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BioRadiations

A Resource for Life Science Research

A HISTORY OF SOLUTIONS

Continued in Current Innovations

In this issue:

BIO-RAD

ribonucleintegrity.

Know your RNA, trust your RT-qPCR.

The success of your real-time qPCR experiments starts and ends with the quality of your RNA. Bio-Rad's Experion™ automated electrophoresis system features the RNA Quality Indicator (RQI), rapidly determining the integrity of your RNA sample and reducing experimental failure.

Continuing our history of providing quality electrophoresis tools, the Experion system's automated separation, detection, and data analysis make your life easier, your research publishable.

Get complete confidence in your real-time qPCR and avoid risking expensive experiments. Please visit **www.bio-rad.com/ad/RNAquality/** for more information.

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If you have comments or suggestions regarding *BioRadiations*, please email us at **bioradiations@bio-rad.com**.

BioRadiations

issue 128, 2009

TO OUR READERS

Bio-Rad Laboratories, Inc., was established more than 55 years ago, when David and Alice Schwartz participated in a game of bridge and conversation veered toward products that should exist but did not. Research scientists themselves, the husband and wife team determined that what was lacking were tools and services to make scientific research processes more efficient and results more reproducible. This mission to discover ways to help scientists contribute to the body of research by removing or minimizing challenges inherent in the experimental workflow continues today. Four Bio-Rad products are profiled to demonstrate how some of our most recent developments can help accelerate scientific discovery.

COVER STORY

DEPARTMENTS

TECHNICAL REPORTS

Legal Notices — See page 32.

ProteoMiner™ Small- and Large-Capacity Kits

ProteoMiner kits for protein enrichment are now offered in formats optimized for use with varying amounts of starting protein.

- **Small-capacity kits** — optimized for use with limited sample material (minimum 10 mg of protein is recommended)
- **Large-capacity kits** — optimized for use with samples in which at least 50 mg of protein is available

ProteoMiner Protein Enrichment Kits

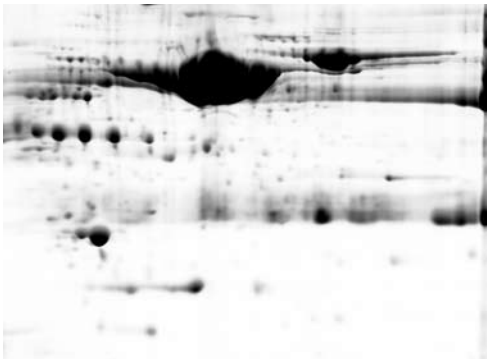
ProteoMiner protein enrichment kits provide all the necessary reagents for accessing low-abundance proteins, utilizing a single elution reagent in a simple, easy-to-perform process. The kits can be used with a variety of biological samples and are compatible with all major downstream proteomics applications. Small- and large-capacity kits are available for processing two or ten samples.

ProteoMiner Sequential Elution Kits

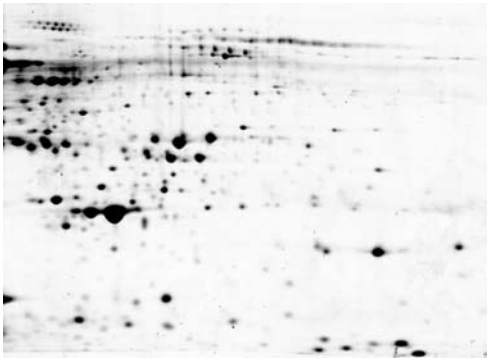
ProteoMiner sequential elution kits utilize multiple elution reagents to sequentially elute proteins based on different properties. These kits fractionate proteins to improve detection and resolution and have been optimized for SELDI experiments.

For more information, go to **www.bio-rad.com/proteominer/**.

Untreated serum



Treated serum



Reduction of high-abundance proteins improves detection and resolution of proteins. Top, untreated serum; bottom, serum treated using the ProteoMiner protein enrichment kit.

Ordering Information

Catalog #	Description
ProteoMiner Protein Enrichment Kits	
163-3006	ProteoMiner Protein Enrichment Small-Capacity Kit, for processing 10 mg of sample, 10 preps, includes 10 spin columns, wash buffer, elution reagents, collection tubes
163-3007	ProteoMiner Protein Enrichment Large-Capacity Kit, for processing 50 mg of sample, 10 preps, includes 10 spin columns, wash buffer, elution reagents, collection tubes
163-3008	ProteoMiner Protein Enrichment Introductory Small-Capacity Kit, for processing 10 mg of sample, 2 preps, includes 2 spin columns, wash buffer, elution reagents, collection tubes
163-3009	ProteoMiner Protein Enrichment Introductory Large-Capacity Kit, for processing 50 mg of sample, 2 preps, includes 2 spin columns, wash buffer, elution reagents, collection tubes
ProteoMiner Sequential Elution Kits	
163-3010	ProteoMiner Sequential Elution Small-Capacity Kit, for processing 10 mg of sample, 10 preps, includes 10 spin columns, wash buffer, 4 sequential elution reagents, collection tubes
163-3011	ProteoMiner Sequential Elution Large-Capacity Kit, for processing 50 mg of sample, 10 preps, includes 10 spin columns, wash buffer, 4 sequential elution reagents, collection tubes
ProteoMiner Kit Accessories	
163-3003	ProteoMiner Sequential Elution Reagents, 10 preps, includes reagents only (columns not included), to be used with 163-3006 or 163-3007

Criterion Stain Free™ Gels

Criterion Stain Free gels have Tris-HCl formulation for PAGE applications and a proprietary compound that facilitates protein visualization by UV imaging. Criterion Stain Free gels are made without SDS. With SDS omitted from the running buffer, the gels can be used to run proteins under nondenaturing conditions for subsequent analysis of native conformation and activity. To run denaturing gels, simply use a running buffer that contains SDS.

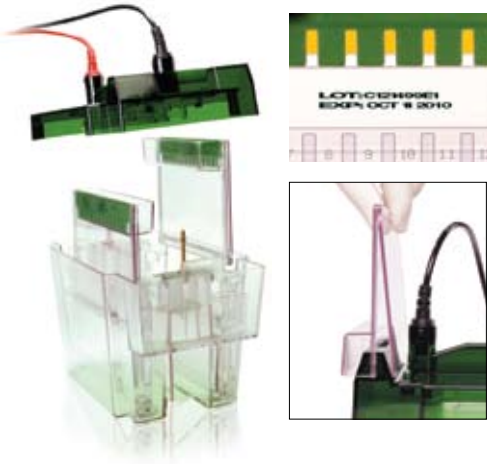
Buffer Recommendations for Criterion Stain Free Gels

Gel Type	Application	Sample Buffer	Running Buffer
Tris-HCl	Native PAGE	Native	Tris/glycine
	SDS-PAGE	Laemmli	Tris/glycine/SDS

Choose from two types of gels:

- **Single-percentage resolving gels** — useful for samples having a limited size range of molecules when your goal is to separate a single band from neighboring bands. These gels will produce the greatest separation between bands with similar molecular weights. Single-percentage gels are cast with a 4% stacking gel to further sharpen protein bands before they enter the resolving gel
- **Linear gradient gels** — useful for when your sample contains a wide range of molecular weights. These gels allow both high and low molecular weight bands to be visualized on the same gel

For more information, go to **www.bio-rad.com/stainfree/**.



Criterion™ electrophoresis system. Features include: locator slots to easily position and guide precast gels into the buffer tank, a built-in wedge on the lid to open the cassette, and lot information printed on each gel.

Configurations of Criterion Stain Free Gels

Gradient	Wells	Sample Volume, µl
10% resolving gel	12+2	45
10% resolving gel	18	30
4–20% linear gradient	12+2	45
4–20% linear gradient	18	30
4–20% linear gradient	26	15
8–16% linear gradient	IPG+1 well	11 cm IPG strip

MyTetraCell.com Instructional Video and Animation

How to Set Up the Apparatus and Run Samples Using the Mini-PROTEAN® Tetra System

An instructional video and animation demonstrating the set up and use of the Mini-PROTEAN Tetra system has been released on **MyTetraCell.com**. The video features a Bio-Rad senior scientist peforming traditional SDS-PAGE using ReadyGel® precast gels in the Mini-PROTEAN Tetra cell. The animation provides step-by-step instructions on how to assemble the Mini-PROTEAN Tetra cell for leakproof operation. Both the video tutorial and animation can be viewed on the **MyTetraCell.com** homepage.

ProteinChip® SELDI Standardization Suite

The ProteinChip SELDI standardization suite is a family of products designed to ensure consistent, reproducible results when using the ProteinChip SELDI system. Standardization products include:

ProteinChip System Starter Kit

- **ProteinChip system starter kit** — includes a complete set of ready-to-use consumables and is a powerful training tool for new customers and experienced ProteinChip SELDI system users who wish to improve their experimental technique
- **Qualification and calibration kits** — allows users to evaluate system performance and ensure that the instrument is operating at specifications
- **Updated software** — provides simplified system evaluation procedures, reduced risk of errors, and greater confidence in the data generated
- **Components upgrade** — optimizes instrument performance and improves system consistency and reliability with the replacement of critical components and subassemblies

SELDI standardization products can be found at **www.bio-rad.com/seldistandardized/**.

ProteinChip Data Manager Software, Version 4, Desktop Edition and 3.x to 4 Upgrade

ProteinChip data manager software, version 4, is the latest release for the ProteinChip SELDI system. Version 4 adds a host of new features to enhance the biomarker analysis workflow, including:

- Analysis wizard for automated data processing, normalization, and clustering
- Plot overlays for visualizing differences in expression levels between spectra
- Trend plots to visualize the expression level of a cluster as a function of time or dosage

The new Desktop Edition provides researchers with full-featured software that can be used while separated from the central data server, useful for collaborative projects and offsite data analysis. Customers can upgrade their existing version 3.x servers through the 3.x to 4 upgrade package.

Ordering Information

Catalog #	Description
SW3-040050	ProteinChip Data Manager Software, Desktop Edition, version 4
SW3-060010	ProteinChip Data Manager Software, version 4, 3.x to 4 upgrade

Biomarker Discovery Using SELDI Technology Guides

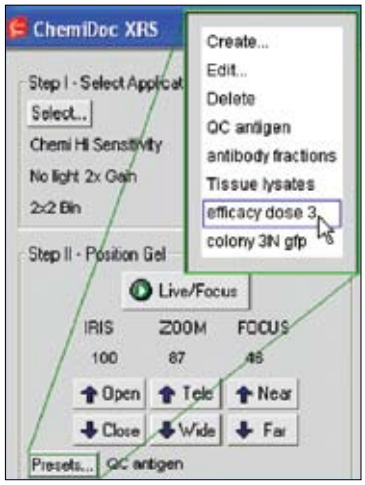
Two new guides address topics critical to successful protein biomarker discovery using the ProteinChip SELDI system:

- **A Guide to Successful Study and Experimental Design** — provides general guidelines for effective study design and planning of experimental workflows, tips on improving reproducibility, and important considerations for sample preparation, data collection, and data analysis
- **A Guide to Data Processing and Analysis Using ProteinChip® Data Manager Software** — includes a description of the data analysis steps beginning with data organization and proceeding through statistical analysis of peak clusters, detailed information about many of the software's analysis features, and recommended system settings for different data sets

The SELDI technology guides (bulletins 5642 and 5814) can be ordered at **www.bio-rad.com/ad/biomarkerguide/**.

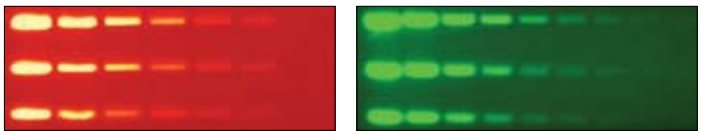
Molecular Imager® Gel Doc™ XR+ and ChemiDoc™ XRS+ Systems

The Molecular Imager Gel Doc XR+ and ChemiDoc XRS+ systems are easy to use for reproducible results. They enable quick visualization, analysis, and documentation of gels, blots, and arrays with a few clicks of the computer mouse. The complete systems are composed of a self-contained darkroom; high-resolution camera with motorized lenses; UV, white, and optional blue illumination; and Quantity One® 1-D analysis software. These are flexible systems that support multiple detection methods, including fluorescence, colorimetry, chemiluminescence, and chemifluorescence. The versatile optics and large imaging areas accommodate a wide array of samples, from large handcast polyacrylamide gels and small ReadyAgarose™ gels to 1-D and 2-D blots. The Gel Doc XR+ and ChemiDoc XRS+ systems are the ideal accompaniment to your PCR, purification, and electrophoresis systems, enabling image analysis and documentation of restriction digests, amplified nucleic acids, genetic fingerprinting, RFLPs, and protein purification and characterization.



Reproducible acquisition settings.

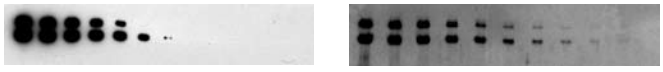
- **Reproducible results** — acquisition settings can be stored for quick imaging of the same or similar samples so you can make fast progress with experiments and projects
- **Flexible, easy-to-use platform** — adaptable to a wide array of samples, from DNA and protein gels to colony plates; from simple documentation to sophisticated sample analysis
- **Safe DNA imaging** — DNA samples are imaged, documented, and analyzed for publication, presentation, team meetings, or laboratory notebooks within a self-contained system using the XcitaBlue™ conversion screen to remove risk of UV exposure to the user and samples



An alternative to UV illumination to better preserve DNA samples. Left, serial dilutions of ethidium bromide–stained precision molecular mass ruler (Bio-Rad Laboratories, Inc.) on agarose gel imaged with UV light; right, serial dilution of SYBR® Safe–stained precision molecular mass ruler on agarose gel imaged with XcitaBlue conversion screen. Lane 1 of 51.2 bp has an initial load of 51.2 ng, and the GelDoc XR+ system detects down to 100 pg. There is no loss in sensitivity when a combination of SYBR® Safe nucleic acid fluorescent stain and less harmful blue excitation is used instead of UV–excitable EtBr. The SYBR® Safe image was taken using the XcitaBlue conversion screen and SYBR® Safe/GFP filter.

XcitaBlue Conversion Screen

SYBR® Safe Emission Filter



Western blot of human serum anti-trypsin and transferrin serial dilutions detected using the ImmunStar™ WesternC™ chemiluminescence kit. The left blot was imaged on film for 300 sec and the right blot was imaged on the ChemiDoc XRS+ system for 60 sec. The ChemiDoc XRS+ system has a lower limit of detection despite overexposure of the blot on film.

For more information, please visit us at **www.bio-rad.com/imaging/**.

Ordering Information

Catalog #	Description
170-8190	Molecular Imager Gel Doc XR+ System, PC and Mac
170-8251	Molecular Imager ChemDoc XRS+ System, PC
170-8252	Molecular Imager ChemDoc XRS+ System, Mac
170-8183	XcitaBlue Conversion Screen, viewing goggles, SYBR® Safe filter

Mini-Sub® Cell GT Model Redesign

The popular Mini-Sub cell GT cell has been redesigned with many updated features to make horizontal electrophoresis even easier.

Mini-Sub Cell GT Cell

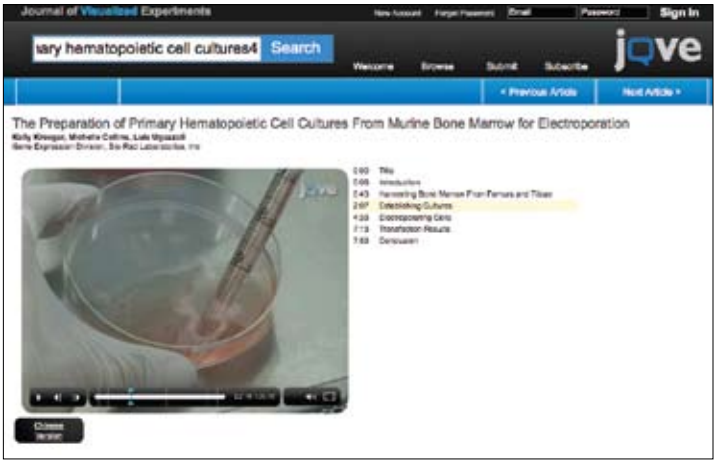
- **Environmentally friendly** — less plastic is used in constructing the redesigned model
- **Easy to clean** — patent-pending QuickSnap electrodes are easily removed
- **Leakproof** — new electrode design
- **Intuitive setup** — printed arrows on each side of the base indicate the direction of the run and ensure proper gel orientation
- **Simple assembly** — color-coded, labeled electrodes and labeled base facilitate the correct positioning of the lid onto the base
- **Easy lid removal** — improved longer tabs on the base prevent incorrect lid positioning and permit easy removal of the lid, reducing buffer spillage
- **Flexible design** — UV-transparent gel tray, combs, and other accessories are compatible with both new and old models

For more information, including ordering information for complete systems and compatible accessories, go to **www.bio-rad.com/horizontalelectro/**.

Ordering Information

Catalog #	Description
164-0300	Mini-Sub Cell GT Cell and PowerPac Basic Power Supply, with mini-gel caster, 7 x 10 cm UV-transparent gel tray
170-4406	Mini-Sub Cell GT System, with 7 x 7 cm UV-transparent gel tray
170-4466	Mini-Sub Cell GT System, with 7 x 10 cm UV-transparent gel tray
170-4467	Mini-Sub Cell GT System, with mini-gel caster, 7 x 10 cm UV-transparent gel tray
170-4486	Mini-Sub Cell GT System, with mini-gel caster, 7 x 7 cm UV-transparent gel tray

Educational Tool Guides Preparation of Primary Hematopoietic Cells for Electroporation



Properly cultured cells are required for transfection experiments, including electroporation. Cell cultures are derived from either primary tissue explants or cell suspensions, and when these cultures are grown in a controlled, sterile environment, they can be assumed to be healthy.

