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2D Imaging with the UVP BioSpectrum® 810 Imaging System

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Two dimensional SDS polyacrylamide gel electrophoresis remains a cornerstone for protein research, giving an array highly resolved proteins by separating a protein sample first by the isoelectric point and second by size (1, 2, 4, 5). The end result is an SDS PAGE gel with 100's to 1000's of "spots," generally circular or ellipsoid in shape with diameters from fractions of a millimeter to 1 cm or more. The individual spots typically represent just one protein, making 2D SDS PAGE an ideal way to isolate proteins for further analysis. In some cases, the spots will contain more than one protein, but the reduction in complexity by using 2D SDS PAGE greatly simplifies further analysis.

Once separated, the proteins need to be visualized prior to image acquisition and analysis (3, 4, 5). The sample can be fluorescently tagged before separation, with the end result of separated proteins which are already fluorescent and ready to be fluorescently imaged with UV or visible excitation light. If the protein is not tagged at the start, then the protein must be stained via Coomassie blue, silver, or SYPRO Ruby fluorescent stains. Coomassie Blue is an easy to use diffusion-based stain which leaves the spots blue and straightforward to image using a visible white light table, as illustrated below.

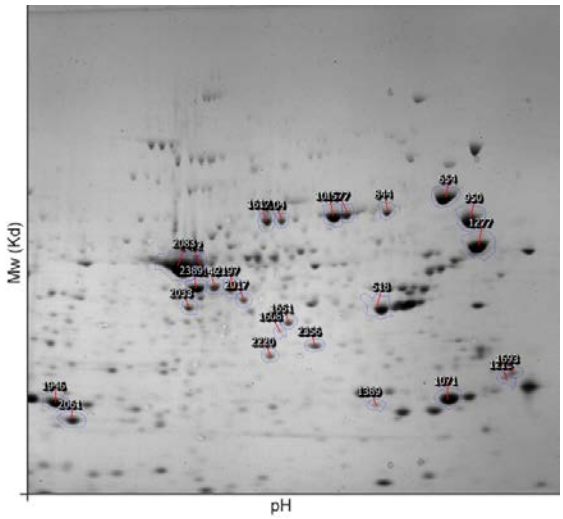

Fig. 1 - Coomassie Blue stained 2D SDS PAGE gel

Applications of 2D SDS PAGE are many (5-14) and include identification of disease or developmental-specific proteins. The samples can be analyzed to identify proteins (or constellations of proteins) that are changing in amount (intensity) due to a treatment or disease. Proteins can also be modified after expression in a way that changes their native charge or size, causing shifts in position on a 2D gel. Post translation modifications of proteins (e.g., phosphorylation, glycosylation, etc.) typically lead to changes in mobility, causing a shift in the position that is straightforward to identify through image analysis.

Once visualized by tagging or staining, 2D SDS PAGE separations are imaged with BioSpectrum 810 and the gel image analyzed with Prodigy SameSpots software for number of spots, intensity and position of spots. Prodigy Same Spots greatly simplifies the process of 2D gel analysis (6-14), using a simple guided process that easily produces comparisons between treatment and control samples to look for changes. Due to the inherent variability of the technique, multiple gels must be analyzed for both control and treatment so that any changes can be validated as real and not due to simple gel-to-gel variability.

BioSpectrum 810 Imaging System

The BioSpectrum 810 system is ideally suited for 2D image analysis:

- 8.1 megapixel camera
- UV, visible, multiple wavelength excitation and emission fluorescence analysis from 300 to 800 nm
- High uniformity LED white light plate
- Coomassie Blue high dynamic range (CBHDR) filter
- VisionWorks®LS Acquisition and Analysis Software
- Prodigy SameSpots 2D analysis software for use with:
  - Windows 8 (32- or 64-bit)
  - Windows 7 (32- or 64-bit)
  - Windows Vista (32- or 64-bit)
  - Windows XP (32-bit)

