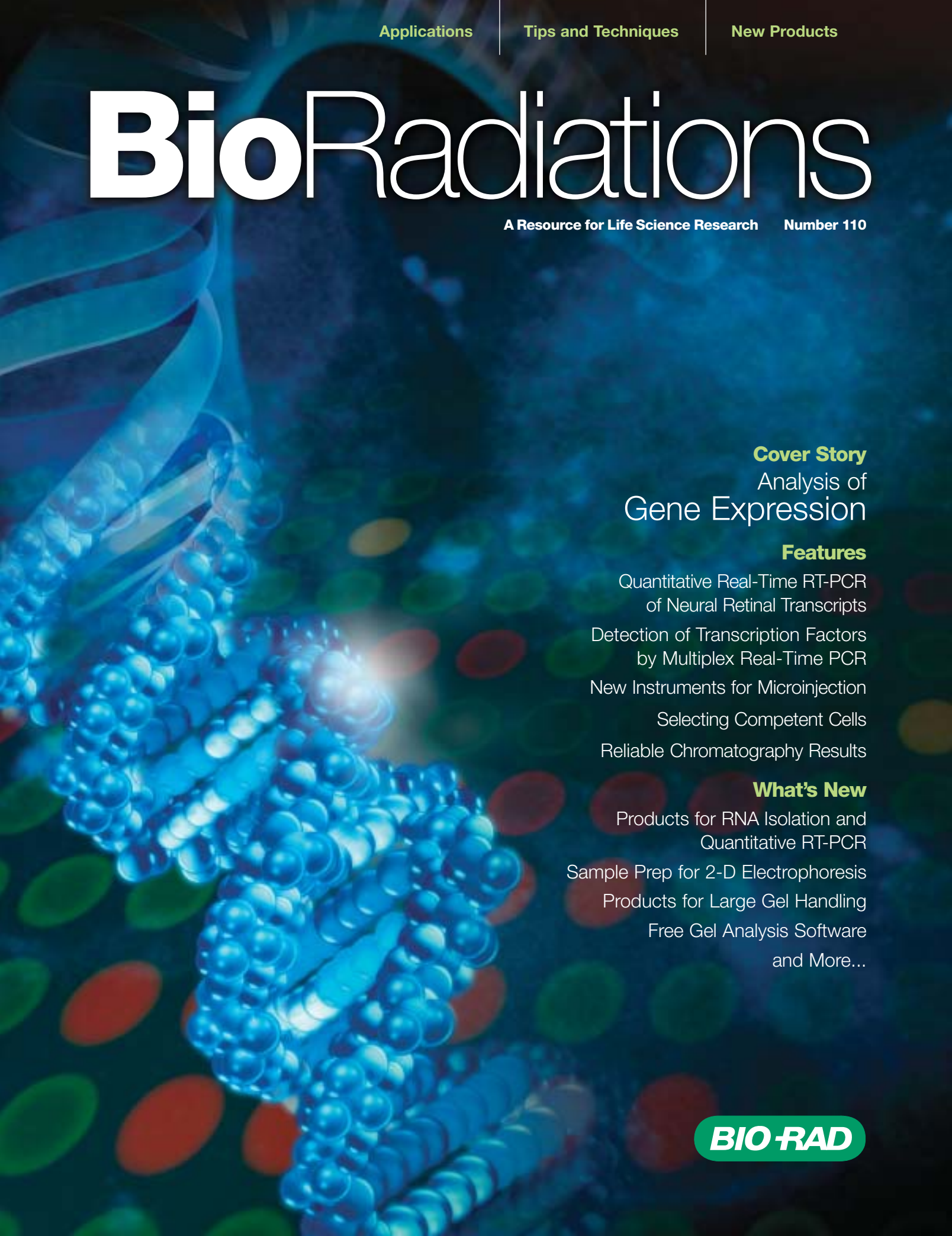
Applications | Tips and Techniques | New Products

BioRadiations

A Resource for Life Science Research Number 110



Cover Story

Analysis of
Gene Expression

Features

What's New

BIO-RAD

Amplification

System Integration

Discover Bio-Rad's range of solutions for all your amplification needs.

iCycler Flexibility
- Intuitive interface
- Interchangeable reaction modules
- Upgrade to real-time PCR (96-well)

iCycler Reaction Module Options
- 96-, 60-, 384-, and dual 48-well formats
- Innovative gradient design (96-well)

Amplification Tools
- Hot start *Taq* polymerase
- Core PCR reagents
- PCR tubes and plates
- Unsurpassed customer service


iCycler™ Thermal Cycler

iTaq™ DNA polymerase


96-well reaction module


60-well reaction module


384-well reaction module

The polymerase chain reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCR process requires a license.

Visit us on the Web at **discover.bio-rad.com.**
Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

BIO-RAD

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Bio-Rad Subsidiaries
Telephone Numbers

Australia 61-2-9914-2800
Austria 43-1-877-8901
Belgium 32-9-385-5511
Brazil 55-21-507-6191
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or 91-124-891-8111
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On the cover:
DNA with a twist.
Illustrated by Audra Geras.

in this issue

BioRadiations 110, 2003

to our readers

In the 50 years since the publication of Watson and Crick's model of the structure of DNA, the scientific community has made incredible progress in understanding genes and their roles in heredity, evolution, development, and disease. With so much new sequence obtained from the completion of the human genome project, the challenging next step is to gain truly meaningful information about our genome, including where and when genes are expressed, and biochemical functions yet to be elucidated. The impressive efforts and technologies that drive genomic studies today enhance progress in other experimental systems, and it is clear that discoveries in biology are growing exponentially.

Bio-Rad's 46-year history has led it to become a leading supplier of products and protocols that facilitate a better understanding of gene expression, including the iCycler iQ™ real-time PCR detection system, XenoWorks™ microinjection system, and VersArray™ microarray products. This issue's cover story gives an overview of many of the most powerful tools used in studies of gene expression, and provides a sampling of further resources in this exciting field.

cover story

departments

technical reports

product focus articles

genetransfer

Transformation Experts

Introducing the emerging standard in high-efficiency competent cells from Bio-Rad, the leader in gene transfer technology.

- Blue-white screening
- Protocol-optimized volumes
- Consistent performance
- Space-saving packaging

For more information, contact your Bio-Rad representative or visit us on the Web at **www.bio-rad.com/genetransfer/**

Chemi-Competent Cells

C-Max™5α	(>1 x 10^9/µg DNA)
C-Max5α F'	(>1 x 10^9/µg DNA)

Electro-Competent Cells

EP-Max™10B	(>1 x 10^{10}/µg DNA)
EP-Max10B F'	(>1 x 10^{10}/µg DNA)

Competent Cells | Lipofection | Microinjection | Biolistics | Electroporation

Visit us on the Web at **discover.bio-rad.com**
Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

BIO-RAD

Competent Cells

Bio-Rad, a leader in gene transfer with technologies such as the Gene Pulser Xcell™ electroporator, Helios® gene gun, and the new XenoWorks™ microinjection system, now offers a new line of competent cells for routine subcloning and for demanding applications such as cDNA and genomic library construction. Both EP-Max™10B electro-competent cells and C-Max™5α chemi-competent cells are available in convenient volumes for simple transformation experiments.

For more information on these competent cell lines and supported applications, see our Product Focus article on pages 44–45.

Ordering Information

Catalog #	Description
Electro-Competent Cells	
170-3330	EP-Max10B Electro-Competent Cells, 5 x 0.1 ml
170-3331	EP-Max10B F' Electro-Competent Cells, 5 x 0.1 ml
Chemi-Competent Cells	
170-3340	C-Max5α Chemi-Competent Cells, 5 x 0.2 ml
170-3342	C-Max5α Chemi-Competent Cells, 10 x 0.05 ml
170-3341	C-Max5α F' Chemi-Competent Cells, 5 x 0.2 ml
170-3343	C-Max5α F' Chemi-Competent Cells, 10 x 0.05 ml

EZ Load™ HT Molecular Markers

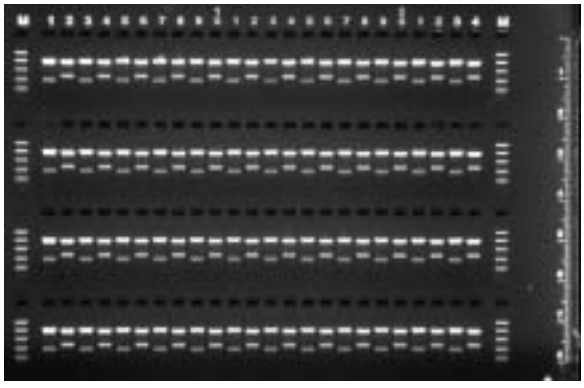
Designed for ReadyAgarose™ 96 Plus Gels

Bio-Rad's commitment to electrophoresis means offering you the standards that work best with your gels!

New EZ Load HT markers complete the ReadyAgarose 96 Plus system, providing a complete solution for high-throughput DNA electrophoresis. EZ Load HT molecular markers are available in two size ranges that meet most researchers' needs. Each set of markers is resolved into five sharp, easily identifiable bands within a short migration distance. EZ Load HT molecular markers are ready to use. They are prediluted to save time and contain Orange G tracking dye for monitoring electrophoresis runs.

Specifications

	EZ Load HT Molecular Marker, 100 bp–2 kb	EZ Load HT Molecular Marker, 500 bp–10 kb
Fragment sizes	100 bp, 200 bp, 500 bp, 1 kb, and 2 kb	500 bp, 1 kb, 2 kb, 4 kb, and 10 kb
Recommended gel type	3% ReadyAgarose 96 Plus gel	1% ReadyAgarose 96 Plus gel
DNA concentration	10 µg/ml	10 µg/ml
Volume/vial	1.6 ml	1.6 ml
Applications/vial	160 lanes or 20 ReadyAgarose 96 Plus gels	160 lanes or 20 ReadyAgarose 96 Plus gels



Separation of DNA fragments on ReadyAgarose 96 Plus gels. A 1% TAE gel with plasmid digest 1 (1,245 bp and 3,661 bp fragments) and plasmid digest 2 (945 bp and 3,962 bp) in alternating numbered lanes and EZ Load HT molecular marker, 500 bp–10 kb, in the "M" marker lanes.

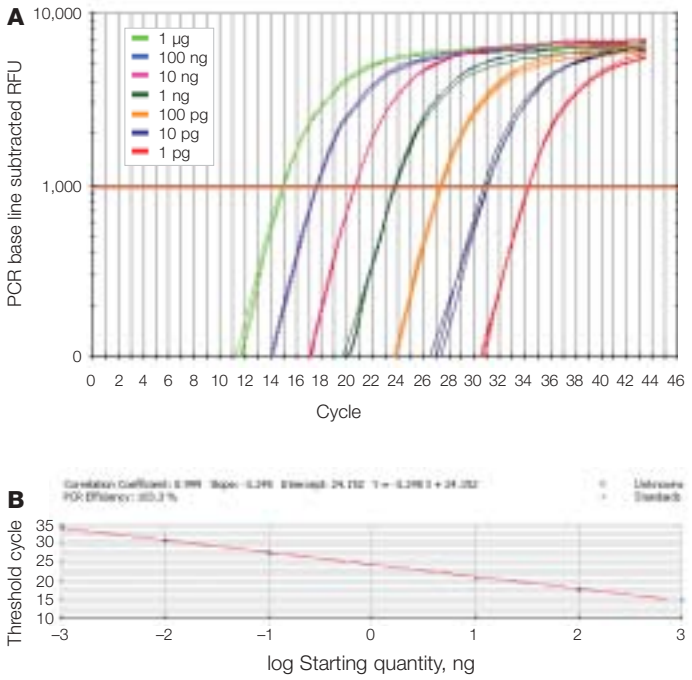
Ordering Information

Catalog #	Description
Molecular Markers	
170-8361	EZ Load HT Molecular Marker, 100 bp–2 kb
170-8362	EZ Load HT Molecular Marker, 500 bp–10 kb
ReadyAgarose 96 Plus Gels	
TBE Gels	
161-3060	1% ReadyAgarose 96 Plus Gel, with ethidium bromide
161-3062	3% ReadyAgarose 96 Plus Gel, with ethidium bromide
TAE Gels	
161-3063	1% ReadyAgarose 96 Plus Gel, with ethidium bromide
161-3065	3% ReadyAgarose 96 Plus Gel, with ethidium bromide

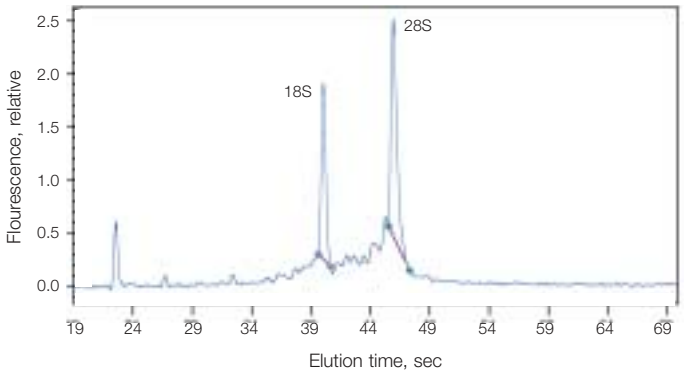
Aurum™ Total RNA Kits

Sample integrity is a primary concern for researchers in fields such as molecular diagnostics, gene expression, and functional genomics. Aurum total RNA kits produce high-quality, DNA-free total RNA from most cell types, meeting the stringent requirements imposed by these research applications.

Aurum total RNA kits are available in mini column and microplate formats for isolation of total RNA from cultured cells, bacteria, and yeast. The mini kit is also suitable for processing animal and plant tissue — up to 20 mg animal hard tissue, 40 mg animal soft tissue, and 60 mg plant tissue. RNase-free reagents, plasticware and lyophilized DNase I are included to ensure successful sample preparation.



Real-time RT-PCR with total RNA isolated using the Aurum total RNA mini kit. Total RNA isolated from a culture of 2 x 10^6 HeLa cells was eluted in Aurum RNA elution solution and a sample used in two-step real-time RT-PCR. Reverse transcription reactions were performed on 10-fold dilutions (1 µg to 1 pg) of the HeLa total RNA using the iScript™ cDNA synthesis kit. cDNA from these reactions was used to detect human β-actin gene expression levels using gene-specific primers and iQ™ SYBR® Green supermix. **A**, real-time data acquired on the Bio-Rad iCycler iQ system; **B**, standard curve showing r = 0.999, efficiency = 103.3%.