Two categories exist when discussing cell cultures: continuous and primary. Continuous cell lines tend to be abnormal and are often transformed, whereas primary cell cultures are typically more delicate with a finite lifespan. The delicate nature of primary cells often translates into a need to adopt precautions during culturing.

To help guide researchers toward successful preparation of primary cell cultures, Bio-Rad has produced a video titled "The Preparation of Primary Hematopoietic Cell Cultures From Murine Bone Marrow for Electroporation." The video features a Bio-Rad application scientist demonstrating first how to culture these cells, and then subsequently how to electroporate murine mast cells using the Gene Pulser MXcell™ electroporation system. Topics covered include:

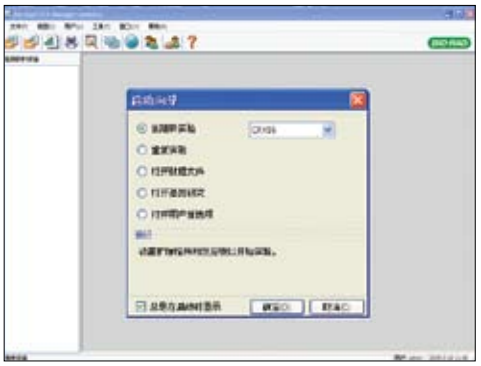
- Obtaining primary cells directly from fresh mouse tissue
- Tissue culture techniques
- Preparation techniques for primary cell electroporation

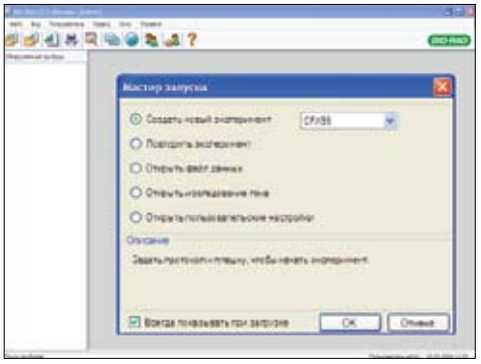
The peer-reviewed video can be found online at the following locations:

- **Bio-Rad website** — click the link to the video from the GenePulser MXcell electroporation system product description page (**www.bio-rad.com/mxcell/**)
- **Gene Expression Gateway** — from the landing page (**www.bio-rad.com/genomics/**), navigate to Products > Gene Transfer > Electroporation, then click the link to the video

CFX Manager™ Software, Chinese and Russian Editions

CFX Manager software provides settings for customization and data analysis in real-time PCR applications. The new, full-featured Chinese and Russian editions of CFX Manager software work with the regional settings of the Windows XP and Windows Vista operating systems to provide localized, language-specific environments.





Features include:

- **Customizable settings** — apply unique log-in names and labels from gene and sample name libraries
- **Intuitive navigation and startup wizard** — quickly learn to use the software and start obtaining real-time PCR results
- **Record keeping on your own schedule** — enter or edit PCR plate information before, during, or after a run
- **Email notification upon run completion** — receive an attached data file or report and analyze results when and where you want
- **Well Groups feature** — analyze multiple experiments from a single plate, each with its own analysis settings
- **Advanced analysis tools** — perform normalized gene expression using multiple reference genes, gene stability values, and individual reaction efficiencies

The Chinese and Russian editions also provide hardware protection to CFX Manager software: a HASP hardware license (HL)-based key must be attached to a USB port on the computer to use the software.

System Requirements

	Minimum	Recommended
Operating system	Windows XP Professional SP2, Windows Vista Home Premium	Windows XP Professional SP2, Windows Vista Home Premium
Processor	1 GHz	2 GHz
RAM	1 GB (2 GB for Windows Vista)	2 GB
Hard drive space	10 GB	20 GB
Screen resolution	1,024 x 768 with true-color mode	1,280 x 1,024 with true-color mode
Drive	CD-ROM	CD-RW
USB port	2.0 Hi-Speed	2.0 Hi-Speed
Internet browser	Internet Explorer	Internet Explorer
Additional software	—	Microsoft Office Suite

Ordering Information

Catalog #	Description
184-5008	CFX Manager Software, Chinese edition, includes 3 user licenses, installation CD, 3 HASP HL keys, instructions
184-5028	CFX Manager Software, Russian edition, includes 3 user licenses, installation CD, 3 HASP HL keys, instructions

amplifantastic.

Bio-Rad and Biogazelle are teaming up.

Combine the unsurpassed performance and flexibility of a CFX96™ or CFX384™ real-time PCR detection system with the impressive analysis tools in qbase^{PLUS} software. Coming soon, every CFX96 or CFX384 system will come with a license for qbase^{PLUS} software. This powerful combination will speed up your real-time PCR and accelerate discovery.

Research. Together.

biogazelle
accelerating your analysis

Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applera's United States Patent No. 6,814,934 B1 for use in research and for all other fields except the field of veterinary diagnostics.

Bio-Rad's real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Nos. 6,767,512 and 7,074,367.

To find your local sales office, visit **www.bio-rad.com/contact/**
In the U.S., call toll free at 1-800-4BIORAD (1-800-424-6723)

Visit us at www.bio-rad.com

BIO-RAD

Testing the Efficacy of Bio-Rad Low-Profile Plates and Tubes on Two ABI Fast Real-Time PCR Systems

Bio-Rad and Fast PCR

It is possible to greatly reduce PCR run times by using what is known as fast PCR, thus maximizing throughput and shortening experimental time. Fast PCR is characterized by run times of no more than half the traditional 90 minute PCR protocol, and is typically achieved with run times of approximately 30 minutes. A majority of the time savings is achieved through changes to the thermal cycling protocol, such as shorter hold times, higher annealing temperatures, and lower denaturation temperatures; fast ramping cyclers provide only minimal additional benefit. These fast PCR conditions present challenges for components of the traditional PCR system, leading to the development of consumables optimized for fast conditions.

Fast PCR requires quick heat transfer between samples and the cycler block. Just as thin-wall tubes helped take advantage of increasing cycling speeds in thermal cyclers a decade ago, today's faster thermal cyclers can benefit from low-profile PCR tubes and plates. Traditional 0.2 ml full-height PCR tubes and plates extend well above cycler blocks. The space beyond the cycler block is not efficient for heat transfer and provides room for condensation. The overall height of low-profile vessels is 5.3 mm less than full-height vessels (Figure 1). The elimination of unnecessary space improves low-volume reactions and fast PCR.

Fig. 1. Low-profile tubes (left) do not have extraneous space above the cycler block compared with full-height, traditional PCR tubes (right).

In addition to reduced vessel height, it is also essential that tubes and plates are made to the highest quality standards. Slight variations in manufacturing can lead to less efficient heat transfer or reduced sealing. Similarly, poor fit between the reaction vessel and a particular instrument platform will result in inefficient and, therefore, variable heat transfer among wells. Even small variances in heat transfer or sealing can affect data quality. Highest quality consumables are necessary for the best uniformity among replicates.

Bio-Rad has a long history of offering high-quality low-profile tubes and plates (Figure 2) that provide superior performance in several of its thermal cyclers and real-time PCR detection systems that can utilize the low-profile format (such as the DNA Engine® and 1000-series cyclers, and the Opticon™, Chromo4™, and CFX96™ systems). More recently, Applied Biosystems (ABI) also adopted low-profile 0.1 ml MicroAmp Fast reaction plates and tubes for their fast PCR and qPCR instruments. To determine if Bio-Rad low-profile plates and tubes can provide excellent performance on ABI's instrument platform, we tested our Multiplate low-profile 96-well unskirted PCR plates (MLL-9601) and low-profile tube strips (TLS-0801) on ABI's StepOnePlus and 7500 Fast real-time PCR systems. We found the performance of Bio-Rad low-profile plates and tubes to be comparable to or better than that of ABI's own MicroAmp Fast reaction plates (4346906) and tube strips (4358293).

Fig. 2. Multiplate™ low-profile unskirted PCR plate and tube strip.

Experimental Results

Bio-Rad low-profile plates and tube strips demonstrated excellent real-time performance on the ABI fast block systems. The well-to-well uniformity across the plates (or 96 wells of tube strips) was comparable to or better than that of the plastic consumables recommended by the instrument manufacturer.

On the 7500 Fast real-time PCR platform, the Bio-Rad Multiplate low-profile unskirted PCR plates had the highest uniformity of all the consumables tested. The standard deviation in C_T values across 96 wells was only 0.055 whether the low-profile plates were sealed with Bio-Rad optical flat caps (TCS-0803) or Microseal® 'B' adhesive seal (MSB-1001) (Figure 3). The ABI MicroAmp Fast plates had C_T standard deviations across the 96 wells of 0.095 and 0.067 when sealed with ABI film (4360954) or caps (4323032), respectively. Bio-Rad low-profile tube strips showed slightly less uniformity in C_T values than the Bio-Rad plates with a standard deviation across 96 wells of 0.074 (Figure 3), but remained comparable to the ABI plates. The ABI tube strips also showed lower uniformity than plates with a standard deviation across 96 wells of 0.19, which is more than twice that observed with the Bio-Rad tube strips.

Bio-Rad plates and tube strips were also comparable or better than the ABI vessels on the StepOnePlus instrument (Figure 4). The Bio-Rad low-profile plate again showed the best uniformity among the vessels tested on the StepOnePlus instrument, although uniformity of all the tested vessels was not as high as that on the 7500 Fast system. Bio-Rad plates

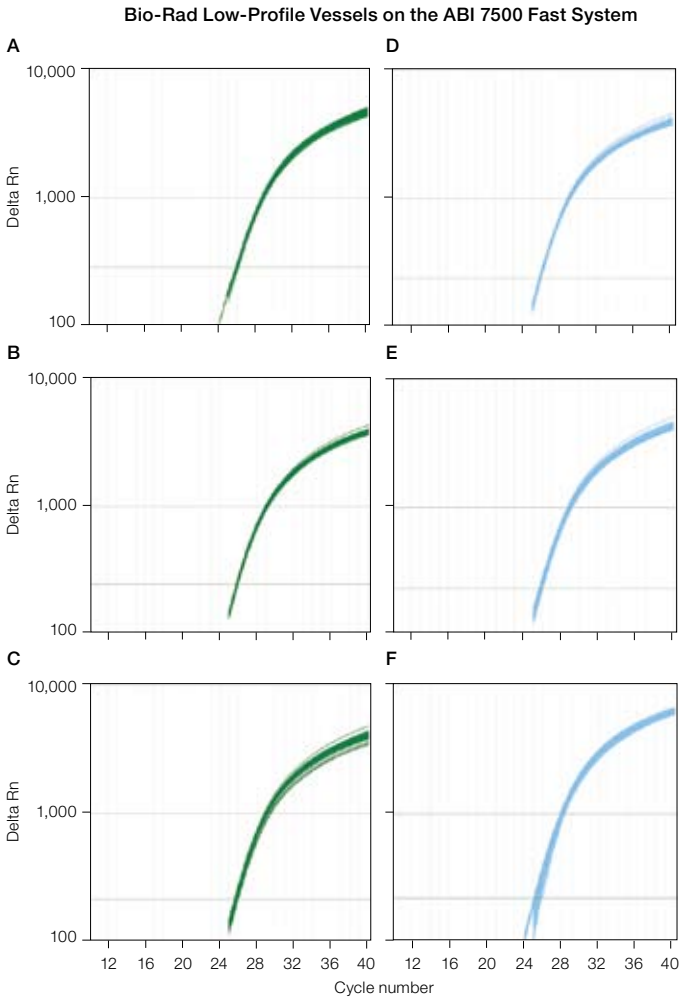


Fig. 3. Real-time amplification of a 68 bp fragment of the IL-1β gene from human spleen cDNA. The 10 µl reactions were amplified using iTaq™ fast supermix with ROX on an ABI 7500 Fast real-time PCR system using either Bio-Rad Multiplate low-profile vessels (**A–C**, —) or ABI MicroAmp Fast low-profile vessels (**D–F**, —). Accumulation of amplification products was monitored with a Cy5-labeled probe normalized to ROX fluorescence. **A**, Bio-Rad Multiplate low-profile 96-well unskirted PCR plate sealed with ultraclear optical flat cap strips; **B**, Bio-Rad Multiplate low-profile 96-well unskirted PCR plate sealed with Microseal 'B' adhesive seal; **C**, Bio-Rad low-profile 0.2 ml 8-tube strips sealed with ultraclear optical flat cap strips; **D**, ABI MicroAmp Fast plate sealed with optical caps; **E**, ABI 0.1 ml MicroAmp Fast plate sealed with optical adhesive film; **F**, ABI MicroAmp Fast tube strips sealed with optical caps.

showed a C_T standard deviation across 96 wells of 0.109 when sealed with Microseal 'B' adhesive seal, and 0.175 when sealed with optical flat caps. The ABI MicroAmp Fast plates had C_T standard deviations across the 96 wells of 0.209 and 0.287 when sealed with ABI film or caps, respectively. Removing one outlier from the ABI plate with caps and reanalyzing across 95 wells resulted in a standard deviation of 0.264. Tube strips again showed slightly lower uniformity than plates from the same manufacturer. Bio-Rad low-profile tube strips had a standard deviation across 96 wells of 0.219, which was again slightly larger than the standard deviation observed with Bio-Rad Multiplate plates, but comparable to the ABI plates. ABI's tube strips yielded a standard deviation of 0.362 across 96 wells and 0.316 after removing two outliers.

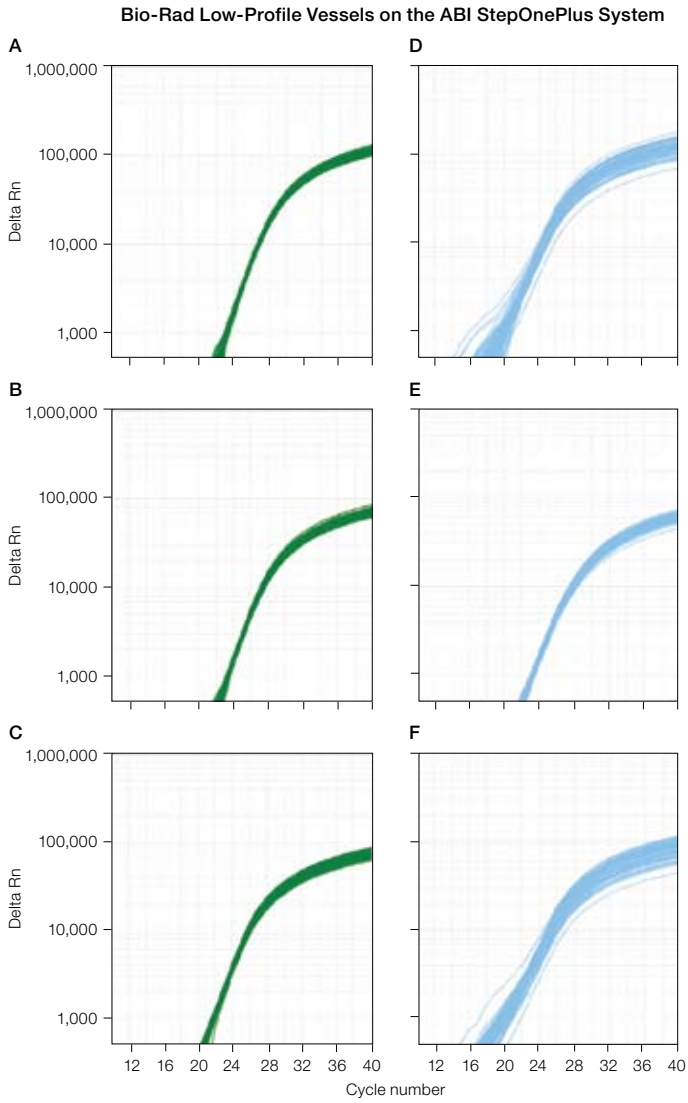


Fig. 4. Real-time amplification of a 68 bp fragment of the IL-1β gene from human spleen cDNA. The 10 µl reactions were amplified using iTaq fast supermix with ROX on an ABI StepOnePlus real-time PCR system using either Bio-Rad Multiplate low-profile vessels (**A–C**, —) or ABI MicroAmp Fast low-profile vessels (**D–F**, —). Accumulation of amplification products was monitored with a FAM-labeled probe. **A**, Bio-Rad Multiplate low-profile 96-well unskirted PCR plate sealed with ultraclear optical flat cap strips; **B**, Bio-Rad Multiplate low-profile 96-well unskirted PCR plate sealed with Microseal 'B' adhesive seal; **C**, Bio-Rad low-profile 0.2 ml 8-tube strips sealed with ultraclear optical flat cap strips; **D**, ABI MicroAmp Fast plate sealed with optical caps; **E**, ABI 0.1 ml MicroAmp Fast plate sealed with optical adhesive film; **F**, ABI MicroAmp Fast tube strips sealed with optical caps.

