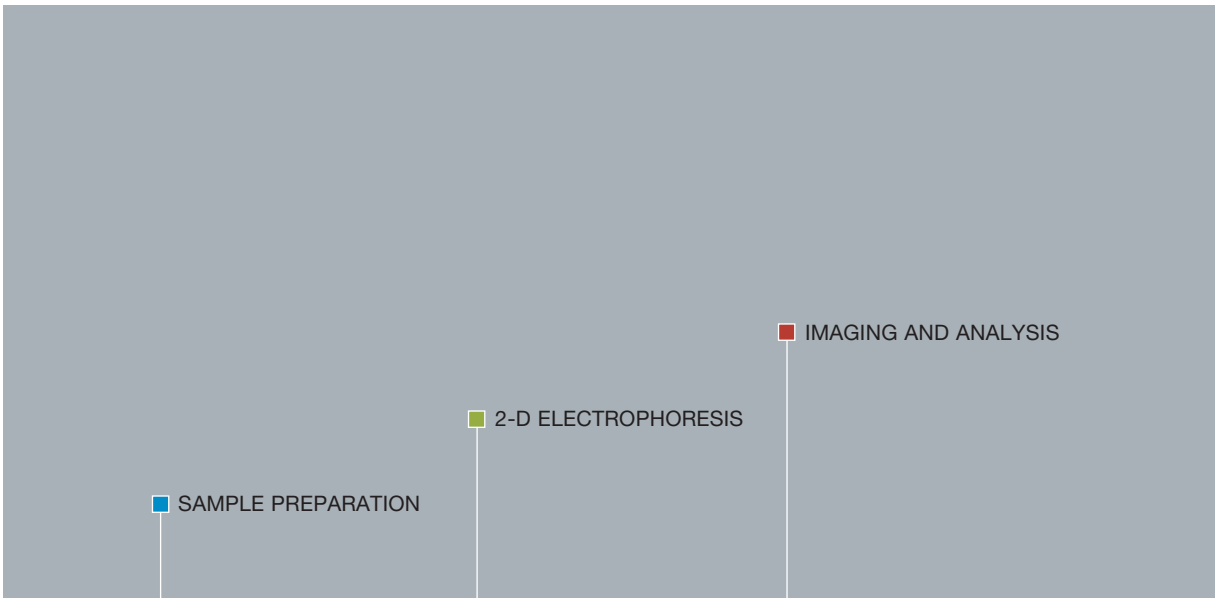


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Overview

Tools for Protein Sample Preparation,
2-D Electrophoresis, and Imaging and Analysis





Proteomics, the study of protein expression in cells or organisms, has become a major focus in the characterization of biological systems. The growing interest in this area of research has stimulated development of new techniques and applications, making it useful to categorize the field into several areas of investigation: expression proteomics, functional proteomics, and structural proteomics.

Expression Proteomics

Expression proteomics defines patterns of proteins expressed in different biological samples. This global quantitative analysis and identification of proteins can generate a map of a given proteome, helping to identify differences in protein expression that result in a particular phenotype. Ultimately, the goal of expression proteomics studies is to reference these changes back to the corresponding genes.

Within the area of expression proteomics, several complementary tools are available for proteome analysis, including the following three technologies: sample preparation, two-dimensional (2-D) electrophoresis, and imaging and analysis. Each set of tools is ideally suited for a 2-D gel electrophoresis-based approach to analysis of sample proteins.

From sample preparation to protein analysis, Bio-Rad's expression proteomics tools provide you with a variety of choices in methodology, protocols, and products. For sample preparation, you can choose from reagent kits, chromatography columns, and preparative electrophoresis cells to clean up or fractionate your sample. For 2-D electrophoresis, Bio-Rad offers flexibility in gel format and size for the optimal combination of speed and resolution while providing reproducible results. For gel imaging and analysis, you can choose from a variety of imaging systems, each with sophisticated image analysis software onboard, to help you organize and interpret your results. Each module is optimized to ensure complete integration to resolve, display, characterize, and quantitate any protein sample.