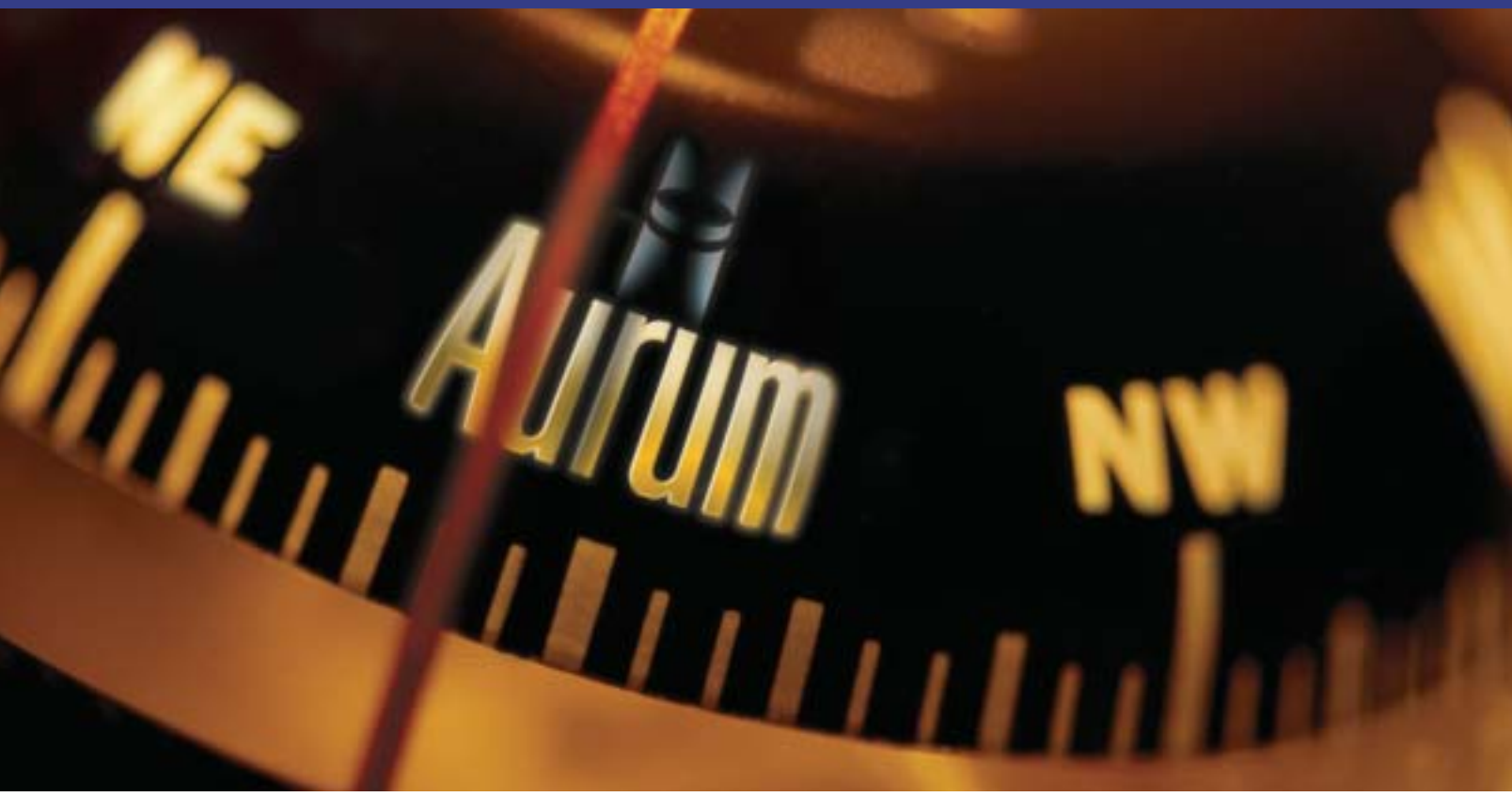
Agilent 2100 bioanalyzer electropherogram of high-quality eukaryotic total RNA. Total RNA was isolated from 30 mg of rat brain using the Aurum total RNA mini kit in the spin format. Start times of the 18S and 28S ribosomal RNAs are 39.6 and 45.5 seconds, respectively. The clearly defined peaks and minimal background fluorescence indicate intact, undegraded RNA.

Ordering Information

Catalog #	Description
732-6800	Aurum Total RNA 96 Kit, 2 x 96-well preps
732-6470	Aurum Vacuum Manifold
732-6820	Aurum Total RNA Mini Kit, 50 preps

Please inquire with your local representative for a trial size of the Aurum total RNA kit.

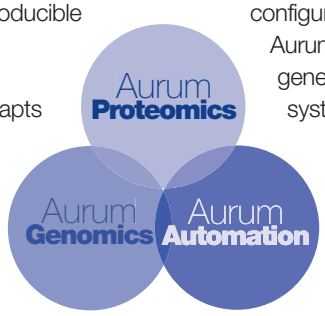
SYBR is a trademark of Molecular Probes, Inc.



Leading You to Great Results

Aurum™ sample preparation kits, the new direction for genomic and proteomic sample preparation.

The Aurum line of nucleic acid and protein purification products will put you on course towards reproducible results. Relying on Bio-Rad's experience in separation science, Aurum kits offer a flexible strategy for sample preparation that easily adapts to your genomic and proteomic research. You'll arrive at meaningful downstream results quickly, whatever your starting material, platform, or preparation format.



Bio-Rad offers guidance with customized packaging configurations and automated protocols. Use Aurum kits alongside our iCycler™ thermal cycler, gene transfer products, and ProteomeWorks™ system to map out a complete solution that delivers high quality, efficiency, and performance every step of the way. For more information, visit us on the Web at **www.bio-rad.com/aurum/**

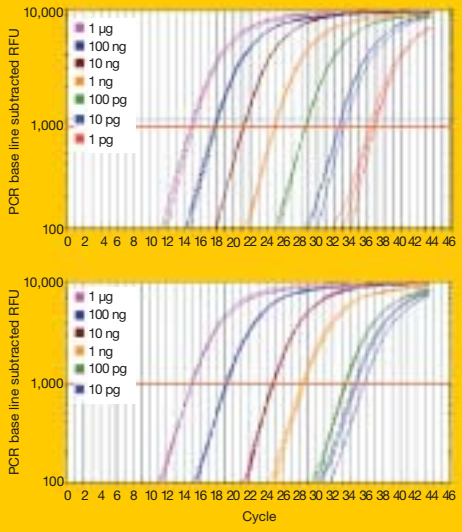
Visit us on the Web at **discover.bio-rad.com**
Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

BIO-RAD

Leader of the Pack

When it comes to quantitative real-time RT-PCR, the iScript™ cDNA synthesis system separates your data from the pack.

- Engineered MMLV RNase H^+ reverse transcriptase delivers highest sensitivity for real-time RT-PCR
- Optimized blend of oligo(dT) and random primers enables complete and unbiased RNA sequence representation
- Easy reaction assembly and streamlined protocol — the most convenient reverse transcription reactions you'll ever do



Comparison of amplification success using iScript and competitor I's reverse transcriptase. Input RNA was reverse transcribed and amplified using iQ™ SYBR® Green supermix with detection on the iCycler iQ™ real-time detection system.

Top: Bio-Rad iScript cDNA synthesis system delivers at least 6 orders of dynamic range while maintaining optimum sensitivity with limited amounts of input RNA.

Bottom: Competitor I's reverse transcriptase fails to deliver resolution over a wide dynamic range or sensitivity with a limited amount of input RNA.

For more information about iScript visit us on the Web at www.bio-rad.com/iScript/

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outside the US, contact your local sales office.

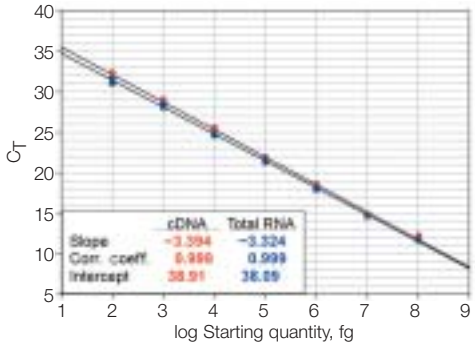
BIO-RAD

iScript™ cDNA Synthesis Kit

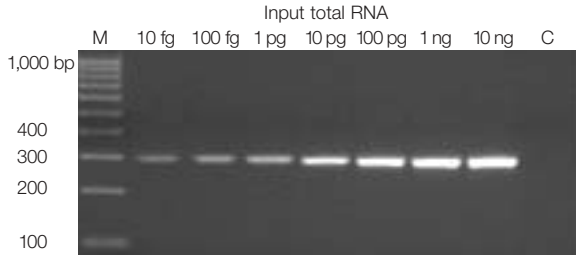
The iScript cDNA synthesis kit is the most sensitive and easy-to-use system for first-strand cDNA synthesis. It contains RNase H^+ iScript reverse transcriptase for sensitivity, a premixed RNase inhibitor to prevent indiscriminate degradation of RNA template, and a unique blend of oligo(dT) and random primers. All components are optimized to yield sensitive, unbiased representation over a broad dynamic range, with minimal setup and reaction time. With the iScript cDNA synthesis kit, great results have never been easier.

Comparison of Reaction Assemblies and Protocols

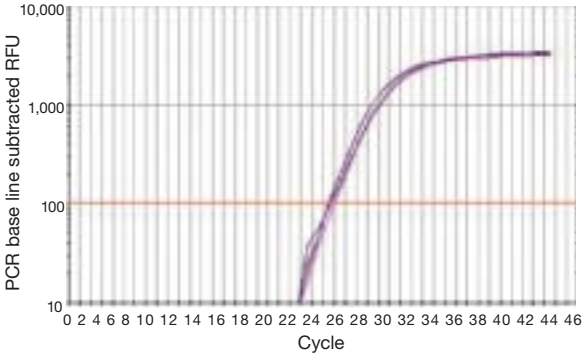
Bio-Rad Laboratories' iScript cDNA Synthesis Kit	**Competitor I's First Strand cDNA Synthesis System**
To a 0.2 ml tube, add:	**To a 0.2 ml tube, add:**
5x cDNA synthesis kit buffer	Oligo(dT) primers
iScript enzyme mixture	Random hexamers
Nuclease-free water	Buffer
RNA sample	$MgCl_2$
	dNTPs
	DTT
	RNase inhibitor
	Reverse transcriptase
	Nuclease-free water
	RNA sample
Next, program the thermal cycler and perform the following protocol:	
5 min at 25°C	5 min at 65°C
30 min at 42°C	Remove, quickly chill on ice
5 min at 85°C	Add additional items
Hold at 4°C	Mix and incubate 2 min at 42°C
	Add reverse transcriptase
	Return reaction to thermal cycler
	50 min at 42°C
	15 min at 70°C
	Hold at 4°C
	RNase H digestion, 20 min at 37°C
Total time, including setup and reaction:	
45 min	>110 min



Efficiency and performance of iScript across a broad range of concentrations in quantitative RT-PCR. Quantitative PCR was performed on serial dilutions of cDNA (●) or on cDNAs from serial dilutions of input total RNA (▲). The resulting standard curves are linear and virtually superimposable.



Sensitivity of the iScript cDNA synthesis kit in RT-PCR. cDNA was synthesized at 42ºC and amplified with iTaq™ DNA polymerase using β-actin primers. Lane M, EZ Load™ 100 bp molecular ruler; C, no-template negative control.



The iScript cDNA synthesis kit demonstrates complete and unbiased coverage of both the 5' and 3' ends of RNA input population. Following reverse transcription with the iScript cDNA synthesis kit, the 5 kb map4 cDNA was amplified using iQ™ supermix and primers specific for the 5' or 3' ends of the transcript. The cDNAs generated with iScript completely covered the gene, as evidenced by the superimposable traces for 5' or 3' end amplification.

Ordering Information

Catalog #	Description
170-8890	iScript cDNA Synthesis Kit, 25 x 20 µl reactions
170-8891	iScript cDNA Synthesis Kit, 100 x 20 µl reactions
170-8874	dNTP Solution, 200 µl of 10 mM of each dNTP
170-8860	iQ Supermix, 100 x 50 µl reactions
170-8862	iQ Supermix, 500 x 50 µl reactions
170-8864	iQ Supermix, 1,000 x 50 µl reactions
170-8880	iQ™ SYBR® Green Supermix, 100 x 50 µl reactions
170-8882	iQ SYBR Green Supermix, 500 x 50 µl reactions
170-8884	iQ SYBR Green Supermix, 1,000 x 50 µl reactions
170-8870	iTaq DNA Polymerase, includes 250 U polymerase, 10x PCR buffer, $MgCl_2$ solution
170-8872	$MgCl_2$ Solution, 1.25 ml of 50 mM $MgCl_2$

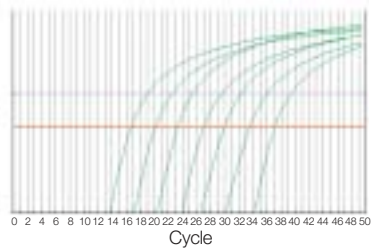
MyiQ™ Single-Color Real-Time PCR Detection System

An Easy-to-Use Real-Time PCR Instrument From the Experts in Quantitative PCR

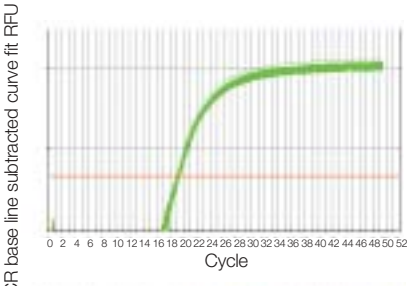
The new MyiQ real-time PCR detection system offers an affordable alternative for the detection of common green fluorescent dyes such as FAM and SYBR Green I. This system interfaces directly with the iCycler™ thermal cycler, and offers superior features such as thermal gradient and Peltier-effect driven performance. The MyiQ real-time PCR detection system is a perfect solution for those just getting started with this technology as well as those looking for additional instruments to handle increasing routine assay demands. This system was developed by the same experts at Bio-Rad who have pioneered performance in real-time PCR. The MyiQ delivers the same excellent-quality data as the iCycler iQ™ real-time detection system. Its features include:

- Innovative real-time PCR thermal gradient
- Easy melt-curve analysis
- Customizable end-point analysis

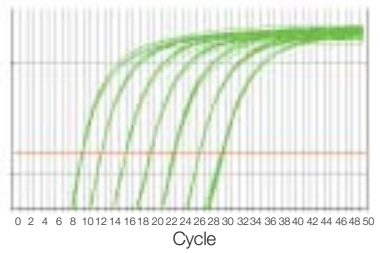
For more information, visit us on the Web at **www.bio-rad.com/ad/MyiQ/**



Sensitivity of detection down to one copy of IL-1β.