Conclusions

Bio-Rad low-profile plates and tubes provide excellent performance on the ABI fast block instrument platforms and are a comparable and cost-effective alternative to the ABI MicroAmp Fast plates and tube strips. The unskirted design of the Bio-Rad low-profile plates is not only convenient and easy to handle, but also provides additional flexibility as the plates can be cut to allow running less than a full plate of samples without sacrificing the entire plate. This added flexibility is particularly advantageous because of the reduced uniformity observed with tube strips relative to plates on the fast block platforms tested.

ProteinChip® Data Manager Software — Dynamic Software for Biomarker Discovery

Introduction

The search for clinical biomarkers entails the analysis of large numbers of patient samples, and when combined with the ProteinChip SELDI system's high-sensitivity, high-throughput capabilities, the result is an enormous amount of data. The ability of clinical researchers to mine this data and uncover possible biomarkers is directly dependent on the quality of the software tools available. ProteinChip data manager software is designed to provide a biomarker discovery toolset that drastically reduces the time required for data analysis. The software encompasses all aspects of the process, from instrument control through creation of a list of candidate biomarkers of statistical significance.

Instrument Control

For most samples, the simplified instrument control interface of the ProteinChip data manager software takes the large number of settings normally required to acquire data and distills them down to the most important input commands. For applications requiring more flexibility, advanced protocols are used to fine-tune parameters.

Sample Tracking

ProteinChip data manager software provides an intuitive, graphical user interface for fast, easy input of sample properties and acquisition parameters using the Virtual Notebook (Figure 1). The Virtual Notebook allows the user to enter comprehensive information on sample processing conditions associated with each spot on the arrays.

During unattended acquisition, the ProteinChip SELDI reader communicates with the data manager server to transfer the sample property and acquisition information from the Virtual Notebook for up to 1,344 unique samples. Spectra are then exported to the data manager software, fully annotated and ready for further downstream analysis.

Fig. 1. Virtual Notebook interface showing sample randomization.

Data Analysis

The search for biomarkers often involves the search for proteins whose expression levels differ from one sample group to another. Analysis features of the data manager software include algorithms to group peaks of similar molecular weight from across samples and plot overlays to visually display differences in expression levels. *P* values and receiver-operating characteristic (ROC) curves (Figure 2) are used to report statistical relevance for individual markers.

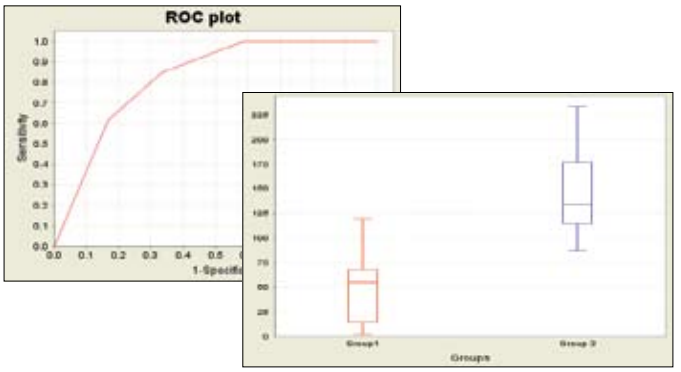


Fig. 2. ROC plot and whisker plot highlighting the differences between sample groups.

The software includes additional statistical tools, such as principal component analysis (PCA) and hierarchical clustering (Figure 3) for analyzing the relationship between samples. Export formats are compatible with other data analysis tools.

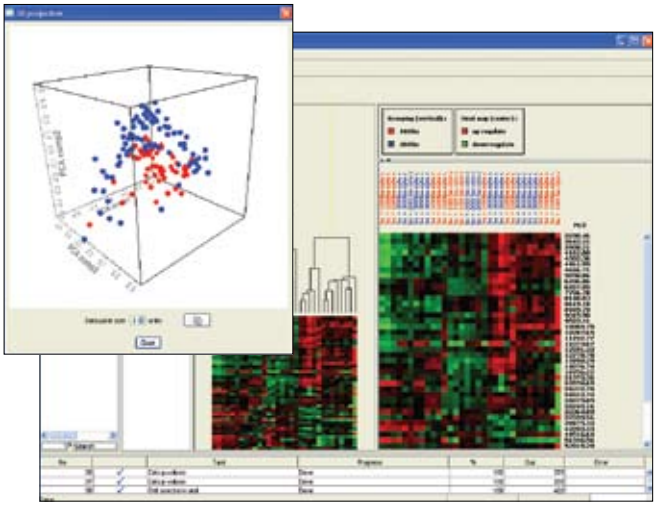


Fig. 3. Multivariate techniques of PCA and hierarchical clustering for visualizing sample relationships.

Operational Qualification

As with any scientific study, the calibration of the platform must be monitored closely at regular intervals to ensure maximum reproducibility. The software works in conjunction with the ProteinChip operational qualification (OQ) kits to provide calibration results for the ProteinChip SELDI reader from a single mouse click. The software automates the application of necessary processing parameters, labels the required peaks, and generates a PDF report of the results (Figure 4).

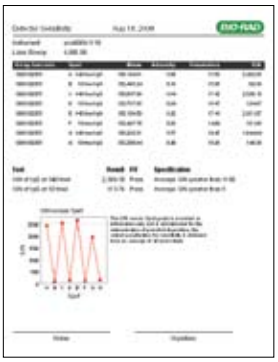


Fig. 4. Report automatically generated using the operational qualification feature.

Server Edition and Desktop Edition

The Server Edition of the software is packaged with the ProteinChip SELDI system; it provides the instrument control interface for running the ProteinChip SELDI reader and includes Virtual Notebook functionality. The Desktop Edition of the software can be installed on any computer or laptop (useful for collaborative SELDI research projects and offsite data analysis).

For more information, go to **www.bio-rad.com/proteinchip/**.

What's New in Version 4?

ProteinChip Data Manager Software

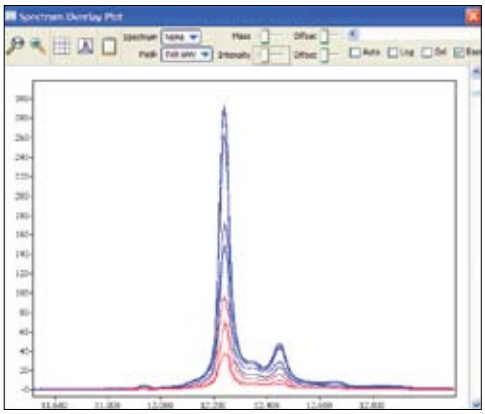
ProteinChip data manager software, version 4, introduces a number of new features that enhance biomarker research by further streamlining data processing and analysis workflows, enabling new data visualizations, and creating a new time point analysis plot.

Analysis Wizards

Previous versions of data manager software had separate dialog boxes for data processing and analysis, and required manual input of parameters for each new data set. In version 4, data processing, normalization, and clustering have been brought together into a single Analysis Wizard, reducing the need to click back and forth between dialog boxes. More importantly, the parameters set in the Analysis Wizard can be saved and rapidly applied to other data sets.

New Look and Feel

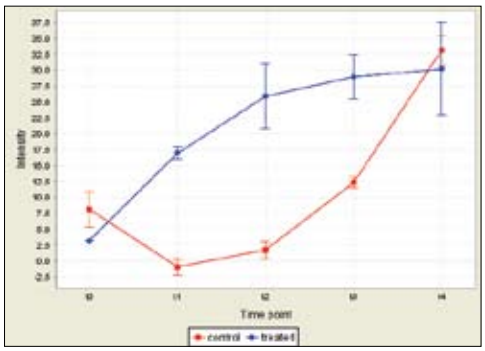
The user interface has been revamped to accommodate a host of new spectrum navigation and plotting features. Graphs have been updated to achieve a presentation-ready quality. Spectrum navigation and zooming have been improved to further enhance the biomarker analysis experience.



Spectrum plot overlay showing expression change between group 1 (—) and group 2 (—).

Plot Overlays

Spectrum plot overlays, combined with the ability to assign colors to spectra, provide researchers with a powerful new plot display for visualizing protein expression changes between sample groups. The plot overlay can even be rotated to produce a landscape spectrum view.



Trend plot showing expression level changes over time between two sample groups across five time points.

Trend Plots

Trend plots introduce the ability to track clustered data along an additional time point axis to plot the expression level of a cluster as a function of multiple readings. This capability enables visualization of the expression level changes of potential biomarkers over the course of multiple patient samplings or varying dosages.

Comparison of Bio-Plex® Magnetic and Nonmagnetic Human Group I Cytokine Assays

Introduction

Bio-Plex assays are multiplexable bead-based assays that utilize xMAP technology and are optimized for the Bio-Plex suspension array system. Each bead set may be conjugated with a unique reactant specific for a different target; the system is capable of analyzing up to 100 biomolecules in a single sample. The capture antibodies used in the original Bio-Plex polystyrene bead-based human and mouse cytokine assays have been coupled to magnetic beads to create the newest members of the Bio-Plex Pro™ family of products.

Why Magnetic Beads?

Magnetic bead-based assays offer workflow flexibility and higher reliability. Using the magnetic format, wash steps can be automated on the Bio-Plex Pro wash station or the user can choose to use traditional vacuum separation. Magnetic separation can reduce the variability often associated with manual washes on a vacuum manifold that is caused by user inexperience, filter plate failure, and uneven vacuum pressure. Thus, the magnetic format can result in higher reliability overall.

Other improvements include an all-in-one kit format (Figure 1). The assay, diluent, and reagent kits previously offered as three separate catalog items can now be ordered using a single catalog number. Human and mouse cytokine targets remain the same as those included in the nonmagnetic assays; however, ICAM-1 and VCAM-1 have been separated from the human cytokine group II panel into singleplex assays, since these require a higher dilution factor. Table 1 provides a comparison between the Bio-Plex Pro magnetic and the Bio-Plex nonmagnetic cytokine assays.

Magnetic vs. Nonmagnetic Human Group I Cytokine Assays

The performance criteria for both magnetic and nonmagnetic assays were verified by running five independent plates for each configuration. Assay performances were validated to ensure consistency in parameters such as precision, accuracy, and sensitivity (limit of detection, LOD). Magnetic assays had comparable precision (intra-assay %CV <20% and inter-assay %CV ≤30%) and accuracy (70–130%) with the nonmagnetic assays. The LODs were comparable for most assays in the panel. Notable improvements in sensitivity were observed for magnetic assays with IFN-γ LOD (nonmagnetic, 19.6; magnetic, 6.4 pg/ml) and eotaxin LOD (nonmagnetic, 14.6; magnetic, 2.5 pg/ml).

The working assay ranges — lower limit of quantitation and upper limit of quantitation (LLOQ and ULOQ) — are listed in Table 2. Of the 27 assays, 21 had similar (<2-fold difference) or higher ULOQ, and 20 of the 27 had similar (<2-fold difference) or lower LLOQ in the new magnetic format. Therefore, a majority of the assay working ranges are comparable; magnetic human group I cytokine assays are comparable in performance to the nonmagnetic polystyrene assays. The human group I assay standard curve performances are comparable between the magnetic and nonmagnetic panels (Figure 2).

A comparison study of magnetic and nonmagnetic human group I cytokine panels was run using 37 plasma, cell culture, and synovial fluid samples. Results that fell within the working

Table 1. Bio-Plex Pro magnetic vs. nonmagnetic cytokine assays.

Feature	Bio-Plex Pro Kits	Bio-Plex Kits
Bead type	Magnetic	Nonmagnetic
Bead size (µm)	6.5	5.6
Bead region	New regions	Old regions
Hu group I	IL-1β (39), IP-10 (48)	IL-1β (32), IP-10 (41)
Hu group II	21-plex: IL-2α (13), TNF-β (20), SDF-1α (22), MIG (14) Singleplex: ICAM-1 (12), VCAM-1 (15)	23-plex: IL-2Rα (24), TNF-β (88), SDF-1α (87), MIG (68), ICAM-1 (17), VCAM-1 (79)
Kit configuration	All-in-one kit	3 kits: assay, diluent, reagent
Standards	Included in all-in-one kit	Part of assay kit
Sample and standard diluents	Included in all-in-one kit	Part of diluent kit
Reagents	Included in all-in-one kit	Part of reagent kit
Bead stock	10x	25x
Detection antibody stock	10x	10x
Bio-Plex Manager™ software		
Protocol	MagPlex	Bio-Plex
DD gates	5,000–25,000	4,335–10,000
No. of bead events/test	50	100
Bead map	100	100

Hu = human; numbers in parentheses correspond to bead region.

Fig. 1. Bio-Plex Pro all-in-one cytokine assay kit.

Table 2. Working assay ranges for magnetic and nonmagnetic assays.

	Magnetic Assay, pg/ml		Nonmagnetic Assay, pg/ml	
Target	LLOQ	ULOQ	LLOQ	ULOQ
IL-1β	3.2	3,261	2.0	4,400
IL-1ra	81.1	70,487	5.5	17,600
IL-2	2.1	17,772	4.3	16,400
IL-4	2.2	3,467	2.0	11,600
IL-5	3.1	7,380	2.0	3,200
IL-6	2.3	18,880	3.1	12,800
IL-7	3.1	6,001	2.0	3,200
IL-8	1.9	26,403	2.0	6,800
IL-9	2.1	7,989	16.0	12,800
IL-10	2.2	8,840	3.1	10,400
IL-12 (p70)	3.3	13,099	4.3	22,400
IL-13	3.7	3,137	2.0	17,600
IL-15	2.1	2,799	4.3	5,600
IL-17	4.9	12,235	10.2	12,800
Eotaxin	40.9	5,824	25.4	12,800
Basic FGF	27.2	7,581	12.5	3,200
G-CSF	2.4	11,565	5.5	12,800
GM-CSF	63.3	6,039	16.0	22,400
IFN-γ	92.6	52,719	11.3	8,000
IP-10	18.8	26,867	5.5	3,200
MCP-1 (MCAF)	2.1	1,820	119.1	2,000
MIP-1α	1.4	836	4.3	800
MIP-1β	2.0	1,726	3.1	5,600
PDGF-BB	7.0	51,933	4.3	27,200
RANTES	2.2	8,617	3.1	4,400
TNF-α	5.8	95,484	16.0	8,000
VEGF	5.5	56,237	2.0	12,800

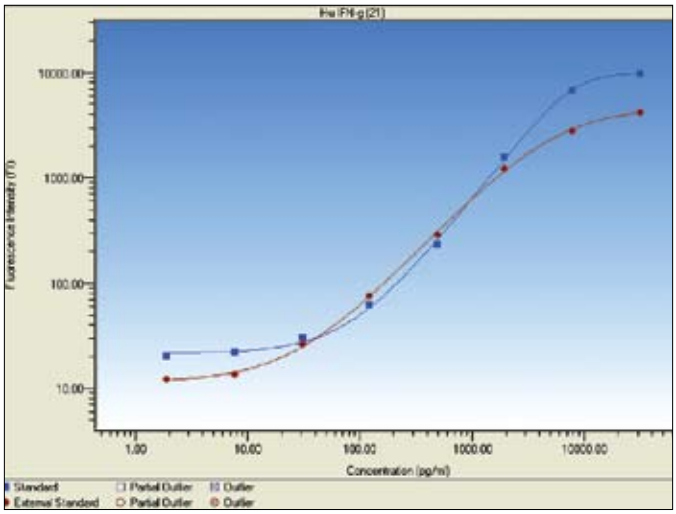


Fig. 2. IFN-γ standard curve. Magnetic (■), nonmagnetic (●).

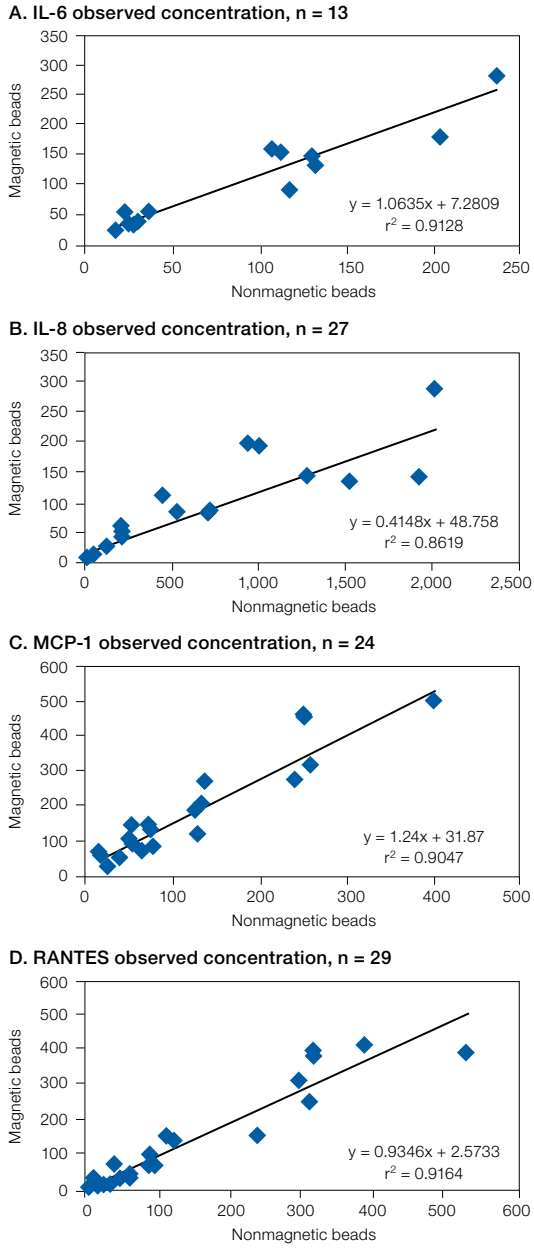


Fig. 3. Correlation plots of magnetic and nonmagnetic assays. The number of samples evaluated for each target is listed at the top of each graph since many of the assays had fewer than 37 samples within working assay range. Assay observed (◆), linear (—).

assay ranges were graphed in correlation plots. There was good correlation for a majority of the magnetic and nonmagnetic assays in the human group I 27-plex cytokine panel. Results for a subset of the assays are shown (Figure 3).