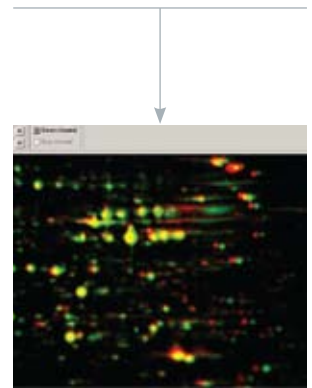
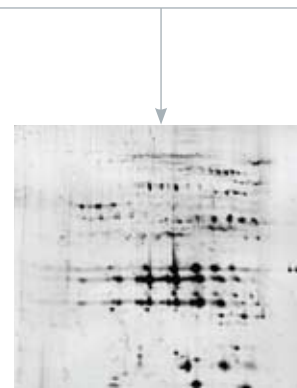
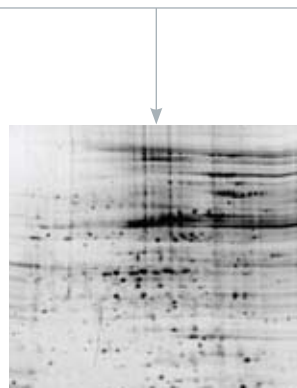
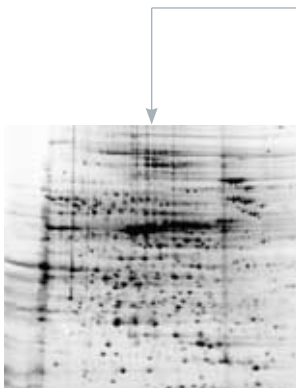
Steps in an Expression Proteomics Workflow

Sample Preparation

2-D Electrophoresis

1. Sample proteins can be enriched using a sequential extraction protocol based on differential protein solubility.

2. Staining the proteins allows visualization and imaging for comparison.



Experimental Design

Differential profiling of sample proteins provides an excellent model to highlight the capabilities of Bio-Rad's expression proteomics tools. In this approach, the effects of changes or differences in experimental conditions on protein expression levels are tracked.

Proteins are resolved using sample preparation tools and high-resolution 2-D gels, then stained for visualization and imaging. The resulting protein spots that show intensity changes are identified and quantitated. These can be excised, then digested for mass spectrometric analysis leading to protein identification. By combining and integrating information from the experimental results with other bioinformatics data, you can identify these changing proteins.

Through the integration of Bio-Rad's expression proteomics technologies — sample preparation, 2-D electrophoresis, and imaging and analysis — your experiments develop from hypothesis to protein discovery.

Sample Preparation

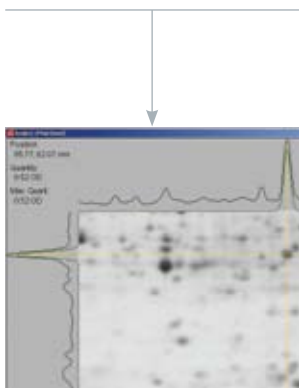
Transforming complex protein mixtures, potentially containing well over 10,000 proteins, into fractions enriched for low-abundance proteins or containing specific subpopulations of proteins is the goal of sample preparation. Finding the optimal sample preparation approach will depend on the biological questions being asked. Whether you use differential solubility to isolate membrane proteins, ion exchange chromatography to resolve basic proteins, or preparative-scale electrophoresis for enrichment of specific protein families, Bio-Rad's expression proteomics tools include the technologies you need.

Visualization

In 2-D electrophoresis, proteins are first separated by isoelectric focusing and then further resolved by SDS-PAGE to generate a 2-D array that can contain thousands of protein spots. Separated proteins are displayed using one of a number of staining methods to reveal proteins in the gel. 2-D electrophoresis provides a reliable, reproducible process for protein separation and analysis. A number of 2-D electrophoresis options offer the flexibility to select the gel size and staining protocol that best fits your needs.

Imaging and Analysis

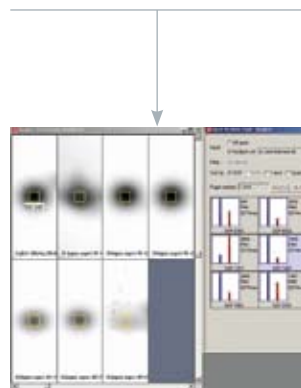
3. Digitizing the gel images ensures accurate quantitation of changes occurring with experimental treatment.



Quantitation

Imaging instrumentation captures protein patterns in 2-D gels. Several factors ensure that the optimum image is gathered from every gel, regardless of its size or the staining method used. Sophisticated digitization and spot-processing algorithms are used to create intensity histograms for each protein in the stained gels. Reliable quantitation of protein spots with PDQuest™ 2-D image analysis software is essential for identifying changes in expression patterns.

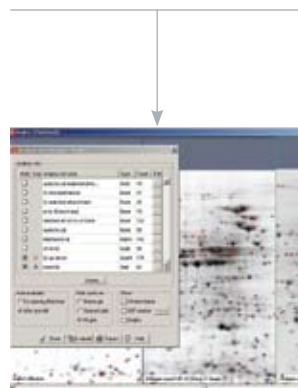
4. Comparative analysis of protein spots matched among gels identifies proteins of potential biological significance.