Superb uniformity of results across all 96 wells in a SYBR Green I uniformity assay.



Excellent resolution over at least 6 orders of magnitude.

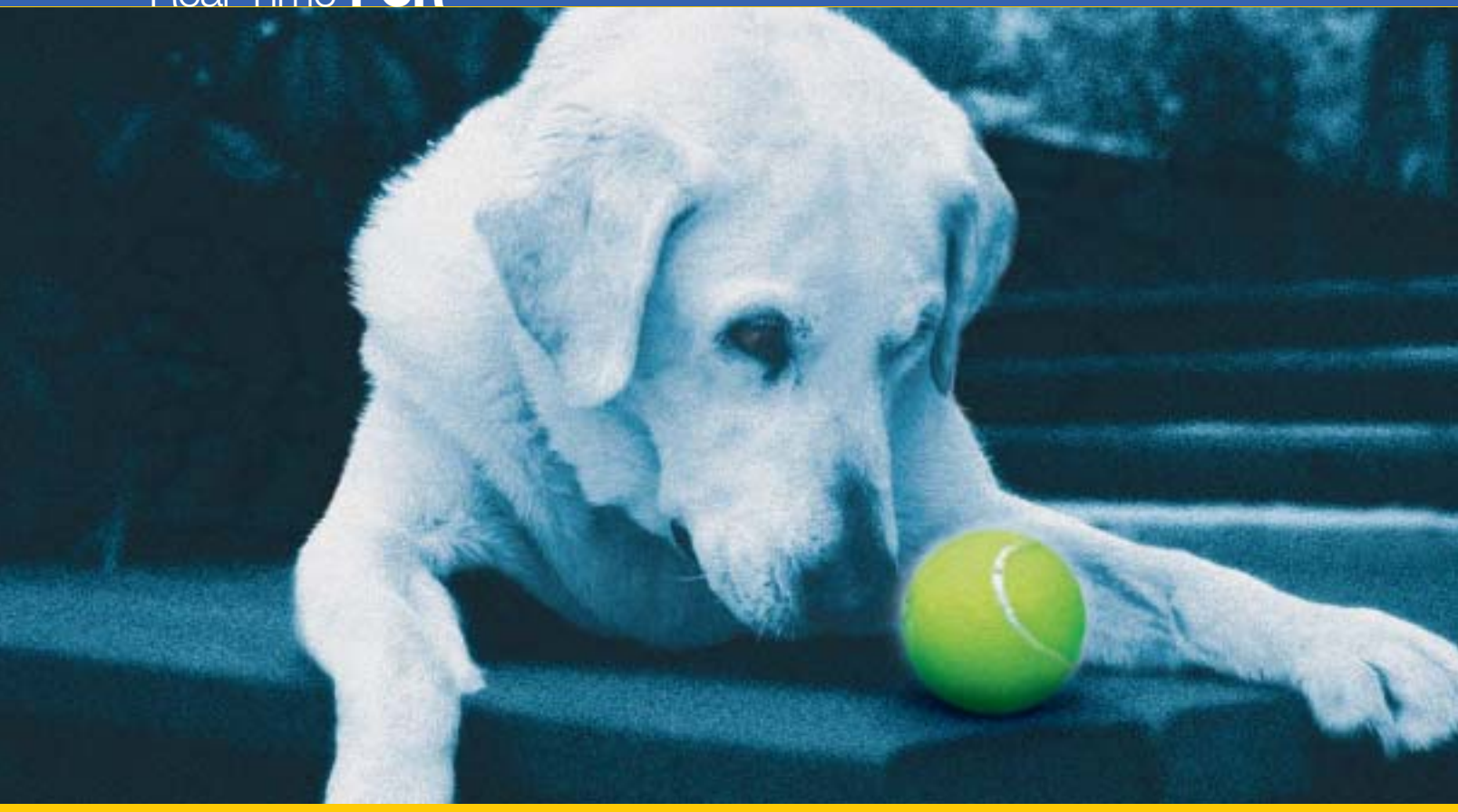
Specifications

Operational	
Heating rate	3.3°C/sec
Cooling rate	2.0°C/sec
Method of heating/cooling	Peltier and Joule
Temperature	
Range	4–100°C
Accuracy	±0.3°C
Uniformity	±0.4°C
Overshoot maximum	<0.5°C
Heated lid temperature	Up to 105°C
Descriptive	
Optical lamp	Tungsten-halogen
Fluorescence detection	FAM and SYBR Green I
Sample capacity	96 wells
Sample size	15–100 µl
Licensed for PCR	Yes
Electrical approvals	IEC, CE
Operating platforms	Windows NT, Windows 2000, Windows XP
Dimensions (including iCycler base unit)	35.6 x 62.2 x 32.7 cm (W x D x H)
Weight	17.6 kg

Ordering Information

Catalog #	Description
170-8720	iCycler Thermal Cycler With 96 x 0.2 ml Reaction Module, includes iCycler base with 96 x 0.2 ml iCycler reaction module, in-sample temperature probe (0.2 ml tube size), PCR tubes, power cord, quick reference card, instructions
170-9740	MyiQ Single-Color Real-Time PCR Detection System, includes optical module, optical power supply, SYBR Green I/FAM filter set, software CD-ROM, 0.2 ml 96-well PCR plates, optical sealing tape, communications cable, power cord, instructions
170-8860	iQ™ Supermix, 100 x 50 µl reactions
170-8862	iQ Supermix, 500 x 50 µl reactions
170-8880	iQ™ SYBR® Green Supermix, 100 x 50 µl reactions
170-8882	iQ SYBR Green Supermix, 500 x 50 µl reactions
170-8890	iScript™ cDNA Synthesis Kit, 25 x 20 µl reactions
170-8891	iScript cDNA Synthesis Kit, 100 x 20 µl reactions
223-9441	96-Well 0.2 ml Thin-Wall PCR Plates, 25
223-9444	Optical-Quality Sealing Tape, 100

Real-Time **PCR**



Focus on Green

Introducing the new MyiQ™ real-time PCR detection system — quantitative PCR just got more lab-friendly and affordable.

New MyiQ Software Includes

- SYBR Green I and FAM detection
- Temperature gradient for assay optimization
- Easy, automated data analysis
- Melt-curve analysis
- Integrated end-point analysis

Unparalleled Real-Time PCR Support

- On-site personalized customer support
- Certified real-time PCR and RT-PCR reagents
- Advanced probe and primer design software

For more information about MyiQ, visit us on the Web at www.bio-rad.com/ad/MyiQ/

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Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

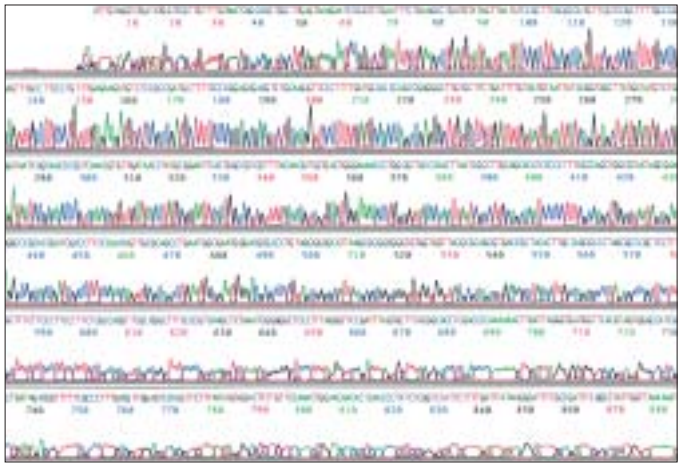
BIO-RAD

SEQueaky Kleen™ H_2O Adaptors

SEQueaky Kleen H_2O adaptors are designed specifically for use with the SEQueaky Kleen H_2O dye terminator removal kit. They allow collection of sequencing samples directly into a sequencing injection plate. By eliminating a tedious and time-consuming transfer step between the collection plate and the sequencing injection plate, these adaptors increase efficiency and throughput in your sequencing workflow. SEQueaky Kleen H_2O adaptors are made of high-strength aluminum alloy anodized to prevent corrosion, and are Teflon coated for a sleek, durable design. Four sequencing injection plate styles are available, ensuring compatibility with the most common automated sequencers.

Injection plates and corresponding compatible sequencers.

Injection Plate	Instrument
Half-skirt	ABI PRISM 3100 genetic analyzer ABI PRISM 3700 DNA analyzer Applied Biosystems 3730(*xl*) DNA analyzer
Unskirted	MegaBACE 1000 DNA analysis system
Full-skirt	MegaBACE 500 DNA analysis system MegaBACE 1000 DNA analysis system BaseStation DNA fragment analyzer
Segmented	CEQ 2000XL DNA analysis system CEQ 8000 genetic analysis system



Sample sequencing result on ABI PRISM 3730 DNA analyzer using SEQueaky Kleen H_2O dye terminator removal kit and adaptors.

	1	2	3	4	5	6	7	8	9	10	11	12
A	18	23	22	23	22	22	22	21	19	23	23	20
B	22	22	22	22	22	20	21	21	21	21	22	22
C	22	22	23	21	21	21	22	22	22	23	22	21
D	22	21	21	21	21	20	20	21	21	20	21	20
E	22	20	22	19	19	21	19	20	20	21	21	21
F	22	21	22	20	21	20	20	23	17	22	22	22
G	22	22	22	21	21	21	22	23	21	20	21	22
H	21	19	23	19	21	22	21	20	18	21	21	22

Consistency of eluate volumes. Ninety-six mock sequencing samples were purified through SEQueaky Kleen H_2O dye terminator removal kit using the adaptors. Sample volumes were 20 µl each. Standard deviation for entire plate was ±1 µl.

Ordering Information

Catalog #	Description
732-6510	SEQueaky Kleen H_2O Adaptors, set of 2
732-6500	SEQueaky Kleen H_2O Dye Terminator Removal Kit, 2 x 96
732-6505	SEQueaky Kleen H_2O Bulk Kit, 50 x 96
732-6530	SEQueaky Kleen H_2O Starter Kit,* for half-skirt plates
732-6531	SEQueaky Kleen H_2O Starter Kit,* for unskirted plates
732-6532	SEQueaky Kleen H_2O Starter Kit,* for full-skirt plates
732-6533	SEQueaky Kleen H_2O Starter Kit,* for segmented plates
732-6540	SEQueaky Kleen H_2O Refill Kit,** for half-skirt plates
732-6541	SEQueaky Kleen H_2O Refill Kit,** for unskirted plates
732-6542	SEQueaky Kleen H_2O Refill Kit,** for full-skirt plates
732-6543	SEQueaky Kleen H_2O Refill Kit,** for segmented plates
732-6520	SEQueaky Kleen H_2O Half-Skirt Plates, 50 pack***
732-6521	SEQueaky Kleen H_2O Unskirted Plates, 50 pack***
732-6522	SEQueaky Kleen H_2O Full-Skirt Plates, 50 pack***
732-6523	SEQueaky Kleen H_2O Segmented Plates, 50 pack***

*Starter kit includes set of 2 SEQueaky Kleen H_2O adaptors, 50 SEQueaky Kleen H_2O dye terminator removal plates, 50 injection plates, and 50 collection plates.

**Refill kit includes 50 SEQueaky Kleen H_2O dye terminator removal plates, 50 injection plates, and 50 collection plates.

***50 pack includes only injection plates.

Aurum™ Ion Exchange Mini Kits and Columns

Fractionation of proteins in a sample is a fundamental step in many research fields, including protein discovery. Ion exchange is a chromatographic technique for selective enrichment of proteins, whether acidic or basic. Easy-to-use Aurum ion exchange mini kits offer the power of ion exchange chromatography without the need for expensive chromatography systems or a sophisticated knowledge of chromatographic techniques.

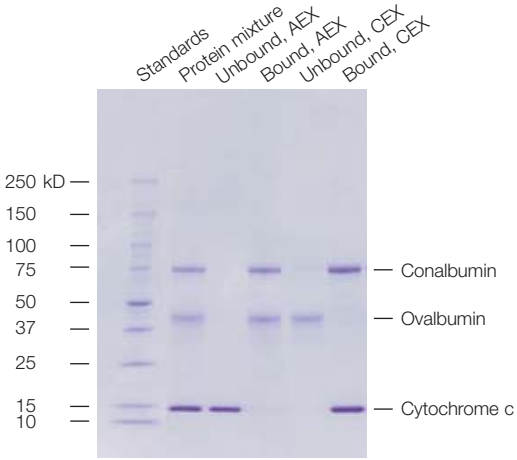
Aurum ion exchange mini kits are available with mini columns for cation exchange (CEX) or anion exchange (AEX). They are suitable for routine prefractionation, purification, and screening of many starting sample types, including serum, bacteria, yeast, and cultured cells. Aurum ion exchange mini kits simplify protein discovery with streamlined protocols and flexible column and buffer options. The purified proteins are suitable for most downstream proteomic applications, including 2-D gel analysis and mass spectrometry.

Media and Buffers

Aurum ion exchange mini kits are packed with UNOsphere S and Q media, a new generation of hydrophilic polymers developed using Bio-Rad's patented* polymerization technology. UNOsphere ion exchange media are specially developed to deliver efficient protein capture and ultrahigh protein binding capacity.

Protein Prefractionation With Aurum Ion Exchange Mini Columns

A mixture of three proteins differing in pI and molecular weight was fractionated on Aurum AEX and CEX columns (see figure below). The complementary fractionation patterns obtained for AEX and CEX columns demonstrate both the principles of ion exchange chromatography and the effectiveness of these mini columns.