Conclusions

Advantages of the new Bio-Plex Pro magnetic human cytokine assays include an all-in-one kit format with simplified ordering, the potential for automation, and robust assay performance. Improvements to these assays have led to a number of modifications, including changes in bead parameters, kit configurations, and software settings. Two separate studies were carried out to verify performance of the magnetic human group I cytokine 27-plex panel as compared to the nonmagnetic panel. In the first study, five plates from each configuration were tested to verify assay precision, accuracy, sensitivity, and working assay range. In the second study, a variety of biological samples were analyzed to test correlation between magnetic and nonmagnetic assays. Both these studies established that performance of the magnetic panel is comparable to the nonmagnetic format.

Authors: Bill Gette and Shawn Miller

REMOVING OBSTACLES TO DISCOVERY

A MORE THAN 55 YEAR TRADITION

Bio Rad Laboratories was founded in 1952 by husband and wife team David and Alice Schwartz. The couple first met at UC Berkeley, where they both graduated, Dave with a degree in chemistry and Alice with a degree in biochemistry. The idea that would soon launch the company came about unexpectedly during a game of bridge. During the card game, the players, which included Dave and Alice, joked about products that were not on the market that maybe ought to be. Someone suggested creating “tobacco mosaic virus,” a virus Alice was using for scientific research and one that required many days to prepare. Dave questioned why no one was manufacturing it. The following day, he and Alice searched their neighborhood in Berkeley for a place to launch a company with a mission to accelerate the scientific discovery process by providing products and tools to researchers.

While the couple quickly discovered that the world was not eagerly awaiting tobacco mosaic virus, they didn’t give up. In the years that followed, they continued to expand their product line. They worked closely with their customers to gain a better understanding of what researchers were trying to accomplish and how. With this feedback, Bio-Rad continued to grow, offering a broader array of innovative solutions designed to meet the needs of researchers. Over 55 years later, Bio-Rad offers thousands of products worldwide that incorporate a broad range of technologies used to separate, purify, analyze, identify, and amplify biological and chemical materials. The four products featured in this article represent a continuity of Bio-Rad’s tradition of providing innovative ways to make research more efficient, more reproducible, and ultimately more effective.

Eliminating the Time and Steps Required For Coomassie Staining

In protein characterization studies, Coomassie dye is a key tool used to visualize separated proteins on SDS- and native PAGE gels. While using Coomassie stain is the standard procedure for imaging protein gels, the mechanisms by which the stain binds to proteins is not well understood. Proteins that are rich in the basic amino acids bind the dye strongly, while others do not. Glycoproteins, which make up more than half of all proteins, also bind the dye poorly, and at least one publication has indicated that using Coomassie stain techniques may result in overestimating relative protein quantities in gels (Ownby et al. 1993). In addition, when using Coomassie stain, researchers often have to choose between sensitivity (G-250 staining) or resolution (R-250 staining). The technique is also time consuming, requires several steps, generates large volumes of organic waste, and the results are frequently not reproducible.

The drawbacks inherent in using Coomassie stain led Bio-Rad researchers to wonder, "What if we could visualize gels within 5 minutes and obtain reliable, reproducible quantitation with the push of a button?" Exploring this possibility is what led to the development of the Criterion Stain Free™ gel imaging system. The technology is based on a UV-induced modification of tryptophan residues contained in proteins after electrophoretic separation (Kazmin et al. 2002). After electrophoresis, the gel is removed from the cassette and placed into the Criterion Stain Free imager, where the separated proteins are activated by UV irradiation, producing a fluorescent signal that is captured by a CCD camera. In as few as 2.5 minutes, the system provides an image of the proteins and automatically estimates the molecular weight and quantity for each detected protein band using a molecular weight standard. The UV-irradiated gel is still compatible with subsequent staining by Coomassie, silver, or fluorescent dyes.

This stain-free technology provides sensitivity equivalent to or better than that of Coomassie stain, while actually improving reproducibility of quantitation for some proteins that lack the basic amino acid structure that tends to bind the dye strongly. For example, quantitation of β-galactosidase using the Criterion Stain Free system provided CVs <10% for quantitation, while the CVs for Coomassie stain techniques were almost 20% (data not shown). While the stain-free method requires tryptophan for detection, it is estimated that proteins without tryptophan represent less than 10% of proteins from 10 to 260 kD (Table 1). In fact, only 7.3% of human proteins that lack tryptophan are larger than 10 kD. The stain-free approach is quite suitable for analysis of a wide range of complex protein samples and cell lysates, often yielding results visually equivalent to techniques utilizing Coomassie stain (Figure 1).

Dr Martina Andberg
Research Scientist
VTT Technical Research Centre of Finland

The VTT Technical Research Centre of Finland is a nonprofit organization that provides research, development, testing, and information services to the public sector, companies, and international organizations. Its objective is to develop new technologies and innovations, thus increasing client competence and ability to compete. Dr Martina Andberg is engaged in research on the production and purification of a wide variety of enzymes generated through genetic engineering, as well as the screening of biological organisms, for applications in biorefinery and utilization of renewable raw materials. She and her staff perform a large number of protein purifications, each requiring PAGE analysis to determine which fractions contain the protein(s) of interest. This process consumes large amounts of technician time in tedious and redundant tasks.

Late last year, Andberg was planning to purchase a new thermal cycler for PCR studies. Aware of the large volume of PAGE gels that Andberg ran and analyzed, her Bio-Rad sales representative suggested that the Criterion Stain Free gel imaging system could greatly improve productivity in her laboratory.

According to Andberg, the system has performed "even better than we expected," providing equivalent or superior sensitivity to the use of Coomassie stain techniques, and greatly reducing the time required to analyze protein fractions. Purifications that previously had to be run overnight can now be completed in the same day. Andberg says that the system "tells you what to do and is very user friendly," so they now routinely obtain molecular weights and percent purities on most fractions. They also use the system for western blotting and have found that it works nicely with their antibodies. Their work sometimes requires protein identification by matrix-assisted laser desorption/ionization time-of-flight analysis, and Andberg was pleased to learn of the recent technical note (bulletin 5810) that demonstrates the compatibility of the system with mass spectrometric protein analysis. The elimination of organic waste has been an additional, unexpected benefit.

Bio-Rad's focus on assisting Andberg in improving the productivity of her workflow enabled her to generate more data, more reliably and in less time.

Table 1. Tryptophan content of the predicted proteomes of several model organisms.*

Species	Total Number of Proteins	Number of Proteins Lacking Tryptophan	% of Proteins Lacking Tryptophan	Number of Proteins >10 kD	Number of Proteins >10 kD Lacking Tryptophan	% of Proteins >10 kD Lacking Tryptophan
Homo sapiens	40,827	4,209	10.31	37,548	2,754	7.33
Rattus norvegicus	12,022	1,081	8.99	11,421	745	6.52
Mus musculus	35,344	3,435	9.72	33,262	2,480	7.46
Saccharomyces cerevisiae	5,815	648	11.14	5,563	491	8.83
Escherichia coli O1:K1 / APEC	4,865	458	9.41	4,754	408	8.58
Escherichia coli O6:K15:H31	4,604	562	12.21	4,147	365	8.80
Escherichia coli (strain K12)	4,181	456	10.91	3,879	325	8.38

* Sequence data was obtained from UniProt (**http://www.ebi.ac.uk/uniprot/**).

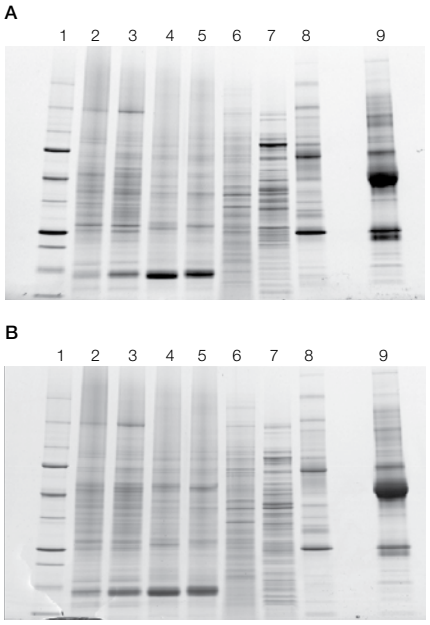


Fig. 1. Comparison of gel visualization techniques after electrophoresis of complex protein samples. A, gel visualized using the Criterion Stain Free system; **B**, gel stained with Bio-Safe™ Commassie (Brilliant Blue G-250) stain. Lane 1, Precision Plus Protein™ unstained standards; lane 2, mouse liver extract; lane 3, rat liver extract; lane 4, mouse thymus extract; lane 5, rat thymus extract; lane 6, HeLa cell lysate; lane 7, *E. coli* lysate; lane 8, human serum (treated with the ProteoMiner™ protein enrichment kit); lane 9, human serum (untreated).

Solving Problems Posed by Sample Complexity in Protein Profiling

Mass spectrometry (MS)-based profiling of intact, undigested proteins has developed into an important biomarker discovery application. The application requires detecting lower-abundance proteins in the presence of those in higher abundance. Proteomics technologies that enable the simultaneous analysis of hundreds of proteins hold the promise of identifying biomarker panels that could be used to accurately detect and predict human disease states. Human biological fluids, especially serum and plasma, contain many thousands of proteins and peptides, with concentrations varying over as many as 11 orders of magnitude. The new proteomics technologies must meet the challenge of being sensitive enough to detect proteins present at low concentrations in the presence of a smaller number of proteins that comprise the majority, as much as 99%, of the protein mass of the sample.

Most MS-based proteomics technologies require distinctly separate sample preparation procedures (including cleanup, depletion, enrichment, and prefractionation) to make biomarker discovery possible. These sample preparation steps can contribute to sample loss, result in data that is difficult to reproduce, and severely impact sample throughput. The need to achieve reliable and reproducible results is an additional, very important challenge to successful biomarker discovery using any MS system. Addressing this challenge requires tools for routine quality control and optimization and validation of the system, as well as rigorous standard operating procedures for study design and sample preparation.

ProteinChip® SELDI Standardization Suite: Achieving Consistent, Reproducible SELDI Results

Quality control, optimization, and validation of any MS technology for its intended use are essential to assure the reliability and reproducibility of biomarker discovery results. Many of the variables that affect the validity of protein profiling data such as appropriate sample preparation, molecular weight calibration, detector sensitivity adjustment, data collection, and data analysis are addressed by the ProteinChip SELDI standardization suite. The various components of this suite of products assure reproducible results from day to day, instrument to instrument, and across multiple operators. The suite provides the ability to effectively train users, validate and optimize system performance, and upgrade software and system components to assure optimal control, data analysis, and system performance (see page 4 for more information on the ProteinChip SELDI standardization suite).

Training — ready-to-use consumables for optimization of sample preparation and data acquisition

ProteinChip System Starter Kit

Validation — operational qualification and instrument calibration ensure system performance

ProteinChip Peptide Mass Calibration Kit

ProteinChip OQ Kit and ProteinChip System Check Kit

ProteinChip Detector Calibration Kit

Upgrades — enhanced system performance and improved data collection and analysis capabilities

ProteinChip Data Manager Software Upgrades

ProteinChip SELDI System Components Upgrade

Bio-Rad's surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) technology meets the biomarker discovery challenge by combining the separation power of on-chip chromatography with high-sensitivity MS. ProteinChip SELDI technology also provides the throughput, reproducibility, and necessary quality control features to process the enormous number of samples required to validate candidate biomarkers and to ensure their analytical utility. Because of these benefits, SELDI technology has been widely used for biomarker discovery and characterization in diverse applications including clinical research, drug development, and basic research.

The distinct advantage provided by the chemistry of the ProteinChip SELDI arrays allows lower abundance proteins and peptides to be enriched and retained directly on the array and then detected by the ProteinChip reader. The functionalized array surfaces (for example, cationic, anionic, hydrophobic, and analyte-specific arrays), coupled with different wash conditions, allow capture and enrichment of proteins with specific biochemical properties (Figure 2). Washing of the spots removes nonspecifically bound proteins and interfering compounds, greatly reducing signal suppression in the mass spectrometer caused by salts, detergents, and overall sample complexity. Sample cleanup directly on the arrays used for analysis enables a simplified and reliable high-throughput protein profiling biomarker discovery workflow.

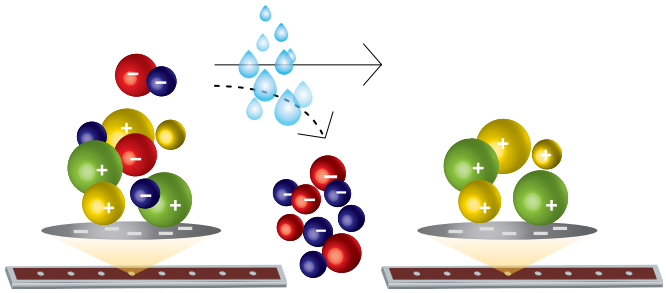


Fig. 2. Proteins captured and enriched on chromatographic ProteinChip arrays are then analyzed by SELDI-TOF MS.

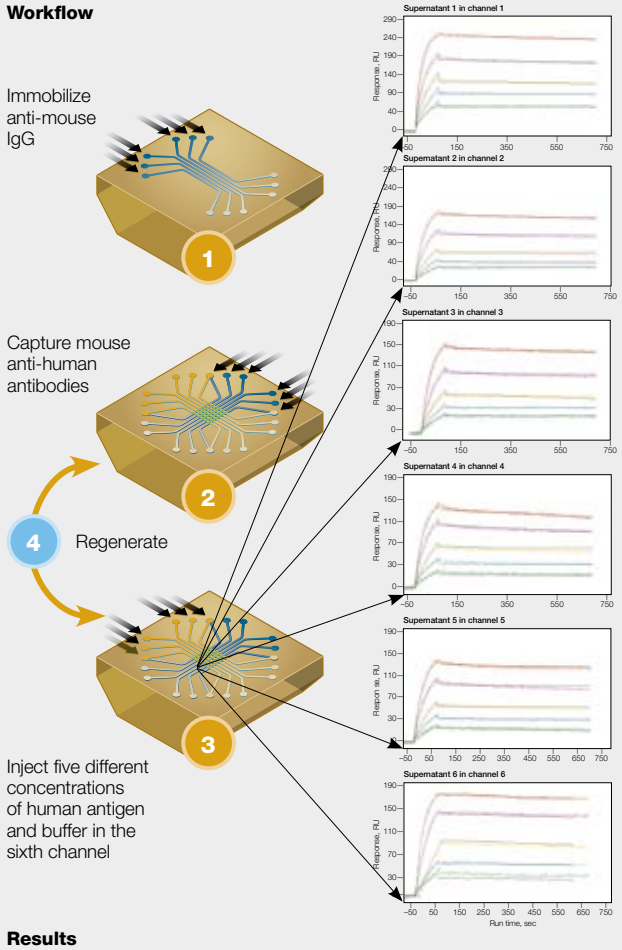
Obtaining Full Kinetic Profiles of Molecular Interactions Without Regeneration

Surface plasmon resonance (SPR) measures molecular interactions on the surface of a chip — in real time and without the need for costly labels that may interfere with binding. SPR has revolutionized the study of protein interactions required for the execution and maintenance of complex biological processes. Such studies have helped elucidate the roles that intracellular concentration, ionic environment, cofactors, and protein conformation play in maintaining those processes. SPR experiments have often been run sequentially, using two flow cells, one as a reference and one for the binding interaction. Ligand is bound to the interaction flow cell, an analyte is injected, and the interaction is measured. The ligand is then regenerated for the study of any additional analytes. No ligand is immobilized in the reference cell; instead, the cell is dedicated to measuring

Case Study: ProteOn™ XPR36 System and Rapid Selection of High-Affinity Supernatant Antibodies

Monoclonal antibodies have become a particularly important resource for medical research, diagnosis, and therapy, as well as for basic science. Here we demonstrate the system for the efficient and rapid screening, ranking, and kinetic binding analysis of hundreds of monoclonal antibody supernatants against the IL-12 human antigen.

Workflow



Results

The screening of 250 IL-12 hybridoma supernatants was completed in about 12.5 hours and yielded full kinetic binding constants for each supernatant. An overview of results obtained:

- IL-12 antigen in five different concentrations was injected simultaneously; running buffer was injected in the sixth channel to correct for decay of the complex (step 3 of workflow)
- Binding curves were fitted to the Langmuir model and the kinetic binding constants of all 250 supernatants were determined
- Affinities were in the range of 0.1–10 nM

Conclusions

- Each cycle determined the kinetic binding of 6 supernatants in parallel; thus, the kinetic analysis of 250 supernatants was accomplished in only 42 cycles
- Kinetic analysis was performed efficiently using supernatant hybridomas without the need for antibody purification
- The parallel mode enabled correction of the drift caused by decay of the supernatant antibodies from the anti-mouse IgG capturing agent
- Local interspot referencing allowed the exact determination of supernatant levels captured by the anti-mouse IgG

nonspecific binding of the analyte to the surface of the chip, bulk effects, and signal drift. The end result of this serial flow cell system is reduced sample throughput, increased time to results, and the risk of losing ligand activity due to repeated regeneration cycles.

