The Experion™ System: Microfluidics-Based Automated Electrophoresis

Blanca Chang, Eric Larson, and Christina Whitman-Guliaev,
Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

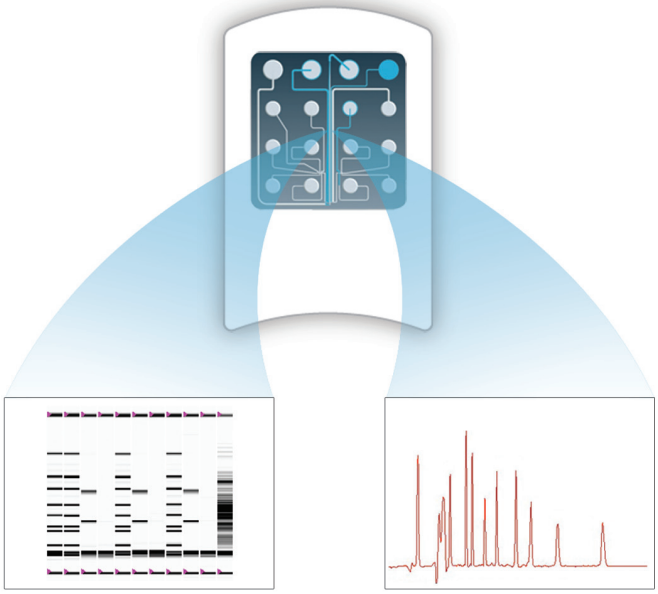
For decades, scientists have gained essential data about their protein and nucleic acid samples from the use of gel-based electrophoresis. Electrophoresis, the movement and separation of charged particles in response to an electric field, results in unparalleled resolution of biomolecules. However, the process is composed of a series of manual steps that require numerous pieces of equipment, various reagents, and several hours to gain the information needed from sample separations. Since this traditional process requires significant time and manual labor, automation has been a key desire of scientists. Bio-Rad's Experion system applies innovative microfluidic separation technology to both automate and accelerate this process.

The growing use of microfluidics technology is very promising for many scientific applications. The Experion system implements and tailors this technology to gel-based electrophoresis, offering fast, accurate, high-resolution separation and analysis of proteins and nucleic acids. The system requires only very small amounts of sample for analysis, allowing fast decision making in experiments that rely on protein or nucleic acid assessments. Because of the many downstream applications that rely on separation and analysis of proteins and nucleic acids, this system presents significant advantages to the research scientist. This article introduces the technology of microfluidics, Bio-Rad's system approach, and the advantages of using the Experion system for research applications.

Microfluidic Separation Technology

The term "microfluidics" refers to the transport and manipulation of very small volumes of liquid. Generally, microfluidics includes not only miniaturization of the channels that the liquid flows through but also the movement and interaction of materials of interest — dissolved or suspended in the liquid — by fluid dynamic, electrophoretic, and chemical principles on a small scale. Microfluidic separation technology brings the advantages of miniaturization and automation to a variety of different chemical and biochemical analyses. These analyses benefit from dramatically reduced time to results, reagent and sample consumption, and user intervention.

Developed by Caliper Life Sciences, Inc., LabChip microfluidic chips perform sample separations within tiny chambers and channels that are fabricated in quartz, glass, or plastic. As with electronic devices, these microchannels and microscopic chambers may serve different functions within a single chip. This allows the integration of several different processes, such as performing sample and reagent dilution, mixing, incubation, and separation all on a single chip. It also enables the introduction and analysis of multiple samples. The microchannels are generally 50 µm wide and 10 µm deep, somewhat smaller than a strand of human hair, and these small dimensions reduce both processing times and the amounts of sample and reagent required for an assay. Microfluidic circuits, coupled with the appropriate software and instrumentation to control separation, detection, and analysis, can be designed to combine the functionalities of several larger benchtop analytical instruments. Depending on their design, these circuits can accommodate virtually any analytical biochemistry process, including separation of nucleic acids and proteins by electrophoresis.