Comparison of protein fractionation characteristics of Aurum AEX and CEX columns. A mixture of three proteins, ovalbumin (pI 4.6, MW 45,000), conalbumin (pI 6.9, MW 77,000), and cytochrome c (pI 10.7, MW 12,000), was run on a Criterion™ Tris-HCl precast gel, 4–20% linear gradient (catalog #345-0033) in Tris/glycine/SDS buffer at 200 V for 60 min. Standards used were Precision Protein™ standards.

Properties of Aurum ion exchange columns.

	Aurum CEX	Aurum AEX
Type of support	UNOsphere S	UNOsphere Q
Binding capacity	12 mg protein/column**	36 mg protein/column**
Column bed volume	0.2 ml	0.2 ml
Binding buffer	20 mM sodium acetate, pH 5.0	20 mM Tris, pH 8.3
Elution buffer	Binding buffer + 1.0 M NaCl	Binding buffer + 1.0 M NaCl

** Binding capacity determined using IgG (CEX) and BSA (AEX). Capacity will differ slightly depending on the protein.

Ordering Information

Catalog #	Description
732-6710	Aurum AEX Mini Kit, 2 pack
732-6705	Aurum AEX Mini Kit, 10 pack
732-6706	Aurum AEX Mini Columns, 25 pack`
732-6707	Aurum AEX Mini Columns, 100 pack
732-6711	Aurum CEX Mini Kit, 2 pack
732-6702	Aurum CEX Mini Kit, 10 pack
732-6703	Aurum CEX Mini Columns, 25 pack
732-6704	Aurum CEX Mini Columns, 100 pack

The ProteomeWorks System™

Aurum ion exchange mini kits and columns are components of the ProteomeWorks system, the global alliance between Bio-Rad Laboratories, Inc. and Waters Corporation (Micromass MS Technologies), dedicated to furthering proteomics research.

* US patent 6,423,666

Simple Steps to Protein Discovery.

1 **2-D Sample Prep**

2 2-D Electrophoresis

3 2-D Gel Imaging and Analysis

Stellar Results

Get brilliant 2-D results with Bio-Rad's constellation of new ReadyPrep™ sample prep kits.

One of the most critical steps in 2-D protein electrophoresis occurs during sample preparation, even before the first-dimension separation begins. Many samples contain impurities that cloud the picture with streaking and spurious spots. Some samples require fractionation to enrich for low-abundance proteins. Bio-Rad's new ReadyPrep kits for sample cleanup and fractionation provide tested tools and proven protocols that put clear 2-D results within easy reach. For a closer look at ReadyPrep, go to **www.bio-rad.com/products/ReadyPrepReagents/**

Visit us on the Web at **discover.bio-rad.com**
Call toll free at 1-800-4BIORAD (1-800-424-6723); outside the US, contact your local sales office.

BIO-RAD

ReadyPrep™ Sample Preparation Kits for 2-D Electrophoresis

One of the most critical steps in 2-D protein electrophoresis occurs even before the first-dimension separation, at the sample preparation step. No sample preparation protocol is universally suitable; each experiment offers unique challenges due to the variety and complexity of sample sources. The ReadyPrep line of 2-D sample preparation kits is a set of complementary procedures and reagents that yield great 2-D results. Each ReadyPrep kit provides simple, optimized protocols without time-consuming steps. Bio-Rad offers products for general-purpose 2-D sample preparation as well as products for fractionation of samples prior to 2-D. The general-purpose kits can be used on any protein sample to remove contaminants (e.g., salts, detergents) and to reduce disulfide bonds that interfere with 2-D electrophoresis. These kits reduce streaking and spurious spot formation, thus improving the quality of information from gels. The cytoplasmic, membrane I, and signal protein extraction kits fractionate proteins based on specific characteristics. The table gives an overview of the extraction methods used for the complete ReadyPrep line of 2-D sample prep kits.

Examples of the kind of improvement possible with these kits can be seen in Figures 1 and 2. For more information, see the article on pages 38–40 and request bulletin 2934.

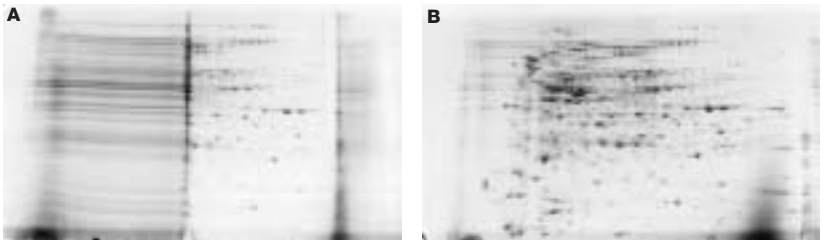


Fig. 1. Effective detergent removal by the ReadyPrep 2-D cleanup kit leads to improved 2-D SDS-PAGE separation. *E. coli* extracts were spiked with 1% SDS. **A**, untreated sample; **B**, sample treated with the ReadyPrep 2-D cleanup kit.

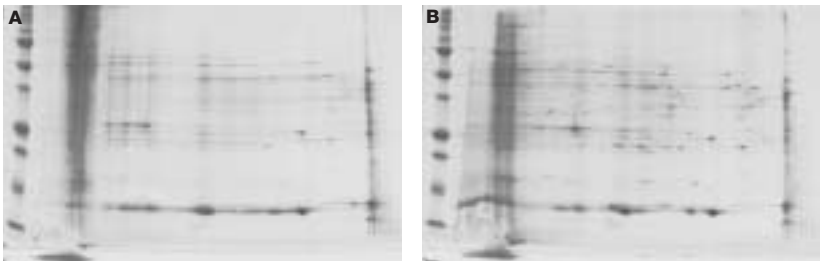


Fig. 2. SDS-PAGE 2-D separation of mouse liver extract with and without treatment using the ReadyPrep reduction-alkylation kit. Both samples were applied by cup loading onto 11 cm ReadyStrip™ pH 7–10 IPG strips. **A**, reduced with 50 mM DTT in rehydration/sample buffer; **B**, treated with the ReadyPrep reduction-alkylation kit.

Application	ReadyPrep Kit	Extraction Method
General-purpose	ReadyPrep 2-D cleanup kit	TCA-like precipitation
	ReadyPrep reduction-alkylation kit	Reduction using TBP followed by alkylation with iodoacetamide
Fractionation	ReadyPrep protein extraction kit (cytoplasmic/nuclear)	Organelle isolation followed by strongly chaotropic extraction buffer
	ReadyPrep protein extraction kit (membrane I)	Temperature-dependent differential solubilization using Triton X-114
	ReadyPrep protein extraction kit (signal)	Differential solubilization using Triton X-100
	ReadyPrep sequential extraction kit	Differential solubility

Ordering Information

Catalog #	Description
163-2130	ReadyPrep 2-D Cleanup Kit
163-2090	ReadyPrep Reduction-Alkylation Kit
163-2089	ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear)
163-2088	ReadyPrep Protein Extraction Kit (Membrane I)
163-2087	ReadyPrep Protein Extraction Kit (Signal)
163-2100	ReadyPrep Sequential Extraction Kit

Triton is a trademark of Union Carbide Chemicals and Plastics Technology Corp.

New PowerPac™ Basic and PowerPac™ HC Power Supplies

Good Looks and Great Performance

The PowerPac Basic and PowerPac HC power supplies, with a sleek new design and unparalleled ease of use, offer the high quality of Bio-Rad power supplies with attractive new features, including:

- Autosensing of AC line voltage with automatic switching between 100–120 and 220–240 VAC — eliminates ordering confusion and allows portability
- Compact and stackable design that saves benchspace
- Flip-down legs for easy viewing at benchtop or shelf level
- New pause/resume run capability to adjust run parameters without resetting the timer

Stacked PowerPac Basic Power Supplies

PowerPac Basic

Ideal for basic electrophoresis applications, including:

- Mini vertical electrophoresis with the Mini-PROTEAN® 3 cell
- Submerged horizontal electrophoresis with any size Sub-Cell® electrophoresis cell
- Blotting with the Mini Trans-Blot® cell

Runs 4 identical mini cells (vertical or Sub-Cell models) simultaneously

For more information, request bulletin 2881.

PowerPac HC

Drives all high-current applications to their maximum performance:

- A perfect choice for western blotting with any of Bio-Rad's extensive line of blotting cells, — get great results in 15–30 min with the new Trans-Blot Plus large format cell
- Ideal for high-throughput vertical cells, such as Bio-Rad's Dodeca™ cells

Larger LCD shows all run parameters at once for monitoring at a glance

Programmable multi-step methods and timed runs with choice of constant voltage, current, or power

For more information, request bulletin 2882.

Specifications

	PowerPac Basic	PowerPac HC
Output specifications	300 V, 400 mA, 75 W	250 V, 3.0 A, 300 W
Type of output	Constant voltage or constant current with automatic crossover	Constant voltage, current, or power with automatic crossover
Output terminals	4 pairs recessed banana jacks in parallel	4 pairs recessed banana jacks in parallel
Timer control	1–999 min, fully adjustable	Up to 99 hr 59 min, fully adjustable
Programability	Single-step methods with timed or continuous operation	Multi-step methods with up to 3 steps
Pause/resume function	Yes	Yes
Display	3-digit LED	2-line, 16-character backlit LCD
Automatic power failure recovery	Yes, user selectable	Yes, user selectable
Safety features	No-load detection, rapid resistance change detection, overload/short circuit protection, input line protection	No-load detection, rapid resistance change detection, ground leak detection, overload/short circuit protection, over voltage protection, over temperature protection, input line protection
Safety compliance	EN61010	EN61010
EMI	Conforms to CE standards for emissions and immunity class A, tested only at 230 V; TÜV EMC certification	Conforms to CE standards for emissions and immunity class A, tested only at 230 V; TÜV EMC certification
Input power (nominal)	100–120/220–240 VAC, autoswitching	100–120/220–240 VAC, autoswitching
Dimensions (W x D x H)	24.5 x 21 x 6.5 cm	28.5 x 25 x 8 cm
Weight	1.1 kg	2.0 kg

Ordering Information

Catalog #	Description
164-5050	PowerPac Basic Power Supply, 100–120/220–240 V
164-5052	PowerPac HC Power Supply, 100–120/220–240 V

PowerPac™ Power Supplies

Ready to Charge.

Introducing Bio-Rad's new PowerPac power supplies. They're packed with naturally easy-to-use features, solid reliability, and, let's face it — a touch of attitude.

PowerPac power supplies meet your electrophoresis needs head-on, with exceptional ease of use and the reliable performance that your applications demand. Choose the compact PowerPac™ Basic for everyday applications such as mini vertical or submerged horizontal cells, or use the high-current PowerPac™ HC for high-intensity blotting with Bio-Rad's extensive line of blotting cells and for high-throughput SDS-PAGE with the Dodeca™ cells. Both power supplies offer exceptional programmability and a range of output options. With their streamlined design, stackable cases, and vibrant, translucent green lids, they'll get a lot of attention — just like your results. For more information, visit us on the Web at **discover.bio-rad.com**

Visit us on the Web at **discover.bio-rad.com**
Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

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Dodeca™ Stainers and Gel Clip

The Dodeca stainers and accessories simplify and improve high-throughput staining and handling of large format protein gels, minimizing hands-on time and helping to eliminate gel breakage. The patent-pending Dodeca stainer comes in two sizes to optimize staining volume. Both sizes accommodate up to 12 gels. The Dodeca stainers match the capacity of the PROTEAN® IEF cell for first-dimension electrophoresis and the PROTEAN® Plus Dodeca cell for the second dimension to streamline your 2-D electrophoresis workflow. The Dodeca stainers ensure high-quality, consistent results, and are compatible with Bio-Safe™ colloidal Coomassie Blue G-250 stain, Coomassie Brilliant Blue R-250 stain, SYPRO Ruby protein gel stain, and a new mass spectrometry compatible Dodeca silver stain kit (see next page).

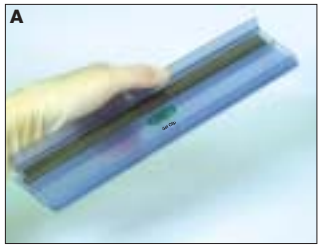
Large Dodeca Stainer
41.3 x 46.2 x 38.9 cm
(W x D x H)

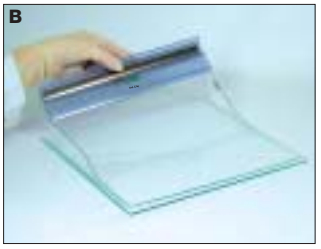
Features

- **Shaker motor** — Attached to the lid, it provides a gentle motion for optimal staining efficiency and to protect gels from breaking
- **Staining trays** — Available in two sizes, these allow gels enough movement for thorough staining without damage. They double as transport trays. Gels easily slide off the trays onto an imager or spot cutter platform. Use the gel clip accessory to lift gels back onto the tray
- **Shaking rack** — Holds the staining trays at an angle to allow air bubbles to escape, ensuring uniform gel staining. Designed with handles for easy placement into the solution tank
- **White tray** — For monitoring the development step of the staining process
- **Reagent access door** — Provides easy access for quickly pouring staining solutions without disturbing the gels

Accessories

- **Storage boxes** — For convenient storage of gels; up to 4 gels, including the staining trays, can be placed in a box for storage at 4°C
- **Gel clip** — Supports large format gels during handling, reducing the risk of gel breakage. The gel clip clamps along one entire edge of a gel, distributing the weight evenly so that the gel can be easily lifted without tearing. A gel can be transferred from a glass plate to a staining tray and then to an imaging or spot cutter platform without ever touching the gel. For more information, see the Tips and Techniques article on page 49.





A, gel clip; **B**, clamping onto a PROTEAN Plus precast gel (25.6 x 23 cm); **C**, lifting the gel off the glass plate; **D**, transferring the gel to a staining tray.

Compatibility of different gel sizes with the Dodeca stainers.