An alternate approach utilizes the ProteOn XPR36 multiplexed SPR device, which integrates a novel microfluidics design with state-of-the-art optics. The system utilizes a unique six channel by six channel interaction array on a sensor chip for the analysis of up to six ligands with panels of up to six analytes, producing as many as 36 data points in a single experiment (see Case Study sidebar). Because multiple conditions can be tested in parallel, robust kinetic analysis of an analyte concentration series can be handled in one experiment (One-shot Kinetics™), on a single sensor chip without the need to regenerate between each analyte concentration.

The ProteOn XPR36 system design eliminates the need for a separate reference cell or channel and allows all channels to be used to collect binding data. The 6 x 6 interaction array pattern not only increases the number of interactions that can be monitored on a single chip, but also creates unique interspot areas directly adjacent to the reaction spots. These interspots can be used to measure any nonspecific interaction of the analyte with the chip surface, bulk effects, and signal drift. The horizontal interspot signal (where no ligand is bound) is then subtracted from the reaction spot signal, providing reference-corrected binding data. Multiplexed SPR enables such applications as the rapid identification of highly specific monoclonal antibodies with high affinity for the analyte of interest (Nahshol et al. 2008).

Dr John Corbin
Senior Scientist
Preclinical Research/Development
XOMA LLC

XOMA is a leader in the discovery, development, and production of therapeutic antibodies. As a senior scientist in the molecular interactions and biophysics group, Dr John Corbin is involved in the early-stage screening and affinity characterization of antibodies produced by phage display, in order to identify those with appropriate properties. He also conducts later-stage characterization of lead antibodies as well as analysis of antibodies during production to assure that their properties have not changed.

Corbin and his team evaluated a number of systems to complement their antibody characterization capabilities, and the ProteOn XPR36 protein interaction array system interested them because of its 6 x 6 array configuration. This system fit their throughput needs and offered high flexibility and capability, making it ideal for assay development. Sensor chips that enable very high ligand densities and the price point for the performance level were also key factors in their decision.

Corbin feels that the ProteOn system has "in many ways accelerated our ability to assess protein-protein interactions," providing data on multiple interactions immediately, in contrast to the laboratory's previous instrumentation. "It allows you to accelerate your research because you can make decisions on the data that you get in a very real-time format," he explains.

As one of the earliest purchasers of this system, Corbin was grateful for the "excellent, incredible" level of support that he received to help him utilize the instrument's capabilities to meet his needs. Bio-Rad personnel were often in his laboratory to provide the software and performance upgrades he needed. His local Bio-Rad sales representative still stops by regularly to make sure that everything is going well with the system. "One of the reasons we chose an instrument that was relatively new was because it was supported by Bio-Rad," says Corbin. "We wouldn't have wanted to buy this expensive an instrument from a start-up company that might not be around in the future."

Generating Immunoassay Results You Can Trust

In the past, ELISAs and western blotting were the primary methods used to measure cytokines and other cell signaling proteins in serum, plasma, and cell culture media. The major bottlenecks attributed to these technologies include the inability to measure multiple analytes in a single sample and the time to results (0.5–2 days/analyte). The Bio-Plex® suspension array system overcomes these limitations, and has become widely accepted as a high-throughput, flexible, and easy-to-use immunoassay platform (de Jager et al. 2003). It allows researchers to quantitate up to 100 different analytes in a single sample, providing a means to decipher complex cell signaling pathways in rare or volume-limited samples. In addition, the microplate platform allows the automated analysis of 96-well plates with a throughput of more than 1,800 assay points in 30 minutes.

When analyzing the results from an immunoassay there are several statistical algorithms that can be applied to the standard (calibration) curve. Bio-Plex Manager™ software contains the Brendan StatLIA 5 parameter logistic (5PL) algorithm, which adds an additional parameter to the 4PL algorithm to account for the asymmetry of the curve (Baud 1993). Accommodating curve asymmetry allows more accurate curve fitting, which yields a broader and more reproducible assay working range (Figure 3). A broad range is especially important for multiplex assays because it allows a single set of standards to be simultaneously applied to multiple analytes.

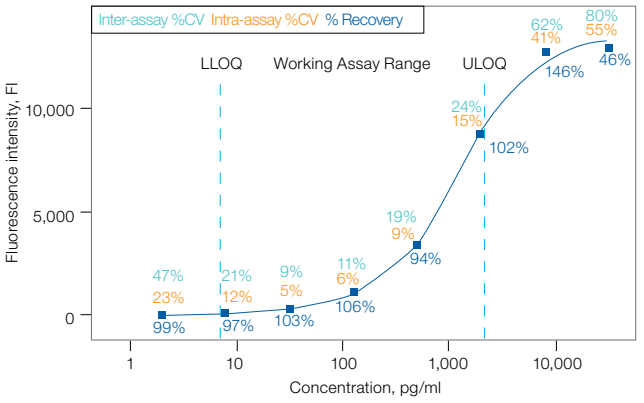


Fig. 3. Working assay range using Bio-Plex Manager software. The working assay range is the range of the standard curve that meets predicted specifications, also referred to as the range between the lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ).

Accurate and reproducible immunoassay results also depend on a properly functioning microplate reader. This is why the Bio-Plex system offers a comprehensive set of quantitative instrument calibration and validation tools. Used daily, the Bio-Plex calibration kit standardizes fluorescence detection from day to day and from instrument to instrument. Used monthly, the Bio-Plex validation kit ensures consistent performance of the fluidics and optics systems. Verifying optical alignment is particularly important since misalignment results in a significant reduction in assay sensitivity (Figure 4). Thus, when used as recommended, calibration and validation procedures should reduce inter-assay variation, as well as maximize assay sensitivity and dynamic range. The net result is robust assay performance.

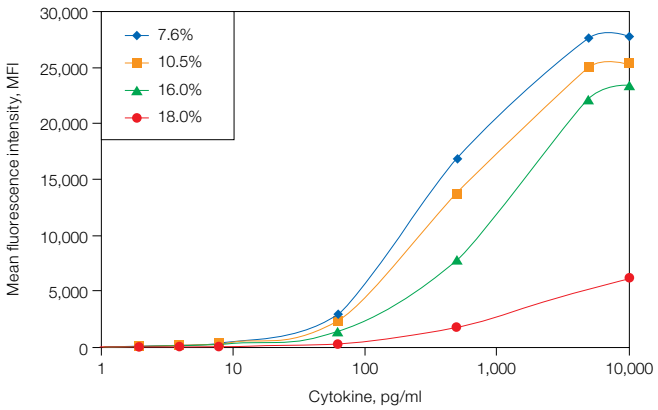


Fig. 4. Detection of the cytokine, IL-6 on a Bio-Plex reader with varying degrees of misalignment.

When combined into a seamless magnetic workflow, the Bio-Plex Pro™ magnetic bead–based assays and the Bio-Plex Pro magnetic wash stations have the potential to reduce assay variability. Precision from well to well (intra-assay) and from washer to washer (inter-washer) was demonstrated using different magnetic wash stations and a custom Bio-Plex Pro human 24-plex cytokine, chemokine, and growth factor panel (Table 2). For each of the 24 analytes detected in a control serum sample, the intra-assay coefficient of variation (%CV) was typically <10% with washers 1, 2, and 3; while the inter-washer variation was <10% for 19 of the 24 analytes tested.

Table 2. Intra-assay precision demonstrated using a Bio-Plex Pro human 24-plex cytokine, chemokine, and growth factor panel with Bio-Plex Pro/ Bio-Plex Pro II magnetic bead wash stations.

	Serum	Intra-Assay Precision*				
Analyte	Cytokine, pg/ml**	Manual Wash	Washer 1	Washer 2	Washer 3	Inter-Washer Precision***
IL-1rα	1959.4	6.3	8.0	7.9	7.8	4.8
IL-8	1449.0	7.3	5.8	4.8	5.8	6.9
PDGF-BB	1444.7	4.5	5.0	5.7	4.8	8.5
IFN-γ	924.2	7.4	9.8	5.8	9.2	12.0
IP-10	483.8	4.4	6.3	4.7	5.2	2.8
GM-CSF	469.6	6.0	9.0	7.4	6.7	6.0
TNF-α	249.4	6.6	10.5	8.1	7.9	6.9
IL-9	116.2	6.3	8.4	6.2	8.7	4.0
IL-2	73.4	5.4	9.5	6.3	7.3	6.1
FGF basic	66.6	6.8	7.3	7.4	8.6	6.2
IL-6	56.1	6.1	8.6	7.7	8.0	5.7
MIP-1β	52.1	6.1	5.4	4.7	4.6	1.6
IL-12 (p70)	48.2	7.8	6.8	5.5	9.2	3.5
G-CSF	47.3	7.9	10.6	8.0	10.3	6.5
IL-17	24.7	4.8	6.5	5.5	7.8	11.6
Eotaxin	23.2	5.0	8.1	7.1	6.3	8.0
IL-1β	19.7	4.8	8.8	7.8	7.8	4.7
IL-13	18.0	9.8	8.5	10.4	10.1	11.1
VEGF	17.1	5.3	8.8	10.8	8.5	9.2
IL-4	12.4	8.3	9.3	9.6	10.1	2.1
IL-7	11.6	7.0	8.0	5.6	8.4	4.3
MIP-1α	8.6	4.5	6.1	5.4	7.4	12.3
IL-10	6.0	6.1	9.3	6.6	8.2	10.2
IL-5	3.9	10.8	10.9	11.9	16.9	3.2

* Calculated as %CV.
** Observed concentrations are represented by the mean of the 18 replicates for 8 microplates using three wash stations and manual washes.
*** Calculated as %CV of assays for three wash stations.

Conclusion

For more than 55 years, Bio-Rad has provided innovative, high-quality products that enhance a wide range of life science research workflows from gene expression, silencing, and modulation to protein separation, purification, interaction analysis, and biomarker discovery. As illustrated by the small sampling of products highlighted in this article, Bio-Rad continues the focus of its founders: producing, supporting, and servicing products that facilitate the quest for highly valuable, reproducible, and reliable data.

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Optimization of Electroporation Conditions for Two Different Burkitt Lymphoma Cell Lines Using the Gene Pulser MXcell™ System

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Introduction

Burkitt lymphoma (BL)-derived cell lines originated from malignant human B cells, and are commonly used as model systems to study the molecular basis of Burkitt lymphoma. Electroporation is the main nonviral method used for gene transfer into these difficult-to-transfect cells. However, electroporation can be challenging, since several parameters must be optimized to achieve good transfection efficiencies, including waveform, voltage, capacitance, and duration. The Gene Pulser MXcell electroporation system is designed to deliver plasmid DNA, siRNA, and other molecules into mammalian cells, especially difficult-to-transfect cells. The enhanced user interface contains optimized preset protocols to easily identify the best possible transfection conditions. Gene Pulser® electroporation buffer is formulated to improve electroporation by minimizing cell death while ensuring highly efficient delivery of nucleic acids.

In this technical report, we describe the optimization of electroporation conditions for the Burkitt lymphoma cell lines Ramos (EBV negative) and Namalwa (EBV positive) using the Gene Pulser MXcell system along with Gene Pulser electroporation buffer. We achieved transfection efficiencies of up to 50% for Ramos and up to 30% for Namalwa cells, while the viability was 50 and 80%, respectively. Our data demonstrate that the Gene Pulser MXcell system is an excellent tool to electroporate difficult-to-transfect cell lines.

Methods

Ramos and Namalwa cells were purchased from the American Type Culture Collection (ATCC CRL-1596, ATCC CRL-1432). For electroporation, cells were washed in PBS and resuspended in Gene Pulser electroporation buffer at a density of 5×10^6 cells/ml. Reporter gene plasmid DNA (gWiz Luciferase or gWiz GFP, both from Aldevron) was added at 20 µg/ml final DNA concentration. The suspension was mixed and transferred to the appropriate wells of a 96-well electroporation plate (150 µl of suspension/well). All transfection reactions were performed in duplicate. Electroporation was carried out on the Gene Pulser MXcell system as indicated. After electroporation, cell suspensions were immediately transferred to growth media (7.5×10^5 cells/ml final cell concentration) and incubated for 24 hr in a cell culture incubator. Lactate dehydrogenase (LDH) levels in the cell culture supernatants were determined using the Cytoscan LDH Cytotoxicity Assay (G Biosciences). Luciferase activity of cell lysates was determined using standard procedures. To determine percentages of viable GFP-positive cells, cells were stained with propidium iodide and analyzed on the BD FACSCalibur instrument using BD CellQuest Pro software (BD Biosciences).

Results

The first set of experiments was devoted to determining the optimal waveform and starting conditions for voltage, capacitance, and pulse duration. The Gene Pulser MXcell system preset 96-well plate protocols (preset protocols 13, Opt 96-well/Exp, Sqr) were chosen. These are designed to test 12 different conditions for both exponential and square waveforms (Figure 1A). These preset protocols are composed of four identical wells per condition, which allow testing of up to four different cell lines on one electroporation plate. Because luciferase assays are ideal for high-throughput experiments, we used the luciferase reporter gene for the first set of experiments.

Twenty-four hours after transfection, luciferase activity was determined in cell lysates for quantitation of reporter gene expression. In addition, LDH levels in cell culture supernatants (a biomarker of plasma membrane damage or rupture) were measured to evaluate cytotoxicity.

As shown in Figure 1B, Ramos cells expressed the highest luciferase levels (light green bars) when they were transfected using exponential-decay conditions at 250 V/350 µF (Figure 1A, conditions 3 and 9). Similar values were obtained with square-wave conditions at 250 V/15 ms (Figure 1A, condition 21). LDH levels (dark green bars) were comparable for all of these conditions, indicating that Ramos cells are amenable to electroporation with both waveforms.

Namalwa cells displayed the highest luciferase activity with the exponential-decay condition at 250 V/350 µF (Figure 1A, conditions 3 and 9). Slightly lower luciferase levels were obtained when the square-wave condition was used (200 V/20 ms; Figure 1A, condition 14), while cytotoxicity was considerably lower than in samples transfected with conditions 3 and 9, indicating that condition 14 may be used for applications when low cytotoxicity is desired. We decided to further optimize the exponential-decay conditions for maximal expression.

Although the setting of 250 V/350 µF was the best exponential-decay condition out of the preset protocols for both cell lines, perhaps better condition sets could be identified in the range between the tested values. Therefore, we tried to further fine-tune these conditions by testing additional capacitance values in the range of 250–500 µF at constant voltage (250 V). We obtained maximal luciferase expression in Ramos cells when the capacitance was set at 400 µF (Figure 2A). Under these conditions, 70% of Ramos cells were viable and 40% of viable cells were

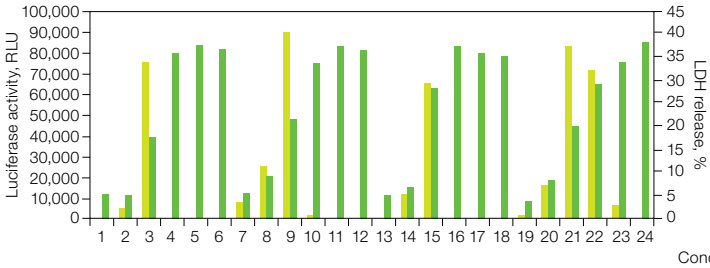
A. Rows A–D, exponential waveform

Column	1	2	3	4	5	6	7	8	9	10	11	12
Voltage (V)	150	200	250	300	350	400	250	250	250	250	250	250
Capacitance (µF)	350	350	350	350	350	350	200	250	350	500	750	1,000

Rows E–H, square waveform

Column	13	14	15	16	17	18	19	20	21	22	23	24
Voltage (V)	150	200	250	300	350	400	250	250	250	250	250	250
Pulse length (ms)	20	20	20	20	20	20	5	10	15	20	25	30

B. Transfection of Ramos Cells (Opt 96-well/Exp, Sqr)



C. Transfection of Namalwa Cells (Opt 96-well/Exp, Sqr)

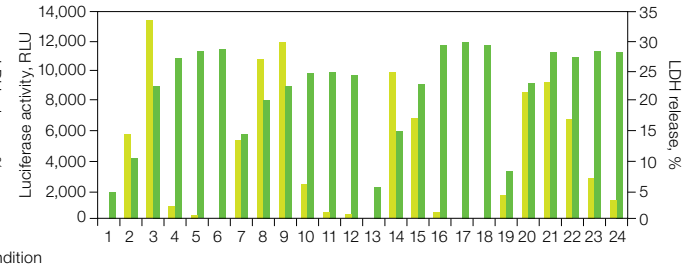


Fig. 1. Optimization of transfection conditions for Ramos and Namalwa cells using preset protocols Opt 96-well/Exp, Sqr. A, electroporation conditions of preset protocols Opt 96-well/Exp, Sqr. The 96-well plate was divided into two sets of conditions, the top four rows were used to deliver exponential-decay pulses and the bottom four rows were used to deliver square-wave pulses. Twenty-four different conditions of voltage/capacitance or voltage/pulse duration were applied to four wells each. Identical conditions are boxed. **B** and **C**, cell viability and luciferase activity of Ramos and Namalwa cells transfected with a luciferase construct using 12 conditions each of exponential and square waveforms as indicated in panel A. Each condition was applied in duplicate. LDH release in cell culture supernatants and luciferase activities in cell lysates were determined 24 hr after transfection. Values represent the average of duplicate samples. Luciferase activity (■), LDH levels (■).

actually transfected, as shown by GFP expression (Figure 2B). Maximal transfection efficiency (50% GFP-positive cells of viable cells) was obtained with 450 µF (Figure 2B). However, only 50% of cells were viable when this setting was used. Depending on whether maximal protein expression, maximal transfection efficiency, or low cytotoxicity is desired, a capacitance setting between 400 and 450 µF can be chosen for Ramos cells.