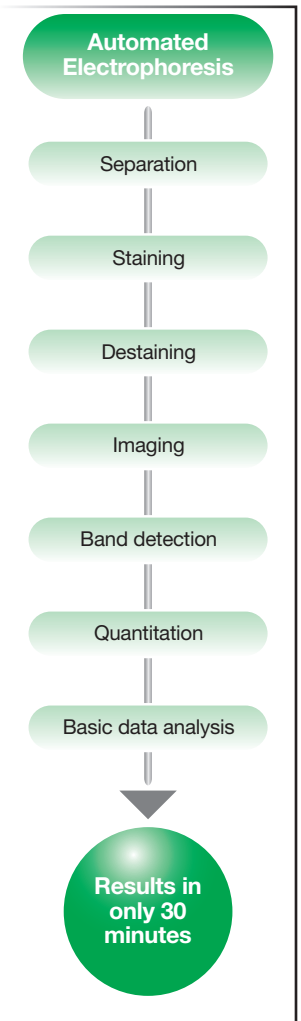
The Experion chip's network of microchannels allows rapid separations using small sample volumes. The results are displayed in an electropherogram and a simulated gel view.

BIO-RAD

LabChip microfluidic chips combine the semiconductor manufacturing processes used in the electronics industry with wet-chemistry laboratory techniques. Designing the optimal configuration and dimensions of microchannels and chambers is a complex process requiring the application of fluid dynamics, biophysics, and principles of biochemistry and chemical and electrical engineering. Microfabrication manufacturing methods are used to produce the small, precise, and intricate patterns of interconnected channels in the Experion chips. First, a photolithographic process is used to etch the channel circuit designs into sheets of glass. Another sheet is then bonded to the first to cover the channels and convert them into closed microfluidic circuits. The end of each channel is connected to an open reservoir through which fluids can be introduced. Individual chips are then packaged into plastic holders, called caddies, to facilitate handling. These microfabrication methods are highly reproducible and are capable of generating large quantities of chips in a relatively short amount of time.

Electrokinetic forces, such as electrophoresis, are typically used to direct the movement of molecules through microfluidic separation devices. Such forces as these result when electrodes are attached to power supplies and placed into reservoirs at each end of the microchannel, generating an electrical current. Charged molecules in solution move and are separated by electrophoresis. For this electrophoresis process, the microchannels are filled with a gel solution, which accomplishes the desired separation, and eliminates electroosmosis to produce reproducible separation results.

The chips are an integrated part of the microfluidic system, working together with specialized computer-controlled instrumentation used to manipulate the timing and sequence of the processes designed into the channel architecture of the chip. The low velocities and small channel dimensions of microfluidic systems lead to predictable motion of fluids and charged molecules. As a result, these systems inherently generate accurate, reproducible data. Nevertheless, chip design must provide optimal channel and sample well dimensions, and system design must provide precise control over temperature, flow rates, and sample injection and separation voltages, to name a few, in order to produce optimal results.



Steps in automated analysis.

Electrophoresis With the Experion System

The Experion system applies Bio-Rad's decades of knowledge and experience in protein and nucleic acid separations to LabChip microfluidic technology. LabChip technology is the leading commercial application of microfluidic principles to laboratory science. With Bio-Rad's contribution of reliable electrophoresis equipment, reagents, standards, and gel solutions, the Experion system provides the performance that is required to obtain meaningful data in a laboratory setting.

When microfluidic chips are used for electrophoresis, the process is, overall, very similar to that of traditional gel-based electrophoresis. The primary difference is that microfluidics enables the miniaturization and combination of multiple steps of gel-based electrophoresis — separation, staining, destaining, imaging, and even basic data analysis — into a single automated process.

The Experion microfluidic chip is the foundation for sample separation and detection. To prepare the chip for separation, gel solution is introduced into the microchannels by a pressure-driven process called "priming". Once the chip has been primed, samples are loaded into the appropriate wells for separation. A preprogrammed series of voltage changes applied at the sample wells allows small amounts of sample to be directed from one channel and injected into another. As a sample moves into a separation channel, its individual sample components begin to separate. In the presence of gel, electroosmotic forces are suppressed and electrophoretic mobility acts as the sole mechanism of sample movement and separation during the entire analysis process.