Stainer Size	Tray Size	Gel Sizes (cm)	Gel Formats
Large	Large	25.6 x 23	PROTEAN Plus precast gels
Dodeca stainer	staining tray	25 x 20.5	PROTEAN Plus handcast gels (require one attachment per tray)
Small	Small	20 x 20.5	PROTEAN Plus handcast gels
Dodeca stainer	staining tray	18 x 20	PROTEAN II XL handcast and precast gels
		16 x 20	PROTEAN II xi handcast gels
		16 x 16	PROTEAN II xi handcast and precast gels
		8.7 x 13.3	Criterion™ gels (up to 24 gels, require one attachment per tray)

Ordering Information

Catalog #	Description
Dodeca Stainers	
165-3400	Dodeca Stainer, large, 100–240 V, includes 13 trays (12 translucent and 1 white), shaking rack, solution tank, lid with shaker, shaker control unit, gel clip, attachments, instructions
165-3401	Dodeca Stainer, small, 100–240 V, includes 13 trays (12 translucent and 1 white), shaking rack, solution tank, lid with shaker, shaker control unit, gel clip, attachments, instructions
Accessories	
165-3414	Gel Clip, 1
165-3429	Storage Box, large, holds up to 5 large staining trays (4 with gels, one to cover top gel)
165-3430	Storage Box, small, holds up to 5 small staining trays (4 with gels, one to cover top gel)

Dodeca™ Silver Stain Kit

Convenient and Sensitive Silver Staining for High-Throughput Applications

The Dodeca silver stain kit is optimized for use with the Dodeca stainer. Proteins stained with this kit are compatible with mass spectrometry for protein identification (no protein-modifying glutaraldehyde is included, only the minimal concentration of formaldehyde required for stain development). The chemistry of the kit ensures reduced background and artifacts, and minimizes formation of silver residue on the Dodeca stainer. The kit provides excellent detection sensitivity (0.25–0.5 ng for BSA).

Ordering Information

Catalog #	Description
161-0480	Dodeca Silver Stain Kit, for large Dodeca stainer tank, stains 12 PROTEAN Plus (25.6 x 23 cm and 25 x 20.5 cm) gels simultaneously
161-0481	Dodeca Silver Stain Kit, for small Dodeca stainer tank, stains 12 PROTEAN and 24 Criterion (20 x 20.5 cm to 8.7 x13.3 cm) gels simultaneously

Trans-Blot® Plus Cell

A Versatile Blotting System for Large Format Gels

The Trans-Blot Plus cell meets the need for a powerful, versatile large format protein blotting apparatus. With the capacity for simultaneous transfer of up to three 26.5 x 28 cm gels, the Trans-Blot Plus cell is the perfect blotter for the large format gels used with Bio-Rad's PROTEAN® II XL and PROTEAN® Plus Dodeca™ cells. The Trans-Blot Plus cell can also accommodate up to 12 Criterion™ gels, making it the ideal accompaniment to the Criterion Dodeca cell, particularly for researchers planning to scale up to a large gel format in the future.

The Trans-Blot Plus cell offers the flexibility of a truly versatile tank transfer system. Transfer conditions can be varied to achieve optimal transfers over a broad molecular weight range. Durable plate electrodes provide a strong and uniform electrical field and their placement within the tank is flexible. Whether you are running one, two, or three gel cassettes, the electrodes can be placed a minimum distance apart for maximum field strength and transfer efficiency. The sturdy gel holder cassettes ensure an even contact along the entire gel and membrane surface, and a cooling coil provides effective temperature regulation during native protein or high-intensity, high-power applications. An optional assembly tray is ideal for gel sandwich and cassette assembly.

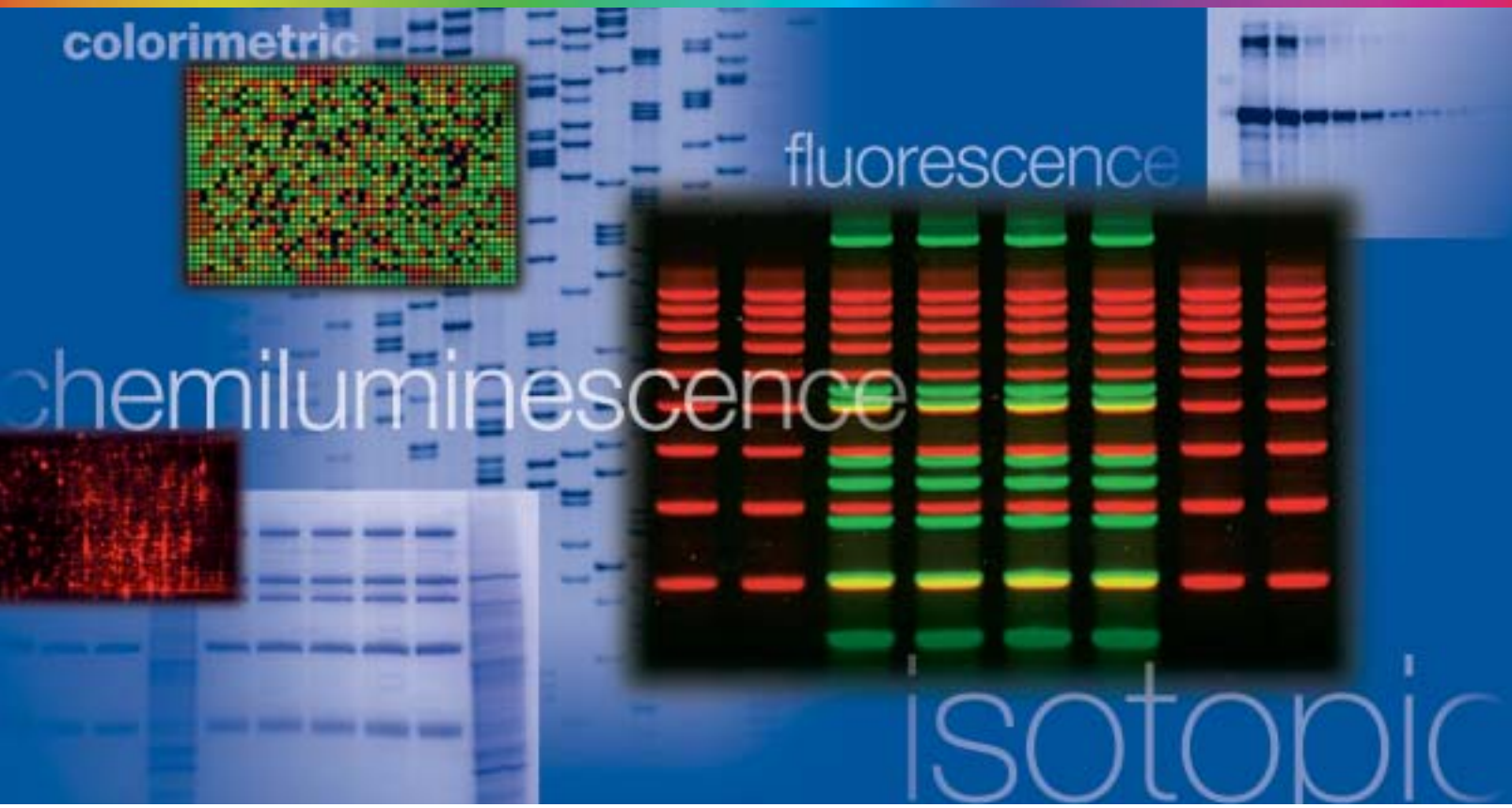
The Trans-Blot Plus cell provides uniform transfers of proteins from large format one- and two-dimensional gels with remarkable efficiency — using the new PowerPac™ HC power supply, most proteins are transferred in as little as 15–30 minutes.

Specifications

Dimensions	30 x 17.3 x 39.4 cm (W x D x H)
Maximum gel size	26.5 x 28 cm
Buffer requirement	10–12 L
Recommended power supply	PowerPac HC power supply (PowerPac 200 also compatible)
Gel capacity	3 PROTEAN Plus gels 3 PROTEAN II xi/XL gels 12 Criterion gels 27 Ready Gel® or Mini-PROTEAN® 3 gels

Ordering Information

Catalog #	Description
170-3990	Trans-Blot Plus Cell With Plate Electrodes and Super Cooling Coil, includes 3 gel holder cassettes, cell with lid and power cables, 6 fiber pads, 1 pack blot absorbent paper (26.5 x 28 cm, pack of 30), roller, stirbar
170-3991	Trans-Blot Plus Cell With Plate Electrodes, Super Cooling Coil, and PowerPac HC Power Supply
170-3994	Trans-Blot Plus Gel/Cassette Assembly Tray
162-0251	Nitrocellulose Membrane, 0.45 µm, 26.5 x 28 cm, 10 sheets
162-0252	Nitrocellulose Membrane, 0.2 µm, 26.5 x 28 cm, 10 sheets
162-0255	Immun-Blot® PVDF Membrane, 26.5 x 28 cm, 10 sheets
162-0256	Sequi-Blot™ PVDF Membrane, 26.5 x 28 cm, 10 sheets



Capture the Full Range.

For a complete selection of imaging systems, look to Bio-Rad.

Imaging never looked better. Single or multicolor fluorescence, chemiluminescence, chemifluorescence, isotopic, or colorimetric. Gels, film, or blots. For proteomics to genomics and everything in between, Bio-Rad has an imaging system to fit your applications and budget. From the powerful Molecular Imager FX™ to the flexible VersaDoc™, Bio-Rad systems feature specialized image analysis software and can be integrated into a single imaging center or a multi-user network. Give your samples the exposure they deserve. For more information on our imaging systems, visit us at **discover.bio-rad.com**

Model	Isotopic	Chemiluminescent	Fluorescent	Colorimetric
Molecular Imager FX™ Systems	•	•	•	•
VersaDoc™ Imaging Systems		•	•	•
GS-800™ Calibrated Densitometer				•
ChemiDoc™ XRS System		•	•	•
ChemiDoc™ System		•	•	•
Gel Doc™ 2000 System			•	•

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outside the US, contact your local sales office.

BIO-RAD

ProteomeWorks™ Plus Spot Cutter System

The ProteomeWorks Plus spot cutter adds backed gel cutting capability and an expanded cutting platform area to the already impressive and highly accurate original ProteomeWorks spot cutter. The original model has performed exceedingly well in proteomics laboratories worldwide, and the new "Plus" model retains all the great features, with additional improvements. The ProteomeWorks Plus spot cutter still is integrated with The Discovery Series™ PDQuest™ 2-D analysis software, which provides the protein spot analysis, spot cutter targeting, and data tracking that benefit any proteomics laboratory.

Maximum Versatility

The ProteomeWorks Plus spot cutter provides:

- Spot cutting from free-standing gels, backed gels, and blots
- A platform area of 30 x 27 cm and cutting tip range of 26 x 21 cm to accommodate larger format gels and blots
- System accuracy of ±0.1 mm, allowing accurate excision of even small, closely grouped spots
- Direct use of PDQuest analysis sets for spot cutter targeting, which minimizes setup of the cut run
- Advanced PDQuest spot automatching to align the analysis image and spot cutter gel image, correcting for gel changes that may occur during storage
- Complete data tracking from gel to microplate within PDQuest software
- Overlapped spot resolution by sophisticated image analysis and spot cutter targeting for better purity of mass spectrometry samples
- Imaging of visible and fluorescent stains including Coomassie Blue, silver, and fluorescent SYPRO Ruby protein gel stain
- Small footprint of only 57 x 73 cm with 72 cm height

With its versatility in cutting formats, along with its complete software integration, the ProteomeWorks Plus spot cutter meets the needs of individual laboratories as well as shared instrument environments.

For more information on the ProteomeWorks Plus spot cutter and PDQuest software, request bulletin 2425, or contact your local Bio-Rad sales representative.

Ordering Information

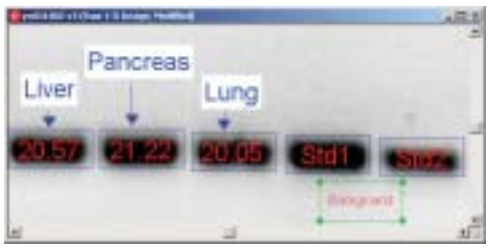
Catalog #	Description
165-7064	ProteomeWorks Plus Spot Cutter System, includes spot cutter, viewer mode PDQuest software for basic excision, gel cutting sheets, accessories, instructions; not included, licensed version PDQuest software
165-7065	ProteomeWorks Plus Spot Cutter With Fluorescent Enclosure System, includes spot cutter, fluorescent enclosure, viewer mode PDQuest software for basic excision, accessories, instructions; not included, licensed version PDQuest software
170-8603	The Discovery Series PDQuest 2-D Analysis Software, Windows

The Discovery Series™ Quantity One® Basic 1-D Analysis Software

Free Software to Promote Sharing of Data Within the Scientific Community

Quantity One Basic is free shareware that allows you to share images and data with colleagues and collaborators, and to submit TIFF images for journal publication. Quantity One Basic can be loaded on an unlimited number of computers, allowing everyone in the laboratory free access to the basic functionality. The basic mode allows you to:

- Acquire and analyze images from all Bio-Rad imaging equipment
- Analyze TIFF images
- View and edit images using 3D Viewer, Crop, Transform, and Plot Density tools
- Add and edit text annotations
- Quantitate bands and objects
- Print and export images and reports



Quantity One Basic software can be installed from a CD-ROM (catalog #170-8605) or downloaded from our web site. For more information, visit us on the Web at **www.bio-rad.com/products/quantityonebasic/**

BioFrac™ Microplate Drophead

Expand your applications with the new microplate drophead for the BioFrac fraction collector. The BioFrac fraction collector, which delivers protein fractions accurately and consistently, now offers a choice of drop volume. Use the standard BioFrac fraction collector drophead for collection of large drops, or replace it with the optional BioFrac microplate drophead for precise collection of small volumes into microplates. With the microplate drophead, fine resolution of fractions can be obtained by a drop size of approximately 25 µl for aqueous solutions. The smaller drop size also improves the uniformity of filling from tube to tube during time- or volume-based collection.