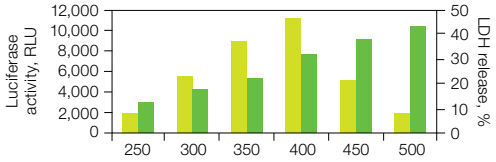
In Namalwa cells, the previously identified condition of 350 µF was still the best setting for maximal luciferase expression (Figure 2C) and maximal transfection efficiency of 30% (Figure 2D), while about 80% of cells were viable. Transfection efficiency could not be further improved by varying the capacitance. We also tried to improve the transfection efficiency by testing voltage settings between 200 and 300 V in increments of 25 V at constant capacitance (350 µF). However, the initial setting of 250 V was still the best condition (data not shown).

Conclusions

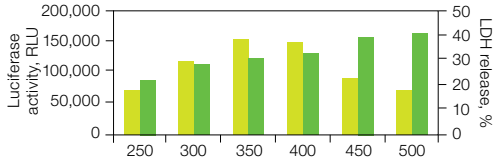
We optimized transfection conditions for the Ramos and Namalwa cell lines, two difficult-to-transfect Burkitt cell lines, using the Gene Pulser MXcell electroporation system. Our results show that both cell lines can be successfully transfected using the exponential waveform. We achieved 30% transfection efficiency for Namalwa cells with a setting of 250 V/350 µF. Ramos cells showed maximal protein expression when they were transfected with a setting of 250 V/400 µF, resulting in 70% viability and 40% transfection efficiency. Maximal transfection efficiency (50% of viable cells) was obtained at 450 µF, with decreased viability at 50%.

Our data show that for some cell lines, optimal conditions can be rapidly identified by screening with the preset 96-well plate protocols. Fine-tuning of the settings can lead to a considerable improvement in transfection efficiencies.

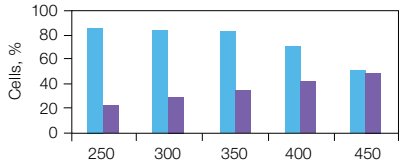
A. Ramos cells (optimization of capacitance)



C. Namalwa cells (optimization of capacitance)



B. Transfection Efficiencies of Ramos Cells



D. Transfection Efficiencies of Namalwa Cells

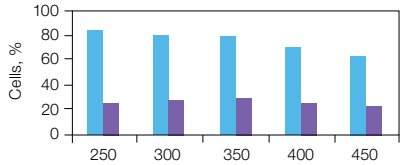


Fig. 2. Fine-tuning of capacitance for maximal transfection efficiencies of Ramos and Namalwa cells. Ramos and Namalwa cells were transfected with luciferase (**A**, **C**) and GFP (**B**, **D**) expression vectors. On the electroporation plate, two rows were used for each plasmid. The settings were an exponential-decay condition of 250 V/1,000 Ω, and capacitance as indicated. Luciferase activity, LDH release, % cell viability, and GFP expression were determined 24 hr posttransfection. Values represent the average of duplicate samples. Luciferase activity (■), LDH levels (■), cell viability (■), GFP transfection (■).

Determining the Binding Kinetics of HIV-1 Nucleocapsid Protein to Six Densities of Oligonucleotide Using the ProteOn™ XPR36 Protein Interaction Array System

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Introduction

The ProteOn XPR36 protein interaction array system is a surface plasmon resonance (SPR) biosensor that can measure the interaction of up to six ligands with up to six analytes, producing 36 kinetic profiles simultaneously (Nahshol et al. 2008). Multiple conditions can be tested in parallel; therefore, a comprehensive kinetic analysis of an analyte concentration series can be performed in one experiment. The ProteOn XPR36 system can be used for comprehensive analysis of biomolecular interactions such as protein-protein and protein–nucleic acid.

In this study, we used the ProteOn XPR36 system to analyze the binding kinetics of the HIV-1 nucleocapsid (NC) protein, with a short deoxynucleotide, $d(TG)_5$. We created a NeutrAvidin capture surface to bind the biotinylated $d(TG)_5$ DNA followed by a single analyte injection of six different concentrations of NC. The data obtained were fit to a 1:1 Langmuir binding model to evaluate which ligand densities are appropriate for detailed kinetic analysis. The results confirmed the complex interaction between these two molecules that was demonstrated in our previous work (Fisher et al. 2006).

Methods

Instrumentation and Sensor Chip

All experiments were performed using the ProteOn XPR36 protein interaction array system (Bio-Rad Laboratories, Inc.) and one ProteOn GLC sensor chip (Bio-Rad).

Immobilization of Biotinylated $d(TG)_5$

The GLC sensor chip was preconditioned with three injections of 50 mM NaOH in 1 M NaCl in the vertical direction and three injections in the horizontal direction, with a contact time of 30 sec and a flow rate of 10 µl/min. ProteOn PBS/Tween (0.005% Tween 20), pH 7.4, was used as the running buffer. All six ligand channels were activated by injection of the amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, 100 mM) and N-hydroxysulfosuccinimide (sulfo-NHS, 25 mM) (from the Bio-Rad ProteOn amine coupling kit) for 300 sec at 30 µl/min in the horizontal direction. NeutrAvidin (200 µg/ml, Pierce Biotechnology, Inc.) in 10 mM ProteOn acetate buffer, (Bio-Rad) was injected in the horizontal direction for 300 sec over the activated channels. Excess reactive amine groups were deactivated with a 300 sec injection of 1 M ethanolamine HCl (Bio-Rad ProteOn amine coupling kit). Using this approach, approximately 6,000 response units (RU) of NeutrAvidin were amine-coupled to the GLC sensor chip. Next, 2-fold dilutions of biotinylated $d(TG)_5$ (biotin-TGTGTGTGTG, Integrated DNA Technologies) ranging from 100–3.12 nM were made in PBS/Tween and injected for 30 sec at 50 µl/min in the vertical direction. Any unbound ligand was removed by regenerating the surface with a 30 sec injection of 0.1% SDS followed by a 30 sec injection of 1 M NaCl.

Nucleocapsid Binding Kinetics

NC protein (a gift from Dr Rob Gorelick, SAIC-Frederick) was diluted to 300, 100, 33, 11, and 3.7 nM in 10 mM HEPES, 150 mM NaCl (pH 7.5), 5 mM β-mercaptoethanol, 100 µM TCEP, 0.005% Tween 20, and 1 µM $ZnCl_2$. The five NC protein concentrations and a buffer control were injected in the horizontal analyte channel with a contact time of 180 sec, dissociation time of 900 sec, and flow rate of 100 µl/min. The ligand channels were regenerated with a 30 sec injection of 0.1% SDS followed by a 30 sec injection of 1 M NaCl. All experiments were run at 25°C.

Sensorgram Analysis

The six sensorgrams from the $d(TG)_5$ ligand channels were x and y transformed, and nonspecific binding was referenced using the interspot reference capability. Sensorgrams were double-referenced by subtracting the buffer response. The six sensorgrams were fit globally to a 1:1 Langmuir binding model, and the refractive index value was kept constant. The kinetic parameters for the association (k_a), dissociation (k_d), and R_{max} were derived from the fitted curves.

Results and Discussion

There was a linear relationship between the $d(TG)_5$ concentration injected and the amount of $d(TG)_5$ captured on each channel (Figure 1). The density of $d(TG)_5$ was determined by taking the mean value across all six interaction spots in the channel between 50–100 sec after injecting the oligonucleotide dilution series. The lowest concentration of oligonucleotide injected, 3.12 nM, gave a similar signal to channel 1.

The data highlighted in red in Table 1 (8.0 and 8.2 RU) represent sensorgrams that correlated well to the 1:1 Langmuir binding model as evaluated by chi^2 values of <5. At higher densities of $d(TG)_5$, the fit is not as tight; this is shown by the increase in the chi^2 values.

The sensorgrams from channels 1 and 4 show that the data obtained overlay the 1:1 Langmuir binding model very well (Figure 2). These sensorgrams correspond to the lowest immobilized levels of $d(TG)_5$, <10 RU. At these densities, the

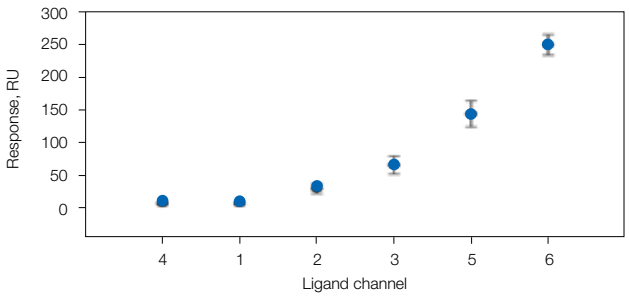

Fig. 1. Oligonucleotide densities. Six different concentrations of $d(TG)_5$, ranging from 3.12 to 100 mM, were injected onto the six channels of a NeutrAvidin-coated chip, creating six different density surfaces. Immobilization of the oligonucleotide on the chip was measured across all six interaction spots in a channel.

Table 1. Kinetic constants and ranking for NC binding to six different surface densities of $d(TG)_5$.

Channel	$d(TG)_5$ injected, nM	$d(TG)_5$ density, RU*	k_a, $M^{-1}sec^{-1}$	k_d, sec^{-1}	K_D, M	R_{max} RU	Chi^2
4	3.12	8.0 ± 1.9	$4.31e^5$	$20e^{-3}$	$4.64e^{-9}$	21.5	3.7
1	6.25	8.2 ± 2.9	$3.65e^5$	$20e^{-3}$	$5.48e^{-8}$	33.65	3.75
2	12.5	30.7 ± 7.3	$2.94e^5$	$10e^{-3}$	$3.4e^{-8}$	85.33	9.87
3	25	66.7 ± 13.6	$2.07e^5$	$8.91e^{-3}$	$4.3e^{-8}$	179.76	47.79
5	50	145.0 ± 20	$1.78e^5$	$4.64e^{-3}$	$2.61e^{-8}$	329.09	313.09
6	100	250.7 ± 15.6	$1.71e^5$	$2.4e^{-3}$	$1.41e^{-8}$	444.3	905.07

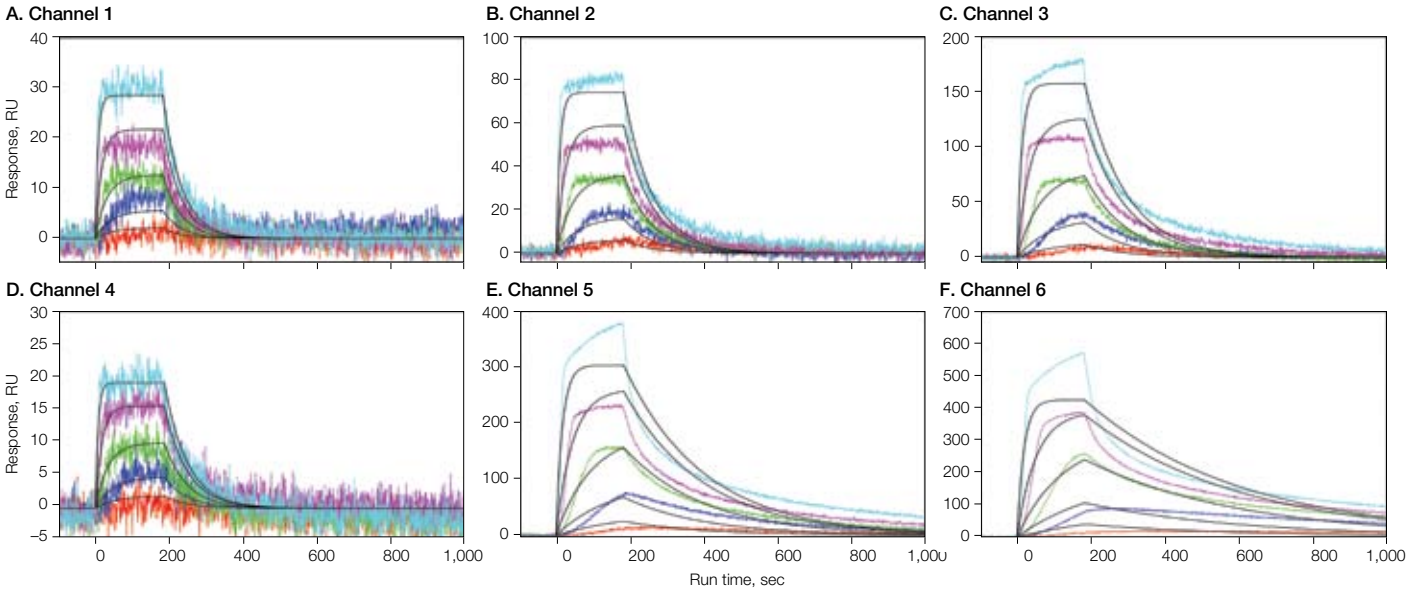

Fig. 2. Interaction kinetics of NC binding to different densities of $d(TG)_5$ are compared to the Langmuir 1:1 model. The black trace represents the global fit of the sensorgrams to the 1:1 interaction model. The interactions between six different $d(TG)_5$ ligand densities with five concentrations (—, 300; —, 100; —, 33; —,11; —, 3.7 nM) of NC were tested.

surface-bound $d(TG)_5$ molecules are sparse enough so that only one NC molecule can bind to each $d(TG)_5$ molecule. In channel 2, the data fit the 1:1 model at only the three lowest NC concentrations. When the oligonucleotide surface reaches higher densities (30, 67, 145, and 251 RU), the data do not fit well to the 1:1 Langmuir binding model because surface-bound $d(TG)_5$ molecules are close enough so that the NC is able to bind to more than one $d(TG)_5$ molecule at a time.

The equilibrium binding constant determined for the two channels with the lowest $d(TG)_5$ densities is approximately 46 nM, which is in the range of values measured in previous work (Fisher et al. 2006, Stephen and Fisher 2009).

Conclusions

The ProteOn XPR36 system performed well in measuring the binding kinetics of the NC protein to the $d(TG)_5$ oligonucleotide. We see a linear response between the amount of biotinylated oligonucleotide that was injected and the amount captured on the NeutrAvidin surfaces.

The NC protein interacted with different binding behaviors to the channels with differing $d(TG)_5$ densities as shown by changes to the closeness of the fit of the experimental data to the 1:1 Langmuir binding model. We were able to produce data on low-density surfaces of $d(TG)_5$ (≤10 RU) that fit well to the 1:1 Langmuir model. These data are consistent with our expectations and previous work (Fisher et al. 2006, Stephen and Fisher 2009). The unique array design of the ProteOn XPR36 system allows the investigator to rapidly evaluate the effect of different density capture surfaces on the binding kinetics of a protein target.

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Affinity Purification of Tag-Free *Shewanella oneidensis* Cystathionine β-Lyase (MetC) Using the Profinity eXact™ Fusion-Tag and Profinia™ Protein Purification Systems

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Introduction

Shewanella oneidensis MR-1 is a nonpathogenic gram-negative bacterium that can grow both aerobically and anaerobically and uses a variety of compounds such as solid metals and radionuclides as terminal electron acceptors (DiChristina et al. 2005). The molecular mechanisms of electron transfer to insoluble metal oxides, however, remain poorly understood due to the difficulty of generating milligram quantities of pure, native proteins for crystallography and in vitro enzymatic studies.

Purification of soluble, cytoplasmic proteins is traditionally performed using affinity chromatography following overexpression of a recombinant fusion protein in *E. coli*. Most common fusion tags, such as glutathione-S-transferase (GST) or polyhistidine (HIS) sequences, allow the target protein to bind an immobilized ligand on an affinity column. However, fusion tags remain attached to the target protein following purification and may inhibit crystallization or lead to artificial results in functional assays. Thus, it is often necessary to introduce an enzymatic cleavage site between the target protein sequence and the fusion tag to facilitate purification of a tag-free protein. This is a laborious and time-consuming multistep process that includes removal of tags and proteases. The Profinity eXact fusion-tag system employs a novel one-step affinity purification and tag-removal process that produces native protein free of the fusion tag, often in less than one hour (Oganesyan and Strong 2007).

The Profinia protein purification system performs automated affinity chromatography of recombinant fusion-tagged proteins with a subsequent integrated desalting step. The Profinia system uses preprogrammed methods and prepacked affinity cartridges and is capable of generating milligram quantities of protein in as little as 30 minutes. The Profinity eXact fusion-tag system is ideally suited for use with the Profinia system, enabling one-step, automated affinity purifications with subsequent cleavage of the fusion-tag and exchange of the native, tag-free protein into the buffer of choice.

In this study, the Profinity eXact fusion-tag was used with the automated Profinia purification methods to obtain sufficient quantities of purified protein for activity assays of cystathionine β-lyase (MetC, CBL), which cleaves cystathionine to generate L-homocysteine, a crucial enzyme involved in methionine biosynthesis (Dwivedi et al. 1982). We demonstrate the effectiveness of using the Profinity eXact technology on an automated chromatography system with preprogrammed methods for the rapid generation of milligram quantities of pure, tag-free protein for downstream functional assays.