A fluorescent dye is incorporated into the sample components during separation. As the sample components pass through the separation microchannel, they are detected by laser-induced fluorescence as they pass by the position of a focused laser. The fluorescence and migration time data are transmitted to the software, which presents them in both electropherogram and simulated gel views (see Protein Analysis sidebar). Software analysis tools simultaneously perform molecular weight sizing, peak detection, or both, as well as automatic quantitation of the individual peaks.

© 2010 Bio-Rad Laboratories, Inc.

The Experion system components — the microfluidic chip, reagents, instrumentation, and software — combine to accomplish separation, staining, destaining, detection, quantitation, and basic data analysis with minimal user intervention in a fraction of the traditional time. The run times for samples applied to a single chip (10–12) are only 30 minutes using the Experion system.

Major Advantages

Rapid, Automated Results

Bio-Rad's Experion system performs the multiple steps of gel-based electrophoresis with automated ease and speed, while generating highly resolved results. It performs reproducible separations of 10–12 samples in just 30 minutes, including data analysis, significantly reducing time to results compared to traditional methods. The unattended process frees up time to improve lab efficiency.

Low Sample Requirements

With detection capabilities down to low nanogram and even picogram amounts of sample, and volume requirements of just 1–4 µl, the microfluidic format enables the Experion system to use a very small amount of sample for separation and analysis. This is especially important in cases where a precious sample is in short supply or must last over many experiments, such as for RT-PCR or microarray experiments. The Experion system not only provides a uniquely comprehensive view of the quality and concentration of the sample, but also requires only a small amount of sample and time, making it an ideal checkpoint for successful completion of gene expression studies.

Familiar Data Output

The Experion system displays separation data in formats that are familiar and easy to use. Each sample separation is displayed as an electropherogram, similar to a chromatogram, and as a simulated gel image (see sidebars). This approach provides optimal information and a convenient means of analysis. The electropherogram is an excellent view for analyzing small peaks among many larger peaks or for close examination of the resolution of two very closely migrating peaks. The gel image is ideal for performing sample comparisons. Furthermore, automatic calculations are displayed in Results tables for improved user convenience.

Automatic Sizing and Quantitation

The Experion system automatically determines the sizes and quantities of protein and nucleic acid sample components. The Protein Analysis and RNA Analysis sidebars provide more detail about how sizing and quantitation are accomplished.

The results of the automatic sizing and quantitation calculations are conveniently displayed in the Results table of the software screen. In protein samples, information is displayed for each detected protein peak, including the molecular weight, quantity, and percent of the total sample. Further user-directed analysis of one or more specific proteins can be accomplished using the Protein table, which allows the comparison of selected protein(s) across all the samples of a chip. The Proteins tab presents the results with a summary of the mean, standard deviation, and coefficient of variation.

For RNA samples, the Results table displays the total quantity of the sample. In a total RNA run, the areas of the ribosomal peaks are shown along with the areas used to calculate the ribosomal RNA ratio, which is a standard indication of RNA integrity. For mRNA samples, the amount of ribosomal contamination is reported along with the total sample quantity.

Conclusions

The Experion system advances electrophoresis automation through the combination of microfluidic technology and electrophoresis expertise. It expands the researcher's ability to produce data quickly, without compromising the quality of results. This affordable, innovative system offers exceptional sensitivity, resolution, and data analysis for protein and nucleic acid samples. In addition, the Experion system offers a variety of advantages over traditional gel electrophoresis, such as faster results, reduced sample and reagent usage, automatic calculations, reduced hands-on time, and elimination of the challenges, tedium, and hazardous materials typically associated with these applications. By automating sample separation and analysis and freeing up researcher time, this integrated system expedites research goals, leading to faster results in a wide variety of applications. It can also be used as a complementary tool to many of the most important and common downstream laboratory experiments that require knowledge of the quality and composition of protein or RNA samples.