For more information on racks available for the BioFrac fraction collector, visit us on the Web at **discover.bio-rad.com**

Ordering Information

Catalog #	Description
741-0088	BioFrac Microplate Drophead Kit
741-0002	BioFrac Fraction Collector, includes rack set F1 (2 x flatpack, for 12–13 mm tubes), BioFrac diverter valve, standard drophead, fittings kit, US power cord, instructions
741-0017	BioFrac Ice Bath/Microplate Rack
224-0096	Costar 96-Well Flat-Bottom Plates, polystyrene, 100

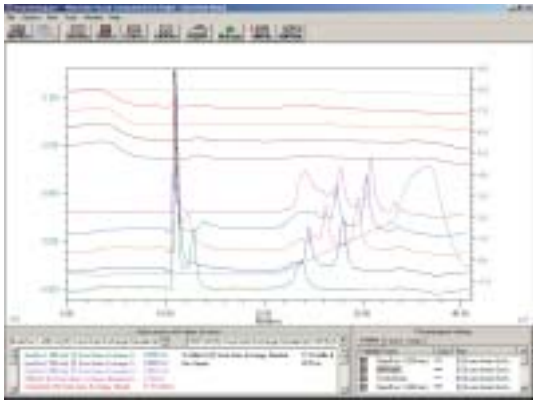
Costar is a trademark of Corning Costar Corporation.

BioLogic DuoFlow™ Version 5.0 Software

BioLogic DuoFlow version 5.0 software offers enhancements to the easy-to-use, intuitive software that has made the BioLogic DuoFlow system so popular with research chromatographers. New features that make the DuoFlow system even more powerful in applications include:

- **Scouting wizard** — Provides simplified setup of scouting experiments. Scouting optimizes purification of a target molecule by changing a variable incrementally with each scouting run
- **Method templates** — Allows easy method creation with predefined chromatography method templates for all commonly used chromatography experiments, including affinity, ion exchange, chromatofocusing, hydrophobic interaction, hydroxyapatite, and size exclusion (gel filtration) chromatography, as well as for more advanced experiments
- **Buffer editor** — Allows creation of user-defined buffer systems for buffer blending experiments with the BioLogic DuoFlow Maximizer™ system
- **Trace compare** — Provides enhanced features such as chromatogram trace shifting and volume-base chromatograms for even easier formatting and viewing of overlaid chromatograms
- **Fraction identification** — Provides BioFrac™ fraction collector numbering schemes that number tubes by collection order or by rack grid number. This is especially useful for microplate collection
- **Threshold collection** — Allows collection of fractions when a detector signal is below a defined threshold. This is particularly useful for desalting and chromatofocusing applications.

Any DuoFlow system already in use can be upgraded to version 5.0. Version 5.0 requires the Windows 2000 operating system. For more information, contact your local Bio-Rad sales representative or technical support.



BioLogic DuoFlow version 5.0 software provides new features such as automated pH scouting for increased performance and ease of use with the DuoFlow system.

Ordering Information

Catalog #	Description
760-2051	BioLogic DuoFlow Software Version 5.0 Upgrade, upgrades existing BioLogic DuoFlow version 4.0 systems, includes version 5.0 software CD, instructions
760-2052	BioLogic DuoFlow Software Version 5.0 and Windows 2000 Package, for new BioLogic DuoFlow users, includes version 5.0 software CD, Windows 2000 operating system, USB Bitbus communicator, instructions
760-2053	BioLogic DuoFlow Software Version 5.0 for Windows 2000 Computers, for new BioLogic DuoFlow users, includes version 5.0 software CD, USB Bitbus communicator, instructions

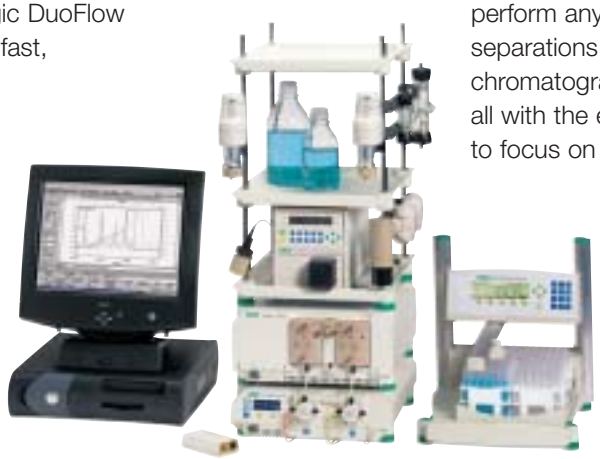
Windows is a trademark of Microsoft Corp.

BioLogic™
Chromatography Systems

Stepwise Solutions

Intuitive BioLogic DuoFlow™ software helps you set up any chromatography experiment with ease.

The BioLogic DuoFlow system is the easiest way to get great chromatographic separations. A big part of the system is BioLogic DuoFlow software, which quickly produces fast, meaningful results because the icon-based programming "thinks" like a chromatographer. The software leads you through the simple steps to create and run a method. Advanced features of this versatile software include method scouting and Trace Compare chromatogram display, which enhance your ability to optimize your experiments.



Together, the BioLogic DuoFlow hardware and software provide a system that can perform any protocol from simple isocratic separations to complex, multidimensional chromatography experiments — all with the ease of use that allows you to focus on results rather than the programming setup.

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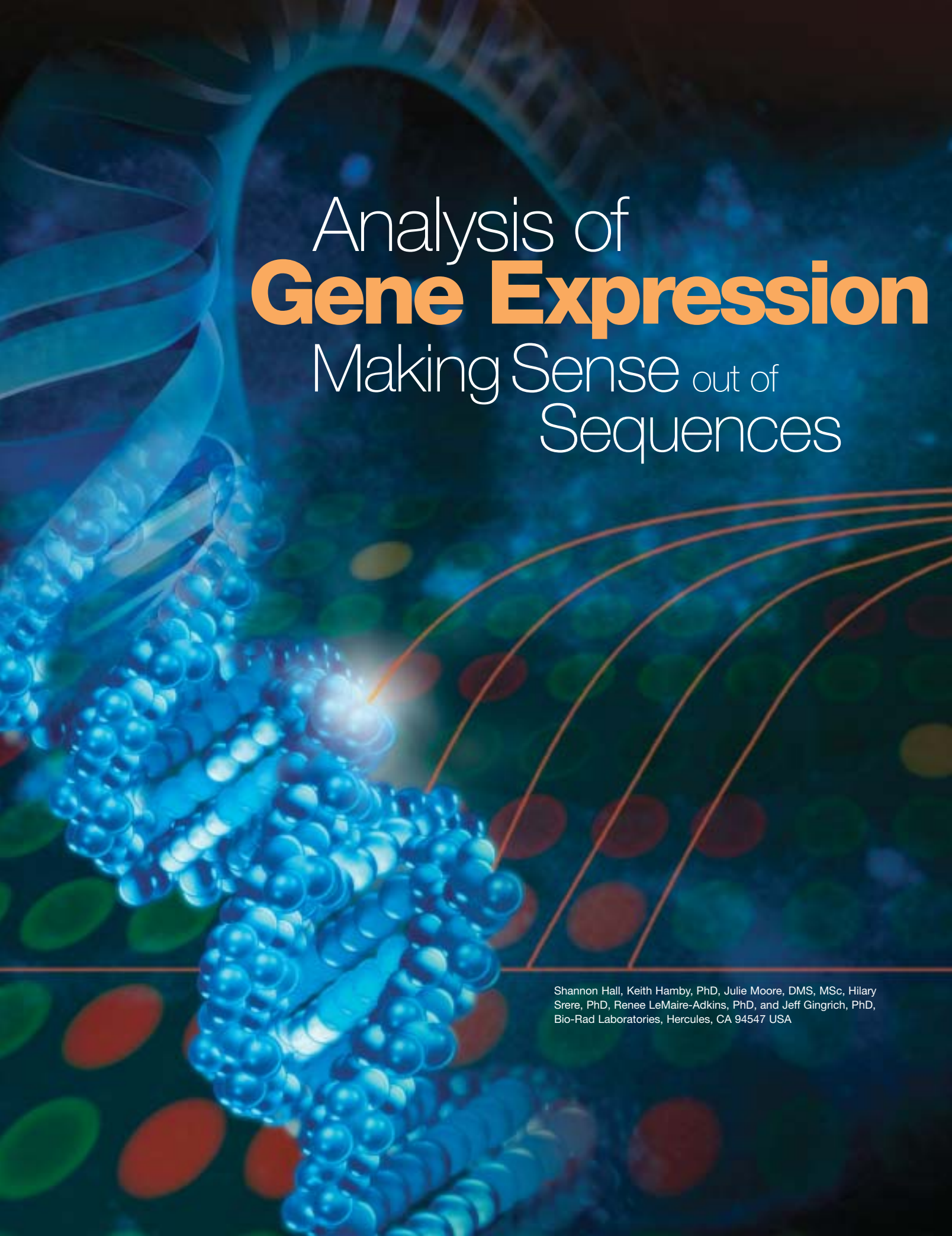


Analysis of **Gene Expression** Making Sense out of Sequences

Shannon Hall, Keith Hamby, PhD, Julie Moore, DMS, MSc, Hilary Srere, PhD, Renee LeMaire-Adkins, PhD, and Jeff Gingrich, PhD,
Bio-Rad Laboratories, Hercules, CA 94547 USA

The rapid progress in scientific research today is nowhere more evident than with the completion of the human genome project. In the 1980s and 1990s, building on molecular techniques developed in earlier decades (see timeline at right), more sophisticated techniques such as the polymerase chain reaction (PCR) and automated sequencing revolutionized the pace of progress, providing robust methods and more reliable results, and vastly increased the amount of genomic information available.

Today a similar revolution is occurring in the range of tools and techniques that can be applied to the study of gene expression. Gene expression analysis not only results in assigning known functions to genes but often identifies the concerted activities and interdependence of multiple genes in a single system. These analyses will help answer fundamental questions of biology, many with direct relevance to human health, nutrition, and aging.

Understanding gene expression, at any scale, relies on three interrelated approaches: 1) profiling expression, 2) quantitating expression, and 3) modifying expression (see illustration below). During the course of an experimental investigation, any or all of these approaches will be informative. Microarray analysis allows unprecedented profiling of thousands of genes simultaneously. For the first time, researchers can observe a range of genes in samples, enabling better insight into biochemical pathways and relationships between pathways. The most reliable technology for measuring gene expression is quantitative real-time PCR, which compares the absolute or relative levels of expression of two or more genes. Gene transfer techniques make possible the modification of gene expression via the introduction of foreign sequences that either supplement or disrupt endogenous genes' functions. Targeting gene function in situ reveals a significant amount of information about gene function in multiple tissues.

Here we provide a brief overview of these and related techniques useful for analysis of gene expression, including some observations on their application based on our 46 years of research and technical support experience.

Interrelated approaches used to study gene expression. Profiling, quantitation, and modification of gene expression give complementary information that can be integrated into a better understanding of the roles of a gene or sets of genes. Depending on the study organism and objectives, the experimental approach may begin with any of these stages and move to any of the other approaches.

General Considerations

RNA Isolation: The Foundation for Further Studies

Gene expression studies typically require RNA isolation, and all downstream experimentation is dependent on the quality of the RNA collected. The isolated RNA must be largely intact and free of contaminating genomic DNA to ensure accurate interpretation. Cell harvesting, reagent preparation, and sample storage can affect the final quality of the isolated RNA. Bio-Rad's Aurum™ total RNA kits isolate RNA of the highest integrity suitable for

Illustration by Audra Geras

Events in Gene Expression Research

1953 Watson and Crick propose model of DNA structure

1957 Founding of Bio-Rad Laboratories

1966 Nirenberg, Khorana decipher DNA triplet code

1971 Bio-Rad publishes first issue of BioRadiations

1973 First gene cloning experiments

1975 Southern develops blotting technique

1976 First reports of gene transfer by microinjection

1977 Gilbert and Sanger publish sequencing methods; first genome (bacteriophage ϕ*X174*) sequenced

1979 Bio-Rad introduces its first sequencing gel dryer

1984 Bio-Rad introduces the first commercial laser-scanning confocal microscope

1986 Bio-Rad introduces the first commercial electroporator

1986 Mullis develops PCR

1988 Bio-Rad receives patent for pulsed field electrophoresis

1988 Start of human genome project

1989 Bio-Rad receives patent for high-voltage transfection system

1992 Bio-Rad introduces biolistic particle delivery system

1995 Microarrays developed for analysis of gene expression

1995 Bio-Rad introduces its first denaturing gel electrophoresis system

1997 Genomes of *E. coli* and *S. cerevisiae* sequenced; Dolly the sheep cloned

1998 First reports of real-time multiplex PCR

1999 Bio-Rad introduces iCycler iQ™ real-time system

2000 Completion of draft human genome sequence

2002 Bio-Rad introduces XenoWorks™ microinjection system and VersArray™ microarray products

2002 Completion of draft mouse genome sequence

2003 Bio-Rad introduces iScript™ cDNA synthesis kit, Aurum™ RNA isolation kits, and MyiQ™ real-time system

2003 Completion of human genome project

The VersArray ChipWriter™ Pro system offers outstanding precision, productivity, and reliable operation.

The VersArray ChipReader™ system offers ADR technology for real-time monitoring and adjustment of dynamic range for optimal image acquisition.

demanding applications such as microarray analysis and quantitative real-time PCR. Generally, to continue with downstream studies, cDNA must be generated from the population of mRNAs. For applications intended to profile or quantitate expression, it is important that the cDNAs created represent the original mRNA population faithfully. The optimal reverse transcriptase for these applications should not demonstrate bias toward a particular sequence, nor toward genes that are present in abundance. Bio-Rad's iScript™ cDNA synthesis kit accurately represents full-length messages and low-abundance genes, ensuring reliability of data interpretation in downstream applications.

Profiling Gene Expression

Microarrays

Fundamental information on the temporal and spatial expression of a single gene is obtained by studying it individually, but ultimately researchers want to know how genes interact and fit into a complex system. Observation of only one or a few specific genes in isolation is usually insufficient to characterize a biochemical or physiological process. Microarrays enable simultaneous assessment of the expression patterns of hundreds or thousands of genes of interest (see sidebar, this page). With this revolutionary technology, researchers can visualize the relative expression of genes under different physiological conditions (e.g., healthy vs. diseased, or two different tissue types) on a large scale. One microarray experiment can reveal not only the relative expression levels of multiple genes within a physiological state, but also each gene's expression level in the two different conditions. Such differential expression patterns help elucidate associations between genes and biochemical pathways, disease states, or developmental states. Because microarray analysis can identify potentially relevant genetic markers based on their distinctive expression pattern in a disease state, microarrays currently play a significant lead identification role in drug discovery.