Methods

Cloning of MetC Gene Into Profinity eXact pPAL7 Supercoiled Expression Vector

The MetC coding region was PCR-amplified with iProof™ high-fidelity DNA polymerase (Bio-Rad Laboratories, Inc.) using primers containing HindIII (5' end) and XhoI (3' end), as directed in the Profinity eXact system manual. Profinity eXact pPAL7 supercoiled expression vector (Bio-Rad) and MetC DNA were double digested with HindIII and XhoI and ligated to form pPAL-MC. A second clone (pPAL-MCL) was constructed in an analogous manner, except that the 5' primer contained an additional six nucleotides coding for a Thr-Ser linker (for optimal cleavage, as recommended). Ligation products were transformed into an *E. coli* host strain (NovaBlue competent cells, Novagen) via electroporation using the MicroPulser™ electroporator (Bio-Rad). Clones were verified via restriction analysis and subsequently transformed into BL21 (DE3) pLysS expression strains (for strict control of MetC expression).

Protein Expression and Extraction

BL21 (DE3) pLysS strains carrying pPAL-MC (BL-MC) and pPAL-MCL (BL-MCL) were cultured in the presence of ampicillin (100 µg/ml) and chloramphenicol (25 µg/ml). Overnight seed cultures were grown at 37°C (200 rpm) and used to inoculate expression cultures. Expression cultures were grown at 30°C (175 rpm) to OD_{600} ~1.0. IPTG was added to a final concentration of 1 mM and cultures were induced for 10 hr. Following expression, cell cultures were harvested via centrifugation (10,000 x g, 4°C, 10 min). For chemical lysis, cells were resuspended in B-PER bacterial protein extraction reagent (Pierce Biotechnology, Inc.) (10 ml/g wet cell weight). Cell suspensions were incubated with benzonase (Novagen) (25 U/ml, 25°C, 10 min) to reduce viscosity. Lysates were cleared via centrifugation (20,000 x g, 4°C, 20 min) and the resulting supernatants were filtered (0.45 µm polyethersulfone [PES] filters). For mechanical lysis, cells were resuspended in the recommended Profinity eXact 1x bind/wash buffer (100 mM Na_2HPO_4, 10 ml/g wet cell weight). Samples were sonicated on ice at 100% intensity in 30 sec pulses (60 W) (total processing time 10 min) using an S4000 sonicator (Misonix, Inc.). Lysates were centrifuged and cleared as described above.

Affinity Purification

Cleared lysates were purified using the Profinia purification system utilizing the preprogrammed Profinity eXact purification methods. These methods consisted of using either the 1 ml and 10 ml or 5 ml and 50 ml Bio-Scale™ Mini Profinity eXact™ and Bio-Scale Mini™ Bio-Gel® P-6 desalting cartridges, respectively (both from Bio-Rad). Buffers were prepared at the following concentrations according to the Profinity eXact system manual: bind/wash buffer (100 mM NaH_2PO_4, pH 7.2); elution buffer (100 mM NaH_2PO_4, 100 mM NaF, pH 7.2); desalting buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 8.1 mM KH_2PO_4, pH 7.4). Samples were applied to Profinity eXact cartridges using the standard flow rate (1.0 ml/min). Fractions for the column flowthrough, washes, and elution were collected via automatic peak detection. Purified protein was collected in a total of 4 ml for 1 ml Profinity eXact cartridge purifications and in a total of 10 ml for 5 ml Profinity eXact cartridge purifications.

SDS-PAGE and Experion™ Pro260 Analysis

SDS-PAGE analysis was performed using Criterion™ Tris-HCl 4–20% linear gradient gels (Bio-Rad). Samples were prepared with a 6-fold dilution into Laemmli sample buffer and 30 µl of sample or 10 µl of Precision Plus Protein™ Kaleidoscope™ standards (Bio-Rad) was loaded per lane. Gels were stained with Bio-Safe™ Coomassie G-250 stain (Bio-Rad) following standard protocols and imaged with a Molecular Imager® Gel Doc™ XR system (Bio-Rad) using Quantity One® 1-D analysis software (Bio-Rad). Protein quantity and purity were determined using the Experion automated electrophoresis system with the Pro260 analysis kit (Bio-Rad) according to the standard protocol. Protein concentrations were independently confirmed using the Bio-Rad *RC DC*™ protein assay kit according to the instructions provided with the kit.

Native PAGE and CBL Activity Assay

Native PAGE analysis was performed using Criterion Tris-HCl 8–16% linear gradient gels (Bio-Rad). Samples were diluted 2-fold in native sample buffer and 45 µl of sample or 10 µl of Precision Plus Protein Kaleidoscope standards were loaded per lane. Gels were stained with Bio-Safe Coomassie Blue G-250 stain following standard protocols (in order to detect the total protein in each lane) or with CBL activity stain (100 mM Tris-HCl, 10 mM L-cysteine, 500 µM $Pb(NO_3)_2$, pH 8.2). For detection with the CBL activity stain, gels were washed three times in dH_2O (15 min, with shaking) then visualized (30 min, 25°C, no shaking). Gels were imaged with a Molecular Imager Gel Doc XR system using Quantity One 1-D analysis software. Positive CBL activity results in an insoluble lead sulfide precipitate in the gel (which appears black in gel images).

Results and Discussion

Native MetC Is Efficiently and Rapidly Cleaved From the Profinity eXact Fusion Tag

Some proteins require a spacer or linker between the Profinity eXact affinity tag and the native protein sequence to promote efficient cleavage of the tag (see the Profinity eXact fusion-tag system manual). To determine if a linker was required, two clones were constructed with one containing only native MetC amino acid sequence (pPAL-MC), and another containing the recommended threonine-serine linker (pPAL-MCL). At the scales tested (250 ml culture volume), protein purification on the Profinia system using the 1 ml Profinity eXact plus 10 ml desalting method from each clone, BL-MC and BL-MCL, resulted in nearly identical yields and purity for the same incubation period (30 min) (data not shown). As native MetC is highly desirable for downstream applications, the clone without the linker region (pPAL-MC) was chosen for further analysis.

Chemical Versus Mechanical Cell Lysis Procedures

In order to determine the optimal method for isolation of total cell protein, both chemical and mechanical (sonication) lysis procedures were compared from two different expression culture volumes (250 ml culture, 10 ml total lysate; 1 L culture, 50 ml total lysate). The resulting chromatograms from the purifications on the Profinia system using the 1 ml Profinity eXact plus 10 ml desalting method (Figure 1) demonstrate that both chemical and sonication lysis methods were equally efficient for isolating soluble MetC protein from lysates prepared at both culture volume scales (250 ml and 1 L). Efficient removal of the Profinity eXact tag from native MetC requires only 12 min (data not shown; see bulletin 5770).

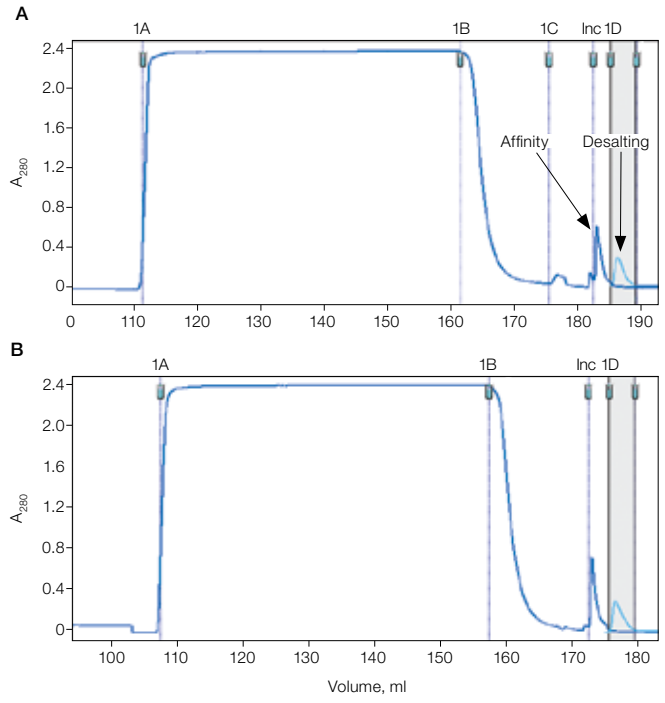

Fig. 1. Comparison of chemical versus mechanical cell lysis procedures using Profinity eXact affinity purification with integrated desalting. MetC from 10 ml of lysate (250 ml culture, data not shown) or 50 ml of lysate (1 L culture) was purified using a 1 ml Profinity eXact cartridge for the affinity step and a 10 ml Bio-Scale Mini Bio-Gel P-6 desalting cartridge for integrated desalting. **A**, chromatogram of lysate prepared by chemical lysis of a 1 L culture. (—), affinity purification (UV1); (—), desalting (UV2); **B**, chromatogram of lysate prepared by sonication of a 1 L culture. (—), affinity purification (UV1); (—), desalting (UV2). Collected fractions are indicated above the graphs: Inc, column incubation step; 1A, flowthrough fraction; 1B, column wash 1 fraction; 1C, column wash 2 fraction; 1D, elution fraction.

Large-Scale Purification of MetC

To obtain sufficient quantities of MetC for further downstream gel activity and mass spectrometry analyses, a large-scale purification of MetC from a 3 L culture was performed. Cells were grown as described above and lysed using sonication. Lysate (200 ml) was prepared as described and loaded onto a 5 ml Profinity eXact affinity column followed by desalting on a 50 ml column using the 5 ml Profinity eXact plus 50 ml desalting method on the Profinia system. The column was incubated for 30 min to allow for sufficient cleavage, and the native MetC protein was eluted (10 ml) using PBS. The large-scale purification resulted in a yield of 8.71 mg total MetC protein (0.871 mg/ml), with measured purity comparable to that observed at the smaller scale (87%) (Figure 2).

Recombinant MetC Is Active In Vitro

Protein fractions from the 5 ml Profinity eXact purification described in Figure 2 were run on both native and denaturing polyacrylamide gels to determine activity, size, and purity, respectively. Activities of wild-type MetC (from *S. oneidensis* crude lysate) and purified, tag-free recombinant MetC recovered from the elution fraction were detected using an in-gel assay. The cleavage of thiol from L-cysteine, and the subsequent reaction with lead nitrate to form insoluble lead-sulfide resulted in black precipitates. Other fractions (load, flowthrough, and wash) from the purification displayed C-S lyase activity at a position in the gel indicating the presence of a tag resulting from the fusion to the Profinity eXact tag (Figure 3A). SDS-PAGE gels were stained for total protein only, and purified MetC displayed a band at the expected size (~40 kD) (Figure 3B).

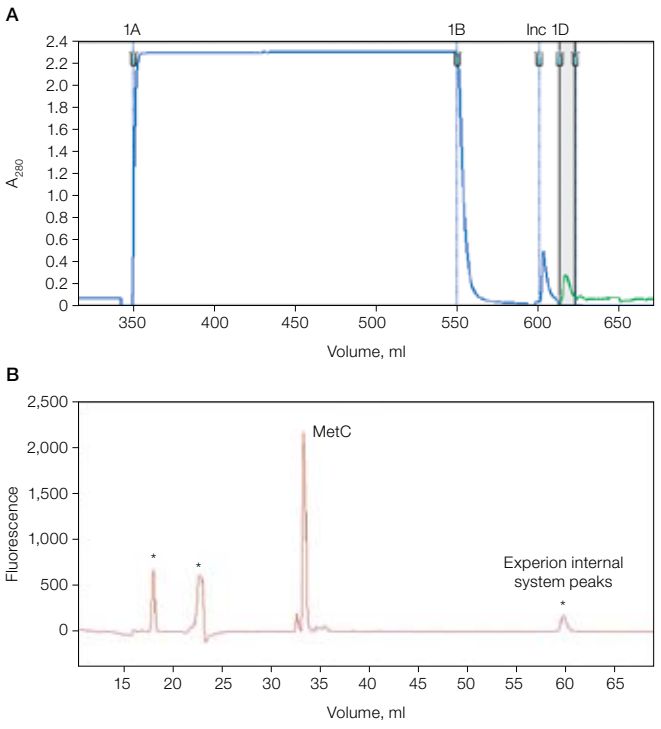

Fig. 2. Large-scale purification of MetC using the 5 ml Profinity eXact cartridge with integrated desalting method on the Profinia system. A, chromatogram of MetC purification from a 200 ml sonicated lysate using a 5 ml Profinity eXact cartridge for the affinity step and a 50 ml Bio-Scale Mini Bio-Gel P-6 desalting cartridge for integrated desalting with recommended buffers. (—), affinity purification (UV1); (—), desalting (UV2). Collected fractions are indicated above the graph: Inc, column incubation step; 1A, flowthrough fraction; 1B, column wash 1 fraction; 1D, elution fraction; **B**, representative Experion electropherogram of eluted MetC protein (87% purity at 0.871 mg/ml protein concentration).

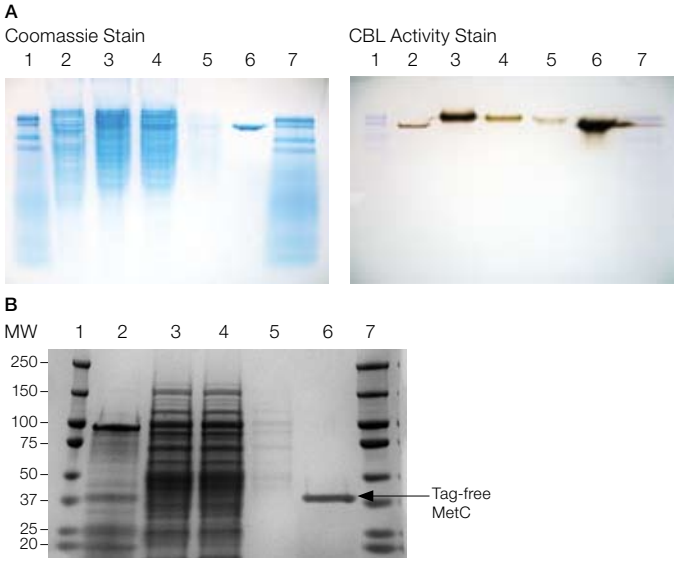

Fig. 3. Gel analysis of recombinant MetC activity. Gel electrophoresis analysis of *S. oneidensis* crude lysate and different fractions collected from purification of 200 ml of lysate. **A**, Native PAGE; lane 1, Precision Plus Protein Kaleidoscope marker; 2, MR-1 crude lysate (endogenous MetC); 3, MetC loading fraction; 4, MetC flowthrough; 5, MetC wash; 6, MetC eluted protein; 7, Precision Plus Protein Kaleidoscope marker. Left panel, Bio-Safe Coomassie G-250 stained gel; right panel, CBL activity assay gel. MR-1 lysate and eluted protein activity bands migrate faster due to absence of the Profinity eXact fusion-tag; **B**, SDS-PAGE, Bio-Safe Coomassie Blue G-250 stained gel of crude lysate and different fractions. Lanes are the same as in panel A.

Conclusions

Purification of tag-free native protein is an important step in elucidating steps in biochemical pathways. Here we report affinity purification of *S. oneidensis* MetC, the enzyme that may play an important role in regulating the redox balance in an organism with a sophisticated respiratory chain that can utilize both soluble and insoluble terminal electron acceptors. The combination of the Profinity eXact fusion-tag and Profinia automated purification systems allowed recovery of milligram quantities of native, tag-free, and functional MetC in a relatively short amount of time (<2 hr), with a purity suitable for use in sensitive downstream assays of protein activity and function.

References

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Dwivedi CM et al. (1982). Cloning, purification, and characterization of beta-cystathionase from *Escherichia coli*. Biochemistry 21, 3064-3069.

Oganesyan N and Strong W (2007). Profinity eXact fusion-tag system performs on-column cleavage and yields pure native protein from lysate in less than an hour. Bio-Rad Bulletin 5652 Rev A.

For an extended version of this article, request bulletin 5770.

Considerations For Processing Samples Using the ProteoMiner™ Protein Enrichment Kits

ProteoMiner protein enrichment technology is a novel protein sample preparation tool for reducing the dynamic range of protein concentrations in complex samples. The technology utilizes a combinatorial library of hexapeptides to enrich low-abundance proteins as opposed to antibodies (which are used in immunodepletion processes). The lack of dependence on antibodies allows the possibility for processing a variety of sample types. ProteoMiner protein enrichment kits have been validated and optimized for use with plasma and serum samples; they have also been successfully applied to urine (Castagna et al. 2005), bile (Guerrier et al. 2007), platelets (Guerrier et al. 2008), red blood cell extract (Roux-Dalvai et al. 2008), and egg white extract (D'Ambrosio et al. 2008). Table 1 summarizes some of the other sample types that have been successfully used with ProteoMiner kits.