Acknowledgements

Bio-Rad R&D Scientists

William Strong, Michael Urban, Marie Nguyen, and Adriana Harbers

Caliper Life Sciences R&D Team

Josh Molho, Stephane Mouradian, and Huan Phan

LabChip and the LabChip logo are trademarks of Caliper Life Sciences, Inc. Bio-Rad Laboratories, Inc. is licensed by Caliper Life Sciences, Inc. to sell products using the LabChip technology for research use only. These products are licensed under US Patent Nos. 5,863,753, 5,658,751, 5,436,134, and 5,582,977, and pending patent applications, and related foreign patents, for internal research and development use only in detecting, quantitating, and sizing macromolecules, in combination with microfluidics, where internal research and development use expressly excludes the use of this product for providing medical, diagnostic, or any other testing, analysis, or screening services, or providing clinical information or clinical analysis, in any event in return for compensation by an unrelated party.

Coomassie is a trademark of BASF Aktiengesellschaft. Practice of the polymerase chain reaction (PCR) may require a license. Some applications may also require licenses from other third parties.

Information in this tech note was current as of the date of writing (2005) and not necessarily the date this version (rev C, 2010) was published.

Protein Analysis

Accurate separation, quantitation, sizing, and analysis of 10–260 kD proteins can be achieved using the Experion system and Pro260 analysis kit. Included in each Experion Pro260 analysis kit are all chips and reagents required to perform protein electrophoresis (available in 10-chip and 25-chip configurations). Each Experion Pro260 chip allows analysis of 1–10 samples with sensitivity comparable to that achieved by colloidal Coomassie Blue staining of SDS-PAGE gels.

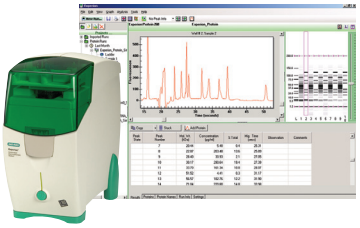
The Experion Pro260 ladder contains nine proteins from 10 to 260 kD, and is a modified version of the Precision Plus Protein™ ladder that has been optimized for automated electrophoresis on the Experion system. The Pro260 sample buffer contains two internal markers to normalize the sample separation to the Pro260 ladder. The upper alignment marker is a 260 kD protein that is also used for relative quantitation of protein samples. A user-defined standard can also be used for absolute quantitation. The precision-engineered internal markers provide clean baselines, and the most accurate microfluidics-based sizing and quantitation analysis available.

Protein analyses on the Experion system are useful for quality control, assessment of protein purity or stability, assessing the effectiveness of purification methods, optimization of protocols, and evaluation of recombinant protein expression, among other applications.

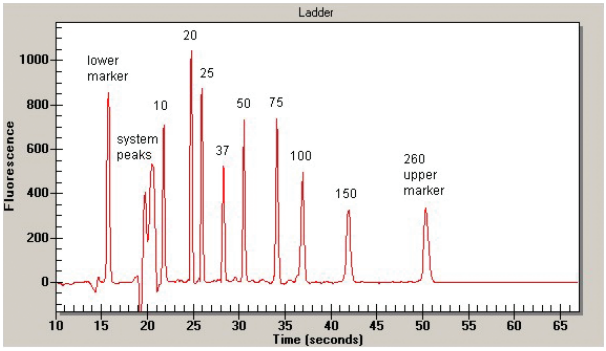
Protein Workflow

Protein Analysis for Drug Discovery

Protein Purification

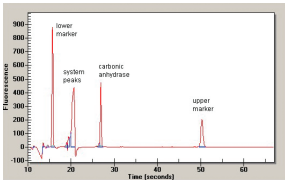


Protein Sizing
Determination of Protein Concentration
Statistical Comparisons

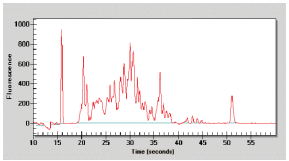


Protein analysis. The Pro260 ladder is used to achieve highly accurate sizing. The nine peaks in the electropherogram above correspond to the nine 10–260 kD proteins in the ladder. The software identifies the first peak as the lower alignment marker at 1.2 kD, and the last peak as the upper alignment marker at 260 kD, which is also part of the ladder.