Serial Analysis of Gene Expression (SAGE)

SAGE is another profiling technique used to compare gene expression in different samples. The technique uses unique short sequences of DNA to capture and determine mRNA sequences within a sample. SAGE captures even low-abundance messages and is not dependent on knowledge of a particular message's sequence in advance. In this way, SAGE enhances the information available from microarray analysis. The number of times a sequence is represented in the final analysis can be compared to those for other sequences. By combining SAGE with microarray and quantitative real-time PCR analysis, researchers are obtaining a more comprehensive representation of expressed sequences.

Quantitating Gene Expression

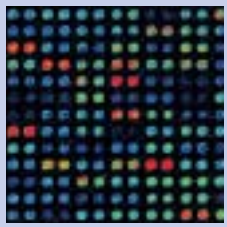
Quantitative Real-Time RT-PCR

While microarrays and SAGE identify target genes of interest among thousands of genes, truly quantitative information relies on real-time PCR. This technique provides more sensitive detection of low-abundance messages and a much wider dynamic range than microarrays for the simultaneous comparison of multiple, but not thousands of, messages (see sidebar, opposite page). Quantitative real-time PCR analysis may be performed on as little as 100 fg of total RNA, making it an attractive method of expression analysis when sample material is limited or total RNA recovered from the sample is small. It is often desirable to quantitatively assess a single sample for multiple genes, for example, genes that participate in the same biochemical pathway together with a housekeeping (reference) gene. It may be of interest to compare one or two genes to more than one housekeeping gene to increase the reliability of the quantitative estimate. Quantitative real-time PCR is also often used to compare gene expression in multiple tissues or in response to some stimulus. Coupled with a general interest in increasing throughput, it is clear that a system optimized for multiplexing the maximum targets for real-time PCR would be ideal for the quantitative aspect of gene expression.

Microarrays

Microarrays are ordered arrangements of hundreds to hundreds of thousands of oligonucleotide or cDNA targets immobilized on a solid support. The targets are generally spotted onto specially prepared glass slides (gene chips) or membranes, at a diameter of less than 200 µm. A pool of mRNA is converted to cDNA with a fluorescent label, and the cDNA pool is hybridized to the microarray. The level of fluorescent signal is representative of the relative abundance of the corresponding message in the sample. By hybridizing one microarray with differentially labeled cDNA from two different sources, the expression profiles of the two sources can be compared simultaneously. Microarrays of DNA sequences from many organisms are commercially available, but it is generally more cost-effective to spot a customized microarray if the number of targets in the array is less than 10,000. Important considerations for creating high-quality microarrrays are highly reproducible spot volume, high spot density, and extremely predictable placement of spots on the glass surface. These factors enable accurate interpretation of results by microarray image analysis software. Bio-Rad's line of microarrayers, the VersArray ChipWriter™ systems, meet the highest standards for spot density and reproducibility, with the added benefit of high throughput.

Recently, imaging of microarrays has been improving rapidly. Since genes are expressed at widely divergent levels, it is important to tune the imaging system to detect and accurately quantitate messages that are present at low levels as well as those that are more abundant. Therefore, in order to achieve the optimal image of the microarray, the dynamic range of the captured image must be considered, and multiple scans are sometimes required. Ideal features of a scanning system are optimization of laser light intensity to allow multiple scans without photobleaching, and software tools that match the dynamic range of the system to that of the array. These requirements are met with the VersArray ChipReader™ systems, featuring adaptable dynamic range (ADR) image optimization.



Modifying Gene Expression — Gene Transfer Techniques

From high-throughput techniques such as microarrays, or from more focused studies characterizing a smaller number of genes, researchers arrive at a list of genetic sequences that could be involved in a particular biological process. These sequences can then be introduced into cells, tissues, or organisms to further observe their regulation and function. The optimal method of DNA or RNA delivery depends on the system being studied, including the target cell or tissue type, and the specific objective of the study. The range of techniques available includes lipid-mediated transfection, viral delivery, electroporation, biolistic particle delivery, and microinjection.

Model Systems

Model systems are very powerful tools for the analysis of gene function. Mice are used frequently as a model system for human studies because the mouse genome is more than 95% homologous to that of the human. Long before genes were understood, geneticists used inbred mice to study inherited characteristics. Now, through the introduction of gene transfer technologies such as microinjection and supporting techniques (see sidebar, next page), exogenous human genes can be introduced into mice, and their effect on phenotype studied.

Change for Good: Benefits of Transgenics

Transgenic animals and plants are being developed using gene transfer technologies to provide benefits to society. Transgenic cows produce milk containing therapeutic human proteins, and transgenic pigs produce humanized organs for transplant.

In addition to animal systems, gene transfer is used to introduce or knock out genes in plants. Since the late 1980s, biolistic particle delivery devices such as Bio-Rad's PDS-1000/He™ system and the Helios® gene gun have become prevailing methods for modifying plant gene expression and producing stably transformed tissues and whole transgenic plants. Some examples of genetically modified plant products with commercial value are fruits modified to reduce spoilage and grains developed to be more nutritious and economical.

Downregulation of Gene Expression

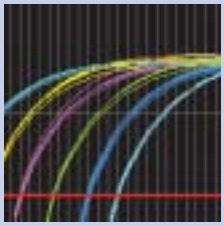
In many instances it is desirable to downregulate rather than completely knock out the expression of a gene. Some approaches for downregulation use RNA interference or anti-sense RNA, for example, to inhibit the function of mRNA. RNA with interfering or inhibitory roles can be delivered into cells in culture using lipid transfection or electroporation, and the effect on target gene expression can be assessed using, for example, quantitative PCR.

Quantitative Real-Time PCR

PCR revolutionized the study of individual gene sequences and genome organization, and now it is a central tool in gene expression studies in the form of quantitative PCR. This technique records the accumulation of PCR product during the progress of the reaction. PCR begins as an exponential process, with a theoretical doubling of template concentration in each cycle. As the reaction continues, however, the cycle-to-cycle accumulation of product becomes linear, and finally a plateau is reached and no more amplification occurs. Because the point at which the reaction ceases to be exponential is not predictable from reaction to reaction, end-point analysis does not permit accurate estimation of starting amounts of templates. Quantitative real-time PCR overcomes this limitation by evaluating product accumulation during the exponential phase of amplification and directly calculating template quantities.

Detection of the accumulation of product is dependent upon the inclusion of fluorescent dyes or a fluorescently labeled primer or probe. Fluorescent dyes such as SYBR Green I and ethidium bromide are easy and inexpensive detection methods; however, they lack the specificity of hybridization probes and do not allow multiplex analysis of a single sample. Fluorescently labeled oligonucleotides can be used as highly specific probes for amplified sequences. The variety of fluorophores available for labeling make multiplex analysis a popular method of evaluating relative gene expression.

Quantitative real-time PCR is commonly used to validate microarray results. SYBR Green I detection chemistry coupled with a thermal gradient enabled system allows fast optimization and accurate quantitation of a subset of genes identified in the initial microarray analysis. Bio-Rad's MyiQ™ real-time PCR detection system offers an affordable alternative for the detection of fluorescein and SYBR Green I reactions. This system is a perfect solution for those just getting started with this technology as well as those looking for additional instruments to handle increasing routine assay demands.



For many gene expression studies, it is advantageous to compare more than two genes or sources simultaneously. Bio-Rad's iCycler iQ™ system enables multiplexing of up to four gene targets in the same tube, greatly expanding possibilities for high-throughput analysis. In addition, multiplexing on the iCycler iQ system saves precious samples and ensures the most valid comparisons between two or more target genes.

Traditional Gene Expression Analysis Techniques

Gene expression studies often include traditional techniques together with the new technologies discussed earlier. These traditional techniques are based upon the same principles, such as the capture of multiple message sequences and the specificity of hybridization, that underlie the newer methods.

Northern blotting — Approaches such as northern blotting allow the comparison of mRNA expression levels under varying conditions. RNA is isolated, separated on a denaturing gel, transferred to a membrane, and detected with a labeled complementary DNA or RNA probe. In addition to the well-known challenges of working with northern blots (e.g., ensuring homogeneous and complete transfer of all mRNAs), there is also the challenge of being confident that the RNA population is accurately represented and detected. Whether the probe is radiolabeled or fluorescently labeled, it is important that the faintest bands as well as the strongest bands can be quantitated in a single image. Imaging methods that provide the greatest dynamic range (ability to detect very faint and very intense signals in a single exposure) for collecting data from northern blots include storage phosphor imaging systems, such as Bio-Rad's Molecular Imager FX™ Pro Plus and Personal Molecular Imager FX™

The iCycler iQ™ real-time PCR system allows multiplexing of up to 4 fluorophores and includes powerful software.

The MyiQ™ system is ideally suited for single-color real-time PCR applications such as SYBR Green I detection.

Microinjection Techniques in Animal Models

Animal models have contributed tremendously to the analysis of gene function. The introduction of microinjection technology in the late 1970s enabled scientists to create specific mutants of their own design. To create such designer mice, exogenous genes are microinjected into the pronucleus of a fertilized mouse egg. The foreign DNA is integrated randomly into the genome, and the first-generation progeny are screened for the presence of the gene of interest. The animals with the modified genome (knock-ins) are bred to produce offspring homozygous for the foreign DNA. The phenotype of the homozygotes is then studied to observe the effects of the exogenous gene.

Later, techniques were developed for targeted mutagenesis and gene replacement. Gene knock-outs are created by transfecting embryonic stem cells with an inactive version of the gene of interest (which undergoes homologous recombination with the endogenous gene) and then injecting those cells into blastocysts that are implanted in a female mouse. The first-generation animals are chimeras, mice possessing a mixture of knock-out and wild-type genotypes in the same animal. These chimeras are bred for progeny homozygous for the foreign gene. These homozygous knock-out animals are then used to study the phenotype associated with lack of the gene of interest. In recent years, improvements to knock-out techniques have enabled even more precise control over the changes that are made to the genome.

Knock-in and knock-out animals allow researchers to observe a complete living system in which specific changes have been made. Creation of these animal models often requires many hours working with microinjection instruments. Such devices must demonstrate great sensitivity of operation as well as features to allow comfortable working for such long periods. Bio-Rad's ergonomic XenoWorks™ system minimizes operator strain and allows fast throughput of modified blastocysts and other tissues. This versatile system is readily customizable to handle a wide variety of microinjection techniques.

The XenoWorks™ microinjection system offers an ergonomic, stable platform for accurate operation.

The CCD-based VersaDoc™ imaging system has excellent sensitivity for a broad range of sample types.

systems, as well as CCD imaging systems such as the VersaDoc™ imaging systems. Both types of imaging systems provide a lower limit of detection than the traditional method of using X-ray film.

In situ hybridization provides a great deal of information regarding the localization of a gene's expression. Expression level may be evaluated within a single cell or an entire tissue. This technique requires the rapid preservation of the cells or tissues to prevent RNA degradation. The specimens are dispersed or sectioned onto slides, and then hybridized with anti-sense RNA or oligonucleotide probes to detect mRNA. Hybridization results are best visualized with a laser-scanning confocal microscope. The ability to perform three-dimensional scans and virtual reconstruction of the cell or tissue into a single image provides important spatial information about localized gene expression. Bio-Rad's Radiance2100™ MP multi-photon confocal imaging systems incorporate cutting-edge optical features to achieve maximal resolution.

Differential display is a common approach to analyzing expression patterns of populations of genes. Samples are amplified by PCR using an anchored primer to reverse transcribe a subset of mRNA and arbitrary-sequence primers to amplify the cDNA. The use of multiple primer combinations ensures a statistically valid representation of all genes expressed in a sample. Like SAGE, this technique allows representation of sequences not defined a priori. The PCR products are labeled with either a radiolabeled nucleotide or a fluorescently labeled primer. The labeled products are separated on a high-resolution polyacrylamide gel and imaged. The results are evaluated for the presence, absence, or relative intensities of bands produced from the different samples. Products that are differentially expressed are potentially transcripts of interest. Results that are reproducible (i.e., amplified consistently in different PCR tubes) are most reliable. The final step is to determine the sequence of a band of interest. One advantage of differential display is that this method detects both abundant and rare mRNAs. Visualization of differential display products requires the flexibility to image either radiolabeled or fluorescently labeled samples, and the dynamic range to detect faint and intense bands in a given gel. Imaging methods that ensure great flexibility and a great limit of detection are CCD- and laser-based systems such as the VersaDoc, ChemiDoc™ XRS, and Personal Molecular Imager FX™ systems.

Keeping Pace With Your Research

Gene expression analysis has never been more dynamic and informative than in today's research environment. The techniques presented here provide a potential toolbox for devising gene expression analysis strategies. For decades, Bio-Rad has worked to develop better products together with technical information for gene expression and protein analyses. This drive to provide the best has resulted in superior devices for pulsed field gel electrophoresis, electroporation, particle delivery, real-time PCR, and microarray and image analysis, together with reagent kits for sample purification and amplification that deliver consistently excellent results. Our product lines provide you with the comprehensive application solutions you need to achieve your research goals.

Where Can You Get More Information About Current Topics in Gene Expression?