Comparison

Many steps in the image analysis process are automated for rapid, accurate comparison of a few gels in a single experiment or many gels within a comprehensive project. Proven statistical analysis tools in PDQuest software are used to identify experimentally significant changes in protein spot patterns. Sets of proteins of interest can be compared among multiple experiments to determine both similarities and differences of protein expression patterns as experimental treatments change. Once protein spots of interest have been selected, PDQuest drives the EXQuest™ spot cutter to precisely excise these spots from the gels.

5. Identification and protein characterization is achieved using mass spectrometric analysis.



Characterization

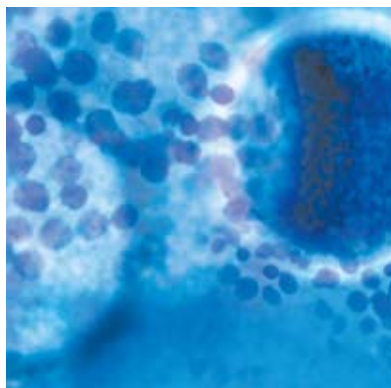
Characterization of excised spots requires digestion to cleave the proteins into peptides, mass analysis to determine peptide molecular weights, and a search of protein databases for sequences that correlate with these masses. Unambiguous identifications may be achieved by mass spectrometry with a MALDI instrument; more detailed characterization may require LC-MS/MS analysis with a Q-TOF instrument. Seamless integration of data tracking between PDQuest software and mass spectrometry results delivers automatic annotations of protein identities on the gel images.

6. Bioinformatic integration links experimental data, sample history, and databases to create a complete archive of experimental results.



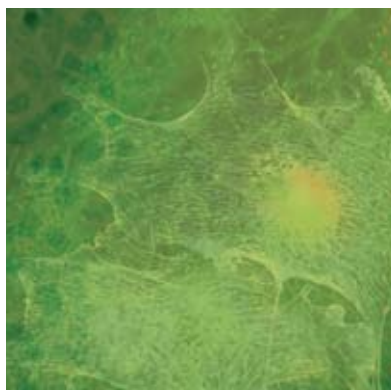
Integration

From sample source information to laboratory protocols and reagents to protein identification, experimental information needs to be tracked and monitored. PDQuest software can store, manage, and record all of the results of your proteomics process and link these results with outside bioinformatics resources.



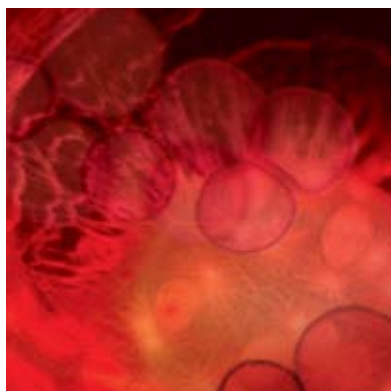
Sample Preparation

Sample preparation prior to 2-D electrophoresis is critical for producing meaningful, reproducible results. Bio-Rad has developed a number of sample preparation products for extraction, cleanup (contaminant removal), fractionation, and depletion. These products include chemical reagents, chromatography columns, and preparative electrophoresis tools.



2-D Electrophoresis

One of the most powerful protein separation techniques available, 2-D electrophoresis has the ability to resolve complex mixtures of thousands of proteins simultaneously in a single gel. Depending on the experiment, requirements for resolution, throughput, and speed may vary. Bio-Rad addresses these varying requirements by offering choices for all components of the 2-D process.



Imaging and Analysis

Proteins separated by 2-D gel electrophoresis are stained for visualization, then imaged and analyzed to compare the protein spot patterns of the different samples. Bio-Rad provides a wide assortment of imaging systems and sophisticated PDQuest 2-D analysis software to simplify the entire analysis process.

Support



Application Support

Bio-Rad's expression proteomics experts offer field support to customers worldwide. Each specialist has a solid understanding of the technology and research experience that will help you find solutions to your experimental needs.

Sales Support

Bio-Rad's trained, knowledgeable customer support staff operates worldwide. They can help you choose the best system to fit your particular needs.

For more information, contact your local Bio-Rad sales representative or visit us on the Web at www.expressionproteomics.com

Global Technical Support

Bio-Rad has over 30 years of experience in 2-D technology. Our worldwide technical support staff is highly trained and can advise you on how to obtain good results. They can help with troubleshooting or with advice on suitable tools for sample preparation or other expression proteomics technologies.

Research and Development

Bio-Rad's expression proteomics R&D team develops ideas into reliable research tools. By continuing to make 2-D electrophoresis a more reproducible and robust technology, R&D helps customers to focus on research, rather than perfecting techniques.

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