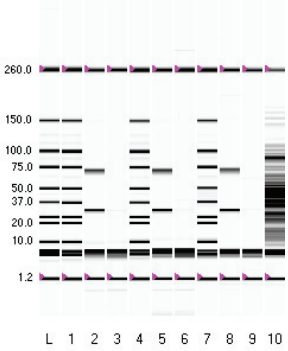
Carbonic anhydrase (100 ng/µl)



E. coli lysate (2 mg/ml)



Various protein samples



Electropherogram (left) and simulated gel (right) views of separations.

© 2010 Bio-Rad Laboratories, Inc.

RNA Analysis

The Experion system is designed for rapid analysis of RNA samples in either picogram or nanogram ranges. Each Experion RNA analysis kit contains the chips and reagents required to perform RNA electrophoresis. The RNA HighSens kit accommodates 1–11 samples, and the RNA StdSens kit accommodates 1–12 samples; both kits are available in 10-chip and 30-chip configurations.

The RNA analysis kits include an RNA ladder that is specifically produced for Experion system applications. The quality and accuracy of the individual RNA transcripts are tested before blending to create a precise ladder, resulting in accurate quantitation of RNA samples. The RNA analysis kits also include loading buffer that contains an internal lower marker, which serves to normalize the separation of the sample to the separation of the RNA ladder.

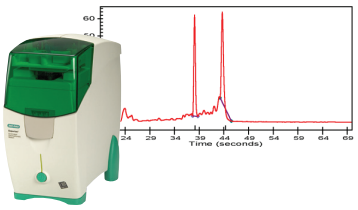
When analyzing mRNA samples, it is important to determine the extent of rRNA contamination. Contaminating RNA can be detected, along with its concentration, using Experion RNA analysis kits. Separation generates a broad mRNA sample peak, and any contaminating rRNA peaks will be visible. rRNA peaks and RNA ratios, such as 28S:18S, are calculated for total RNA samples.

The Experion system offers single-step analysis for RNA samples. It performs both automatic calculations of quantity and a qualitative visual assessment of the RNA sample in a single step. The results are displayed in an electropherogram and simulated gel view, which indicates if the sample has been degraded or has genomic DNA or rRNA contamination. Thus, the Experion system obviates the need to run a gel for visual assessment or to take spectrophotometer readings for determination of concentration.