Steps for 2D analysis

Place the gel on the LED white light plate

With VisionWorks software:

- Set the BioSpectrum 810 camera to 1x1 binning with high quality focus
- Set the filter to Coomassie Blue high dynamic range (CBHDR)
- Adjust the lift height to fill the image frame with the gel
- Adjust exposure time to just saturate the uncovered white light surface
- Adjust the lens at f/5.6 to 8 and focus for maximum sharpness
- Save the settings as a template so they can be repeated
- Image all the gels at the same settings
- Save images to archive folder and create duplicate for analysis
- Analyze with Prodigy SameSpots following the simple workflow
  - Image quality control
  - Image alignment
  - Image alignment review
  - Prefiltering and SameSpots analysis
  - View Results and ID interesting spots
  - Report

Results

Two gels were imaged with VisionWorksLS and analyzed with Prodigy.

Fig. 1 Coomassie Blue staining 2D SDS PAGE gels. 150 ug of mouse liver was separated by 2D SDS PAGE, imaged with the BioSpectrum 810 system with VisionWorksLS and analyzed using Prodigy SameSpots software.

Fig. 2 Rank 518 Example of a protein which, based on Prodigy analysis, increased amount 1.6 fold.

Fig. 3 Rank 1389 Example of a protein which increased amount 1.2 fold

Fig. 4 Spot table generated with analysis of the specified proteins in figure 1, showing the rank based on fold change.

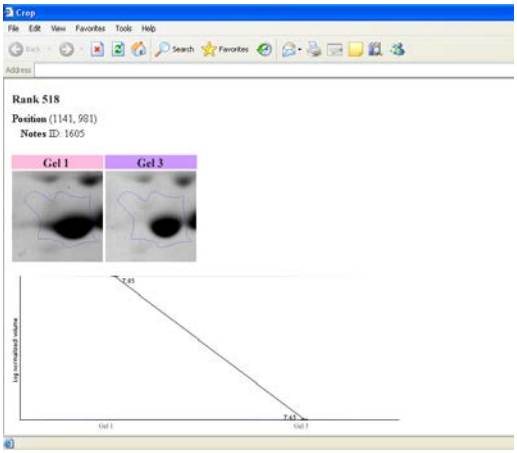

Fig. 2 - Rank 518

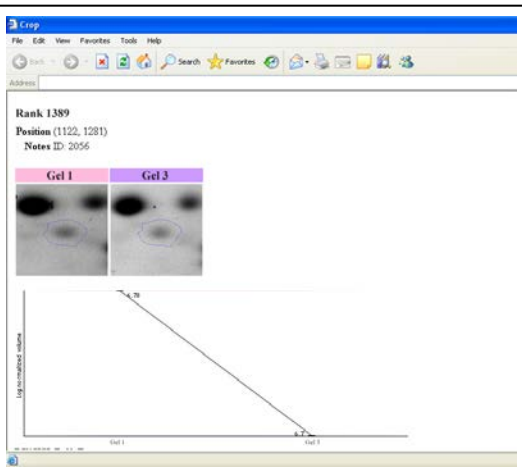

Fig. 3 - Rank 1389

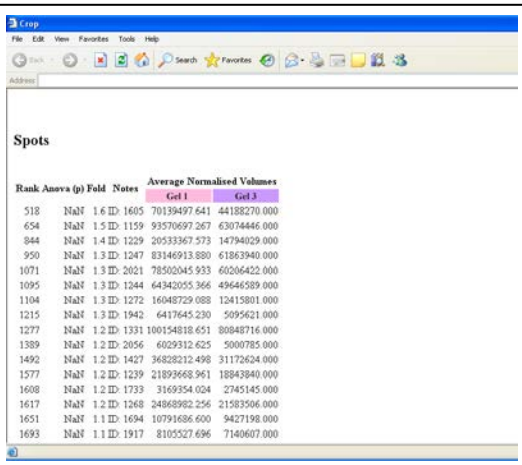

Fig. 4 - Spot Table

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