
Biological Areas of Antibody Specificities

Figure 1



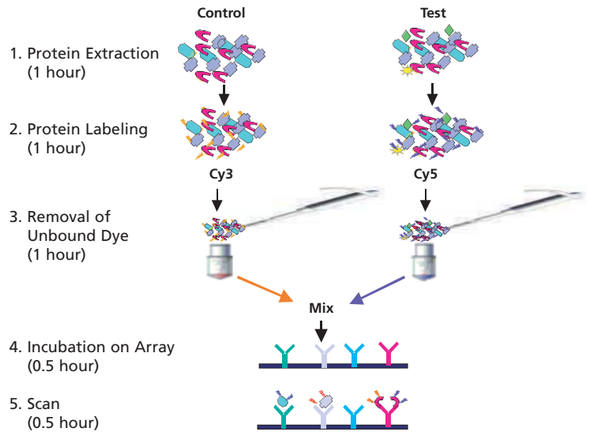
Spotting Format

Figure 2



Experimental Procedure

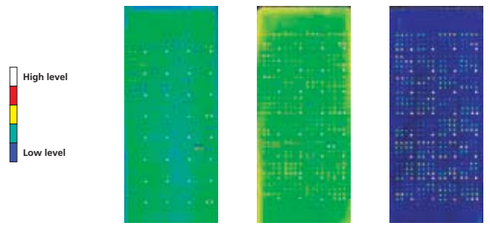
Figure 3



The assay takes 5-6 hours

Background

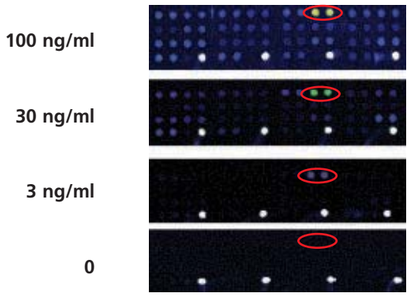
Figure 4



Cy3 labeled NIH-3T3 cell extracts were applied on slides treated by different blocking solutions after spotting.

Specificity and Sensitivity

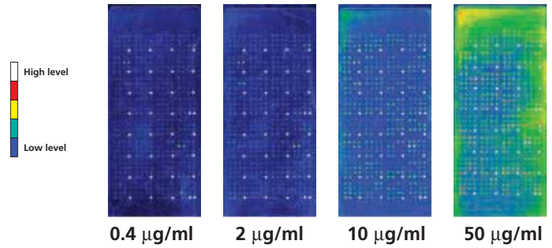
Figure 5 Caspase 9



Cy3 labeled recombinant Caspase 9 was applied on array slides at different concentrations. The position on anti-Caspase 9 antibody on the array is circled.

Protein Concentration Applied Is Important

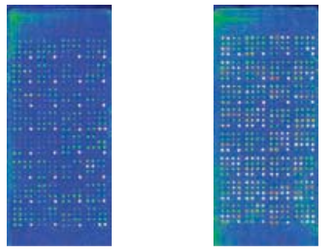
Figure 6



Cy3 labeled NRK cell extracts at different concentrations were applied on array slides.

Dye/Protein Molar Ratio Should Be > 2

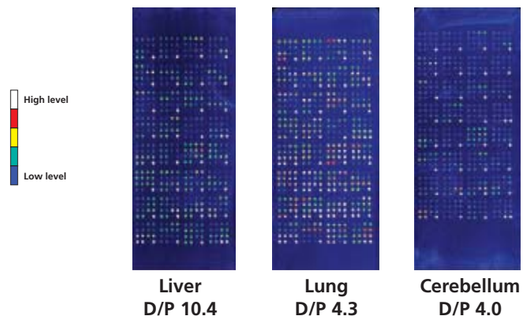
Figure 7



Cy3 labeled NRK cell extracts were labeled at different dye-to-protein molar ratio. Equal concentrations were applied on array slides.

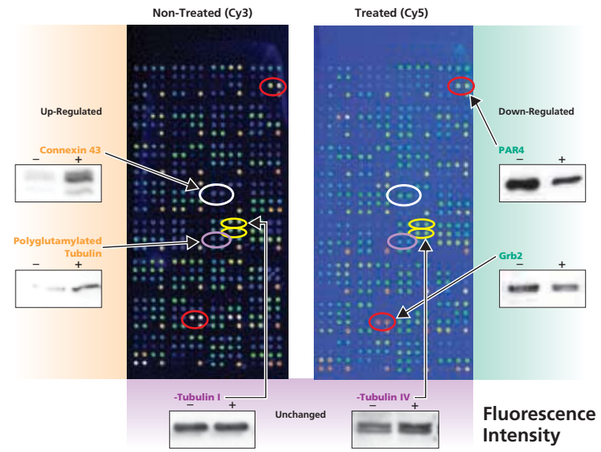
Differential Protein Expression in Mouse Tissues

Figure 8



Protein Expression in F9 Mouse Stem Cells Induced by Retinoic Acid

Figure 9



Mouse F9 cells were stimulated with retinoic acid for 96 hours with trans-retinoic acid (10^{-7} M). Extracts were prepared from treated and untreated cells using Extraction/Labeling Buffer and labeled with Cy5 and Cy3, respectively. A mixture containing equal amounts of each labeled extract (5 µg/ml) was incubated on the array.

The same slide is shown at the two fluorescence emission wavelengths for Cy3 and Cy5.

Changes in expression level were confirmed by immunoblot, as indicated.

Equal amounts of protein extract (20 µg per lane) from treated and untreated cells were separated by SDS-PAGE and blotted onto nitrocellulose membranes. The proteins were probed with the monoclonal or polyclonal antibodies corresponding to the array and visualized using chemiluminescence.

Summary of Array Properties and Usage

1. The detection limit of the array is at the level of ng/ml of protein.
2. The variance of duplicate spots on the same slide: < 8%.
   The variance of spots between slides: < 10%.
3. Molar ratio of the dye (Cy3 or Cy5) to protein should be higher than 2.
4. The concentration of labeled protein applied on the array should be between 2-10 µg/ml.
5. Freshly prepared material should be used.
6. Genomic DNA should be eliminated by DNase treatment.
7. Protease and phosphatase inhibitors should be added.

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