RNA Workflow

RNA Purification and Analysis
for Differential Gene Expression

RNA Isolation and Purification

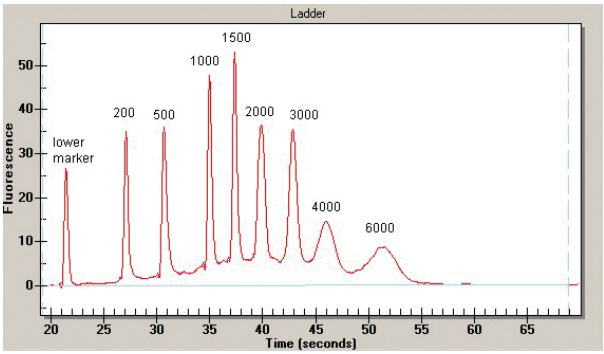


RNA Purity or Quality Assessment

Determination of RNA Concentration

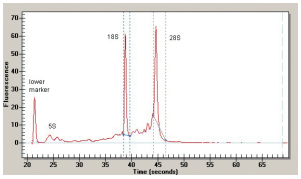
Real-Time PCR

DNA Microarray Analysis

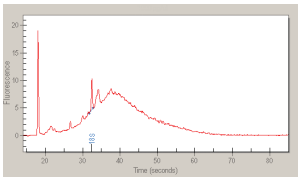


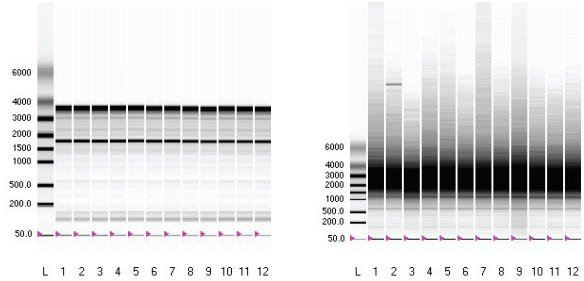
RNA analysis. The Experion RNA ladder contains eight 200–6,000 bp transcripts that generate the eight peaks shown in the electropherogram.

Rat brain total RNA (with RNA StdSens kit)



Rat brain mRNA (with RNA HighSens kit)





To right, electropherogram (top) and simulated gel (bottom) views of separations at two sensitivity levels.

© 2010 Bio-Rad Laboratories, Inc.

Experion System Tour

Instrumentation

The Experion electrophoresis station is the comprehensive sample running apparatus, power supply, and detector, all built into one unit. Electrophoresis occurs by delivering voltage to each well in an analysis chip. The electrode manifold is carefully engineered to align 16 platinum electrode pins with the 16 wells in a chip. The electrophoresis station is built to provide consistent separation performance while remaining an affordable benchtop apparatus.

Eliminating the most likely source of error, incorrect or inconsistent priming, the automated priming station is designed with preset pressure and time settings that are optimized for the different Experion analysis chips. A “priming code” printed on each chip is used for setting the priming station pressure and time. A unique automatic release stops priming at the end of the timed cycle, which lasts 30–60 seconds, depending on the analysis chip. This provides highly consistent chip priming without the need to monitor and manually stop the priming cycle. Because of the viscosity of the gel solution, ambient temperature can have a significant effect on the priming. The Experion priming station has a unique temperature sensor and automatically adjusts the pressure to compensate for the temperature, ensuring reproducible results. These features combine to consistently deliver the right amount of pressure run after run, ensuring error-free automated priming.

For effective separation and analysis of RNA samples, samples and reagents must be mixed in the chip prior to separation. This is achieved with the vortex station, which is designed with preset speed and time settings for precise mixing of samples and reagents for effective sample runs. The specially designed vortex adaptor provides easy chip access and securely holds the chip during the 1 minute vortex cycle.

Analysis Kits

The Experion analysis kits include chips and reagents that are optimized for electrophoresis of particular sample types (protein or RNA). The analysis kits include standards to allow quantitation and sizing of the sample components. Two chip and reagent kit designs are available for RNA analysis, the Experion RNA StdSens kit for RNA detection at the nanogram level, and the RNA HighSens kit for detection at the picogram level. The Experion Pro260 kit is used for protein separation, molecular weight sizing from 10 to 260 kD, and quantitation of protein samples.

Software

The Experion software controls the voltages applied by the electrophoresis station for sample injection and separation, analyzes fluorescence data from the detector, and provides a display of the separation results. The software clearly displays extensive information, including sizing, quantitation, and visual comparisons of sample components, on a single screen. Additional analysis functions allow the flexibility to quickly sort and compare information within the chip. For example, a specific protein peak can be selected and its characteristics compared across the wells of the chip. Electrophoresis is a powerful resolving tool and, like a gel or chromatogram, the Experion data display is an informative tool for reference. Various reports can be generated, either by printing or exporting the data in image or table format. The Experion software offers a powerful combination of the resolving power of a gel with the ease of digital data access.

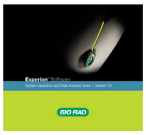
For more information on the Experion system, request bulletin 3140.

Experion Automated Electrophoresis Station

Experion Priming Station

Experion Vortex Station

Experion Analysis Kits



Experion System Software

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 31 3689 6600* ***Canada*** *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Malaysia*** *60 3 2117 5260* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3170* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 6518311* ***United Kingdom*** *020 8328 2000* ***Vietnam*** *84 8 38131140*