Table 1. Sample types that have been tested with ProteoMiner kits.*

Sample Type	Required Pretreatment**	Bead Volume, µl	Sample Size, ml	Protein Load, mg
Urine	pH adjustment, centrifugation	100	400	40
Saliva	—	50	40	40
CSF	Concentration	100	75	80
Cervical lavage	Centrifugation	20	0.7	0.14
Subcellular lysates (cytoplasmic, nuclear, membrane)	Centrifugation	20	0.7	5
Spinach	Ammonium acetate precipitation, solubilization with HFIP	100	50	70
Milk >sweet-whey	Ultracentrifugation	1,000	500	5,000
Egg yolk	Dialysis at pH 5 and lyophilization	500	50	1,000
RBC lysate	RBC lysis	1,000	95	5,700
Follicular fluid	—	100	1	50

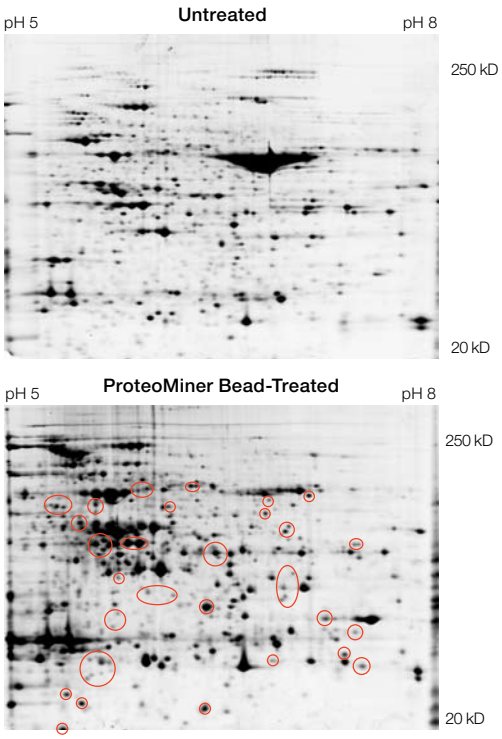
* Data is based on publications. Bead volume and sample load information are provided for reference only and may be adjusted. It is recommended to use ≥50 mg of protein with 100 µl of beads (as provided in the large-capacity kits) or ≥10 mg of protein with 20 µl of beads (as provided in the small-capacity kits).
** After lysis.

Results with sample types other than plasma and serum will vary depending on the concentration of protein in a sample. The dynamic range of the protein concentration in the sample is reduced when the high-abundance proteins saturate their ligands and the low-abundance proteins bind to a sufficient number of ligands to allow enrichment. Therefore the ratio of protein to beads is crucial for optimal results using ProteoMiner kits. Best results are obtained with greater than 50 mg of protein for the large-capacity kits and 10 mg of protein for the small-capacity kits.

In addition to ensuring the proper amount of protein is loaded on the columns, some consideration needs to be taken regarding the sample conditions prior to processing. This especially holds true when attempting to use the kits with sample types other than serum and plasma. Serum and plasma samples require minimal pretreatment prior to processing; plasma samples should be nonheparanized (coagulation should be prevented using EDTA or citrate), both sample types should be free of precipitate, and caution should be taken to avoid aggregate proteins and lipids. When testing the ProteoMiner kits with samples other than serum or plasma, these general guidelines will help ensure proper binding of proteins to the hexapeptide library and avoid interfering with affinity based binding mechanisms:

- Ensure samples are free of urea
- Ensure sample does not contain high levels of ionic detergents such as SDS or NP40 (amount of NP40 should be <0.5%)
- Ensure salt concentration of sample is 50–150 mM
- Ensure sample has a neutral pH (6–8)
- Remove acidic polysaccharides, nucleic acids, lipids, and polyphenols from sample
- Solubilize hydrophobic proteins using fluoroalcohols like hexafluoroisopropanol (HFIP) and trifluoroethanol

For more information, visit **www.bio-rad.com/proteominer/**.



2-D separation of denatured protein extract from spinach. First-dimension separation using large format, 24 cm pH 5–8 IPG strips, followed by second-dimension SDS-PAGE separation using 12.5% hand-cast gels. ○, proteins that have been enriched in the ProteoMiner bead-treated sample.

Fixing Proteomics Campaign: A Global Quest for Reproducibility

The Need

Techniques such as two-dimensional electrophoresis (2DE) have been used for nearly 30 years to separate proteins. However, it wasn't until tools such as the immobilized pH gradient and mass spectrometry were developed in the early 1990s allowing large-scale protein identification and characterization that "proteomics" (as coined by then PhD student Marc Wilkins) emerged as an independent, robust field of study.

Researchers have long experienced a love-hate relationship with 2DE, and even today there is a debate as to whether it is art or science. In a sense, creating the perfect 2DE image is like art but the science is in reproducing the experimental results. Technological developments over the past decade have made this technique a more acceptable protein separation method to support biomarker discovery and protein characterization.

Like any other protein separation method, 2DE does have certain limitations, especially regarding the applicability to membrane and hydrophobic proteins, and in dealing with high molecular weight proteins. Critically important is the reproducibility of the experimental data. This is influenced by several factors ranging from properly preparing and handling samples and using qualified equipment and reagents, to following protocols precisely, as well as optimizing parameters for imaging and data analysis. Hence the quest for reproducibility study, aptly termed "Fixing Proteomics."

The Fixing Proteomics Campaign

Fixing Proteomics is a noncommercial, technique-independent campaign dedicated to solving the experimental challenges that prevent proteomics from delivering on its potential. A list of all participants and coordinators of the initiative can be found in Table 1. Encouraging reproducibility of results has emerged as the key factor toward achieving this end. In fact, one of the basic scientific principles emphasized in Fixing Proteomics literature is "if your results cannot be reproduced, then they should not be published." The organization's website (**www.fixingproteomics.org**) provides background on the importance of and tips for producing work that is reproducible, whether using mass spectrometry, 2DE, or any quantitative proteomics technique.

Table 1. Enrolled participants and coordinators of Fixing Proteomics.

Participant	Lab	Country
Ben Herbert	University of Technology, Sydney	Australia
Toni Posch	Bio-Rad Laboratories, Munich	Germany
Ravi Srideshmukh	Center for Cellular and Molecular Biology	India
Mike Dunn	University College, Dublin	Ireland
Julie Polden	University College, Dublin	Ireland
Aisling Robinson	University College, Dublin	Ireland
Alexander Archakov	Institute of Biomedical Chemistry	Russia
Stanislav Melnik	Institute of Biomedical Chemistry	Russia
Sergei Moshkovskii	Institute of Biomedical Chemistry	Russia
Maxey Chung	National University of Singapore	Singapore
Jose Bermudez	Universidad de Santiago de Compostela	Spain
Francesc Canals	Vall d'Hebron University, Barcelona	Spain
Peter James	Lund University	Sweden
Kathryn Lilly	University of Cambridge	UK
Glen Kemp	NEPAF, Newcastle Upon Tyne	UK
Kevin Emani	NEPAF, Newcastle Upon Tyne	UK
Jun Wheeler	NIBSC, Hertfordshire	UK
Iolanda Vandrell	NIBSC, Hertfordshire	UK
Aran Paulus	Bio-Rad Laboratories, Hercules	USA
Katrina Academia	Bio-Rad Laboratories, Hercules	USA
Amrita Cheema	Georgetown University	USA
Wayne Chadwick	National Institute on Aging, Baltimore	USA
Aldrin Gomes	University of California, Davis	USA
Melissa Sondej	University of California, Los Angeles	USA
Philip Andrews	University of Michigan	USA
Jim Malone	Washington University, St. Louis	USA

Project Objectives and Deliverables

The objectives of Fixing Proteomics include development of standardized protocols to validate the performance and reproducibility of 2-D processes, 2DE test standards, and kits, and establishment of global education (wet lab training) programs (see sidebar). The initiative will also support creation of a central image repository with matching functions for web-based QC of gels.

Cross-lab reproducibility has been successfully demonstrated by the Fixing Proteomics team in a phase I reproducibility study presented at the 2008 Human Proteome Organization (HUPO) meeting. Study participants applied a HeLa cell lysate reference sample to a 2DE protocol developed by the Fixing Proteomics team, and were able to generate 2DE gel images comparable to that which the group has identified as the "gold standard" (Figure 1). One of the objectives of the initiative is to identify factors that contribute to deviations from this gold standard and make recommendations to overcome deviations (Figure 2).

Proteomics Training Program Launched in Toronto

Katrina Academia, Steve Freeby, and Dr Ning Liu, three of Bio-Rad's proteomics experts, recently concluded the first training developed in response to the Fixing Proteomics campaign's ongoing effort to help researchers improve their proteomics results. This three-day training course on 2-D gel electrophoresis took place February 16–18, 2009, at the Medical and Related Sciences (MaRS) center of the University Health Network, Canada's largest hospital and a major teaching hospital at the University of Toronto. Dr Andre Siegel from the MaRS center hosted the event for 14 scientists from eastern Canada interested in both the practical and theoretical aspects of 2-D gel electrophoresis.

The Proteomics Workshop Training covered all aspects of 2-D gel electrophoresis and included a mix of lectures, demonstrations, and question-and-answer sessions. Topics included 2-D cleanup, IPG strip loading and focusing, equilibration, and the second-dimension SDS-PAGE gel. Advice from field experts on how to generate publication-quality 2-D gels from every experiment included the importance of good sample preparation, the secrets of successful sample application onto the IPG strip, and best practices for focusing. During the hands-on portion of the training, participants separated *E. coli* proteins on a 2-D gel, then stained, imaged, and analyzed results using imaging software. Selected spots were then excised with a spot cutter for in-gel digestion and mass spectrometric identification of the protein.

By the end of the MaRS course, participants were able to see the fruits of three days of hard, but fun, work. All gels, produced under strict adherence to a detailed protocol advocated by the Fixing Proteomics initiative, displayed good resolution and reproducibility with more than 0.94 correlation coefficiency. Participants were surveyed at the conclusion of the training, with comments expressing an appreciation for the hands-on nature of the training, as well as "the interaction with the knowledgeable instructors," the "good organization," and "troubleshooting tips." All participants rated the three-day experience as either "excellent" or "good."

For more information on hosting a basic or advanced 2-D gel workshop, visit **www.expressionproteomics.com/training/**.

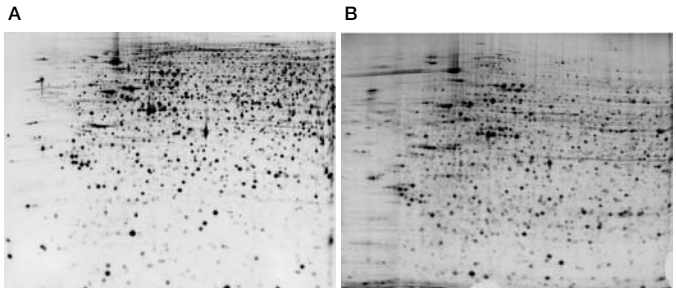


Fig. 1. Gold standard gel image (A) compared to an image from participant laboratory 1 (B). This study participant followed the standard protocol and generated a 2-D gel image that is comparable to the gold standard.

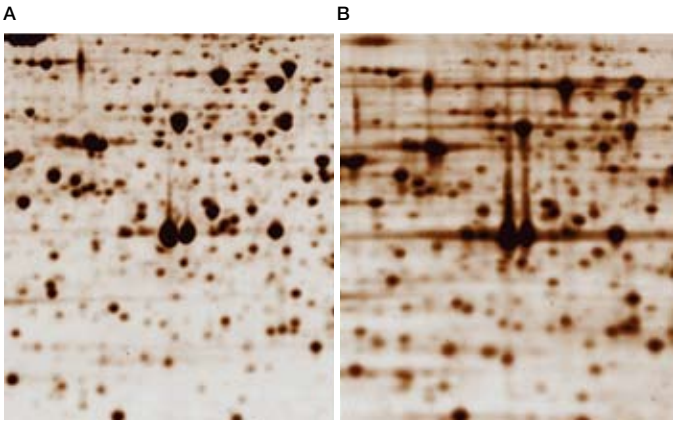


Fig. 2. The gold standard image (A) and deviations from the standard observed in an image generated by participant laboratory 2 (B). Undesirable variations include under-focusing and streaking of spots.

Gold Standard Defined

The reference gel image defined as the gold standard was generated by Hans Voshol by following the standard protocol starting with HeLa cell lysate. Details of the protocol can be obtained by going to **www.fixingproteomics.org/docs/General_Protocol_for_2DPAGE.pdf**. The gold standard image was generated using a 24 cm IPG strip, pH 4–7, and an IEF cell for the first-dimension analysis, followed by separation using 12% SDS-PAGE on the Criterion™ Dodeca™ cell for the second-dimension analysis. Proteins were stained with SYPRO Ruby protein gel stain and imaged. Spot analysis was performed with software from Nonlinear Dynamics.

Project Timeline

Following presentation of results at HUPO 2008, the study was expanded to global participants. Results from the expanded reproducibility study are currently being qualified and will be presented at HUPO in September 2009. The timeline for completion of this phase of the reproducibility study is:

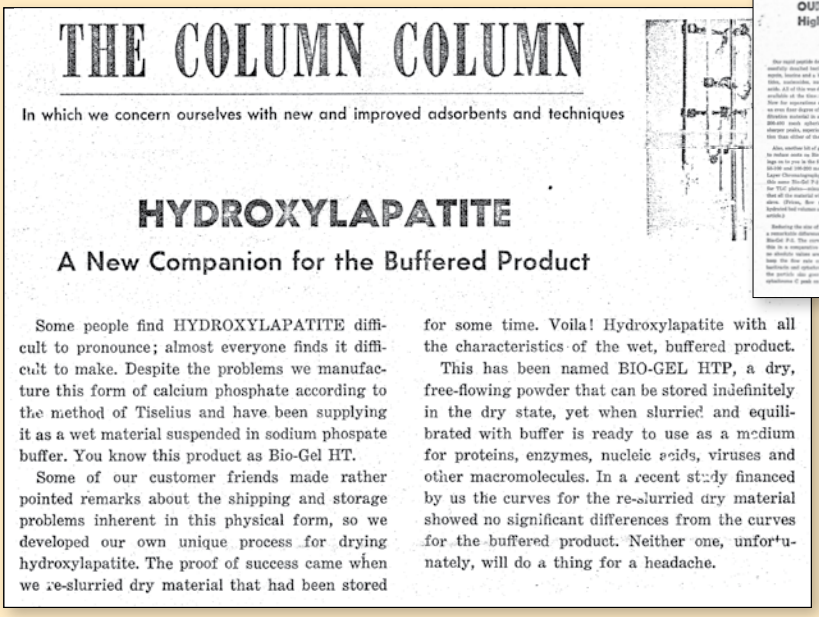
- **Obtain and qualify participant data** — March 31, 2009
- **Present results at HUPO 2009** — September 2009
- **Publish study in peer-reviewed publication** — December 2009

Through completion of these efforts and overall campaign objectives, participants of Fixing Proteomics ultimately hope to further advances in proteomics by enhancing the reproducibility and credibility of its techniques.

BioRadiations 1965: Introducing Bio-Gel HT and HTP Gel

In this column, we look back at previously published issues of BioRadiations to see how tools and applications have changed since Bio-Rad first began publishing the journal in 1965.

Then!



THE COLUMN COLUMN

In which we concern ourselves with new and improved adsorbents and techniques

HYDROXYLAPATITE

A New Companion for the Buffered Product

Some people find HYDROXYLAPATITE difficult to pronounce; almost everyone finds it difficult to make. Despite the problems we manufacture this form of calcium phosphate according to the method of Tiselius and have been supplying it as a wet material suspended in sodium phospate buffer. You know this product as Bio-Gel HT.

Some of our customer friends made rather pointed remarks about the shipping and storage problems inherent in this physical form, so we developed our own unique process for drying hydroxylapatite. The proof of success came when we re-slurried dry material that had been stored for some time. Voila! Hydroxylapatite with all the characteristics of the wet, buffered product.

This has been named BIO-GEL HTP, a dry, free-flowing powder that can be stored indefinitely in the dry state, yet when slurried and equilibrated with buffer is ready to use as a medium for proteins, enzymes, nucleic acids, viruses and other macromolecules. In a recent study financed by us the curves for the re-slurried dry material showed no significant differences from the curves for the buffered product. Neither one, unfortunately, will do a thing for a headache.

BIO-RADIATIONS

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Higher Resolution
at
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Now!

Bio-Gel HTP gel remains a key tool for separating ssDNA from dsDNA. But over the years, the line of chromatography media has grown with advances in purification techniques (ion exchange, affinity, ceramic hydroxyapatite and fluoroapatite, size exclusion, hydrophobic interaction) — headache benefits, however, remain elusive (so media should not be injested)!

enzymagic.

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Introducing SsoFast™ EvaGreen® supermix — a unique Sso7d fusion polymerase combined with EvaGreen dye and optimized buffer that delivers unrivaled speed and performance for a variety of qPCR applications. Using our patented* Sso7d fusion protein technology, SsoFast EvaGreen supermix offers:

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- Instant polymerase activation and rapid polymerization kinetics for fast qPCR results in less than 30 min
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* U.S. patent 6,627,424.

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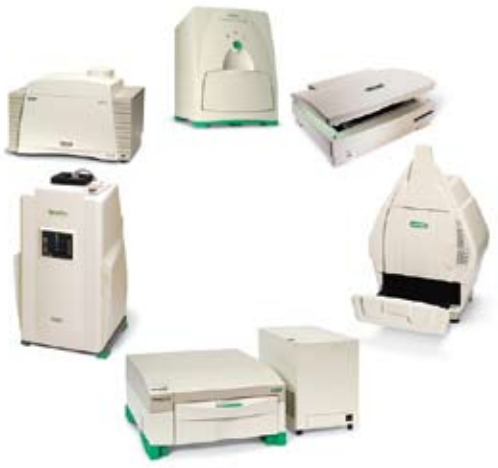

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