NCBI Gene Expression Omnibus Home Page
http://www.ncbi.nlm.nih.gov/geo/

Standardization and Normalization of Microarray Data
http://www.mged.org/
http://www.ifti.org/

Discussions of Real-Time PCR Results
http://www.wzw.tum.de/gene-quantification/
http://allserv.rug.ac.be/~jvdesomp/genorm/
http://www.tataa.com

To Learn More About...
SAGE: http://www.embl-heidelberg.de/info/sage/

RNA interference: http://www.nature.com/nrg/journal/v2/n2/animation/

Selected Bio-Rad Products and Literature for Gene Expression Studies

For more information, visit us on the Web at **discover.bio-rad.com**; for information on laser-scanning microscopy systems, visit **cellscience.bio-rad.com**

Catalog/Bulletin #	Product/Title

Nucleic Acid Sample Preparation

732-6800	Aurum™ Total RNA 96 Kit, 2 x 96 well preps
732-6820	Aurum Total RNA Mini Kit, 50 preps
732-6400	Aurum Plasmid Mini Kit, 100 preps
732-6460	Aurum Plasmid 96 Kit, 2 x 96 well preps
732-6340	AquaPure™ Genomic DNA Isolation Kit, 100 preps
732-6343	AquaPure Genomic DNA Tissue Kit, 100 preps
732-6345	AquaPure Genomic DNA Blood Kit, 100 preps

Microarray Equipment for Genomics Applications

169-0001	VersArray ChipReader™ 10 µm System, 100/240 V
169-0002	VersArray ChipReader 5 µm System, 100/240 V
169-0003	VersArray ChipReader 3 µm System, 100/240 V
169-0004	VersArray ChipWriter™ Compact System, 110/120 V
169-0005	VersArray ChipWriter Compact System, 220/240 V
169-0006	VersArray ChipWriter™ Pro System, 110/120 V
169-0007	VersArray ChipWriter Pro System With Picking, 110/120 V
169-0008	VersArray ChipWriter Pro System, 220/240 V
169-0009	VersArray ChipWriter Pro System With Picking, 220/240 V
169-0010	VersArray™ Colony Arrayer System, 110/120 V
169-0011	VersArray Colony Picker and Arrayer System, 110/120 V
169-0012	VersArray Colony Arrayer System, 220/240 V
169-0013	VersArray Colony Picker and Arrayer System, 220/240 V
170-7430	VersArray Analyzer Software

Amplification and Real-Time PCR

170-8720	iCycler™ Thermal Cycler With 96-Well Reaction Module
170-8722	iCycler Thermal Cycler With 2 x 48-Well Reaction Module
170-8724	iCycler Thermal Cycler With 60 x 0.5 ml Reaction Module
170-8726	iCycler Thermal Cycler With 384-Well Reaction Module
170-9703	MyCycler™ Thermal Cycler
170-8740	iCycler iQ™ Multi-Color Real-Time PCR Detection System
170-9740	MyiQ™ Single-Color Real-Time PCR Detection System
170-8870	iTaq™ DNA Polymerase
170-8860	iQ™ Supermix, 100 x 50 µl reactions
170-8862	iQ Supermix, 500 x 50 µl reactions
170-8864	iQ Supermix, 1,000 x 50 µl reactions
170-8880	iQ™ SYBR® Green Supermix, 100 x 50 µl reactions
170-8882	iQ SYBR Green Supermix, 500 x 50 µl reactions
170-8884	iQ SYBR Green Supermix, 1,000 x 50 µl reactions
170-8890	iScript™ cDNA Synthesis Kit, 25 x 20 µl reactions
170-8891	iScript cDNA Synthesis Kit, 100 x 20 µl reactions

Related Literature

2955	Four-Color Multiplex PCR Assay for Allelic Variants
2806	Quantitation of Lymphangiogenesis Using iCycler iQ System and Scorpions Detection
2805	Real-Time Immuno-PCR on the iCycler iQ System
2593	Real-Time PCR: General Considerations
2568	The iCycler iQ Detection System for TaqMan Assays
2804	iCycler iQ System for Evaluating Reference Gene Expression

Other Nucleic Acid Quantitation Instruments

170-9500	Ultramark™ Microplate System
170-2525	SmartSpec™ Plus Spectrophotometer
170-2402	VersaFluor™ Fluorometer

Gene Transfer

Biolistics

165-2431	Helios® Gene Gun System, 100/120V
165-2432	Helios Gene Gun System, 220/240V
165-2257	PDS-1000/He™ System
165-2258	PDS-1000/He Hepta™ System

Related Literature

2658	Single-Cell Complementation of Barley *mlo* Mutants
2768	Biolistic Gene Transfer to Generate Transgenic Schistosomes
2726	Delivery of pCMV-S DNA in Balb/c Mice
2410	Detection of Reporter Gene After Particle Bombardment

Electroporation

165-2660	Gene Pulser Xcell™ Total System
165-2661	Gene Pulser Xcell Eukaryotic System
165-2662	Gene Pulser Xcell Microbial System
165-2100	MicroPulser™ Electroporator

Catalog/Bulletin #	Product/Title

Gene Transfer (cont.)

Electroporation

165-2086	Gene Pulser® Cuvette, 0.2 cm gap, 50
165-2088	Gene Pulser Cuvette, 0.4 cm gap, 500
165-2089	Gene Pulser Cuvette, 0.1 cm gap, 500
170-3330	EP-Max™10B Electro-Competent Cells, 5 x 0.1 ml
170-3331	EP-Max10B F' Electro-Competent Cells, 5 x 0.1 ml

Related Literature

1029735	Electroprotocols Online (only available online)

Chemically Competent Cells

170-3340	C-Max™5α Chemi-Competent Cells, 5 x 0.2 ml
170-3341	C-Max5α F' Chemi-Competent Cells, 5 x 0.2 ml
170-3342	C-Max5α Chemi-Competent Cells, 10 x 0.05 ml
170-3343	C-Max5α F' Chemi-Competent Cells, 10 x 0.05 ml

Lipid Transfection

170-3250	Cytofectene™ Transfection Reagent, 1.0 ml
170-3251	Cytofectene Transfection Reagent, 5 x 1.0 ml
170-3252	Cytofectene Transfection Reagent, 0.2 ml

Related Literature

2532	Efficient RNA Transfection of *D. melanogaster*
2606	Transfection of Fibroblast, Epithelial, and Lymphoid Cells

Microinjection

165-2801	XenoWorks™ Micromanipulator (Left), 110/240 V, 50/60 Hz
165-2802	XenoWorks Micromanipulator (Right), 110/240 V, 50/60 Hz
165-2805	XenoWorks Digital Microinjector, 110 V, 60 Hz
165-2806	XenoWorks Digital Microinjector, 240 V, 50 Hz
165-2808	XenoWorks Analog Microinjector, 500 µl

Imaging Products

Gel Documentation Systems

170-8100	Gel Doc™ 2000 System, PC, 110 V
170-8101	Gel Doc 2000 System, PC, 220 V
170-8615	Gel Doc 2000 System, Mac, 110 V
170-8616	Gel Doc 2000 System, Mac, 220 V
170-8102	ChemiDoc™ System, PC, 110 V
170-8103	ChemiDoc System, PC, 220 V
170-8616	ChemiDoc System, Mac, 110 V
170-8617	ChemiDoc System, Mac, 220 V
170-8070	ChemiDoc™ XRS System, PC
170-8071	ChemiDoc XRS System, Mac

CCD-Based Multiimaging Systems

170-8010	VersaDoc™ Model 1000 Imaging System, PC
170-8011	VersaDoc Model 1000 Imaging System, Mac
170-8030	VersaDoc Model 3000 Imaging System, PC
170-8031	VersaDoc Model 3000 Imaging System, Mac
170-8050	VersaDoc Model 5000 Imaging System, PC
170-8051	VersaDoc Model 5000 Imaging System, Mac

Laser-Based Systems

170-7800	Molecular Imager FX™ System, PC
170-7801	Molecular Imager FX System, Mac
170-7856	Molecular Imager FX™ Pro Fluorescent Imager, PC
170-7857	Molecular Imager FX Pro Fluorescent Imager, Mac
170-7850	Molecular Imager FX™ Pro Plus MultiImager, PC
170-7851	Molecular Imager FX Pro Plus MultiImager, Mac
170-9400	Personal Molecular Imager FX™ System, PC
170-9401	Personal Molecular Imager FX System, Mac

Related Literature

2597	Three-Color Imaging With Molecular Imager FX Pro Plus System and Quantity One Software
2429-03	Imaging Differential Display Using Molecular Imager FX
2429-05	Nucleic Acid Gel Analysis on Molecular Imager FX

Laser-Scanning Microscopes

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Related Literature (Online at cellscience.bio-rad.com)

9MRC10AN08	Non-Isotopic in Situ Hybridisation
T MRC AN001	Three Colour Confocal Imaging
T MIS AN009	Non-Fading Confocal Fluorescence Imaging for in Situ Hybridization and Immunocytochemistry

Simultaneous Detection of Multiple Transcription Factors in Hematopoietic Progenitors Using iCycler iQ™ Multiplex Real-Time PCR

Hao Wang, Huijuan Zhou, Xiangyi Zhao, and R Michael Wolcott, Department of Microbiology and Immunology, Center for Excellence in Arthritis and Rheumatology, Louisiana State University Health Science Center, Shreveport, LA 71130 USA

Introduction

The development of committed B cells from multipotent progenitors requires the coordinated activity of several transcription factors (TF). Not only is the temporal pattern of expression of TFs important, but also a number of studies have confirmed that the level of expression, as well, is important in the cell fate decisions of the developing progenitors (Kee and Murre 2001). Semi-quantitative analysis of TF gene expression by traditional reverse transcription polymerase chain reaction (RT-PCR) methods, while widely used, often lacks sufficient sensitivity to discern small differences in mRNA levels. The introduction of real-time RT-PCR provided a method for quantitatively measuring small differences in mRNA expression (Boeckman et al. 2001). However, particularly with small samples, the ability to assess the relative levels of multiple TFs is limited.

Multiplex real-time PCR, which can measure up to four target cDNAs simultaneously in a single tube, provides a method for comparing relative TF gene expression levels based on one sample of mRNA. Currently, a number of different fluorophores are available for real-time PCR, and color multiplexing is possible with oligonucleotide probes labeled with different fluorophores. Here we describe the development of a multiplex four-color real-time PCR method to detect the TFs EBF, E2A, and PU.1 using the iCycler iQ system.

Methods

cDNA Preparation

IgM$^-$B220$^+$ cells and Mac-1$^+$ cells were harvested from bone marrow and spleens of 8-week-old male C57Bl/6J mice by magnetic cell sorting with indirect MicroBeads (Miltenyi Biotec, Auburn, CA). RNA was prepared using an RNA extraction kit (QIAGEN, Valencia, CA), and cDNA was then generated using the ThermoScript RT-PCR kit (Invitrogen Life Technologies, Rockville, MD), both according to the manufacturers' instructions.

Multiplex Real-Time PCR

External well factors were collected before each run using iCycler iQ external well factor solution (Bio-Rad). Collection of well factor data optimizes fluorescent data quality and analysis in multiplex PCR.

Reactions for multiplex real-time RT-PCR contained primers and probes for the three TF cDNAs, as well as for GAPDH cDNA, which served as an internal standard for calculation of relative expression levels of the TFs. Reactions were prepared for each cDNA sample as follows:

12.5 µl iQ™ supermix (Bio-Rad)
1.0 µl 50 mM $MgCl_2$
0.5 µl 5 U/µl iTaq™ DNA polymerase (Bio-Rad)
2.5 µl 10 mM dNTP mix
2 µl cDNA template
1 µl PCR-grade ddH_2O
0.5 µl 10 µM GAPDH forward primer (5'-CCCCAATGTGT-CCGTCGTG-3')
0.5 µl 10 µM GAPDH reverse primer (5'-GCCTGCTTCAC-CACCTTCT-3')
0.25 µl 10 µM GAPDH probe (5'-HEX-CGTGCCGCCTGG-AGAAACCTGC-BHQ-1-3')
0.5 µl 10 µM EBF forward primer (5'-CTTGCTAACACTTC-GGTCCAT-3')
0.5 µl 10 µM EBF reverse primer (5'-ACCTTGATTGGTGG-CTTGTG-3')
0.25 µl 10 µM EBF probe (5'-6-FAM-TGCCTCCGAGACA-TTCACAGCCAG-BHQ-1-3')
0.5 µl 10 µM E2A forward primer (5'-CCCGGATCACTCC-AGCAATAA-3')
0.5 µl 10 µM E2A reverse primer (5'-TGGAGACCTGCAT-CGTAGTTG-3')
0.5 µl 10 µM E2A probe (5'-BHQ-3-TCTCACCTAGCCC-CTCAACGCCT-Cy5-3')
0.5 µl 10 µM PU.1 forward primer (5'-GGGCATCCAGAA-GGGCAA-3')
0.5 µl 10 µM PU.1 reverse primer (5'-GGTAGGTGAGCTT-CTTCTTGAC-3')
0.5 µl 10 µM PU.1 probe (5'-Texas Red-TCTTCACCTCGC-CTGTCTTGCCGT-BHQ-2-3')

Duplicate 25 µl samples of each reaction were added to individual wells of a 96-well thin-wall PCR plate. PCR conditions were 3 min at 95°C followed by 60 cycles of 10 sec at 95°C and 60 sec at 55°C.

Results

Expression of three TFs (EBF, E2A, PU.1) in IgM$^-$B220$^+$ and Mac-1$^+$ cells from both mouse bone marrow and spleen (see figure) was detected using multiplex real-time PCR. The relative amount of each TF cDNA was calculated by determining its ΔC_T value: ΔC_T = (threshold cycle for the TF) – (threshold cycle for GAPDH); i.e., the number of additional cycles required for a cDNA to reach the threshold value after the GAPDH threshold cycle. A ΔC_T value of 1.0 represents a 2-fold lower expression relative to GAPDH. The table shows the ΔC_T value of TFs in each group of cells. The results of these analyses show that multiplex PCR can be used to determine the relative expression of multiple genes in a single sample.

Table. ΔC_T value of TFs in different groups of cells.

Sample	EBF	E2A	PU.1
Bone marrow IgM$^-$B220$^+$ cells	7.1	13.4	7.4
Bone marrow Mac-1$^+$ cells	6.4	11.9	6.6
Spleen IgM$^-$B220$^+$ cells	5.2	11.8	7.3

Discussion

The data in the figure show that the iCycler iQ system can be used to quantitate cDNAs simultaneously even though their relative expression levels differ by as much as 2^8, as indicated by the difference between the highest and lowest ΔC_T values shown in the table. Points that are critical for successful analysis of multiple cDNAs include: 1) increasing the concentration of DNA polymerase, dNTPs, and $MgCl_2$; 2) adjusting the primer/probe concentrations in order to yield C_T values of the target genes that are not significantly different from the value calculated in a single-gene real-time PCR reaction (data not shown); 3) testing primer sets with SYBR Green I to generate a melting curve to detect primer-dimers before use in multiplex real-time PCR; 4) titrating the probe and generating a standard curve to ensure that the efficiency of the reaction with the probe is >90%.

References

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Kee BL and Murre C, Transcription factor regulation of B lineage commitment, Curr Opin Immunol 13, 180–185 (2001)

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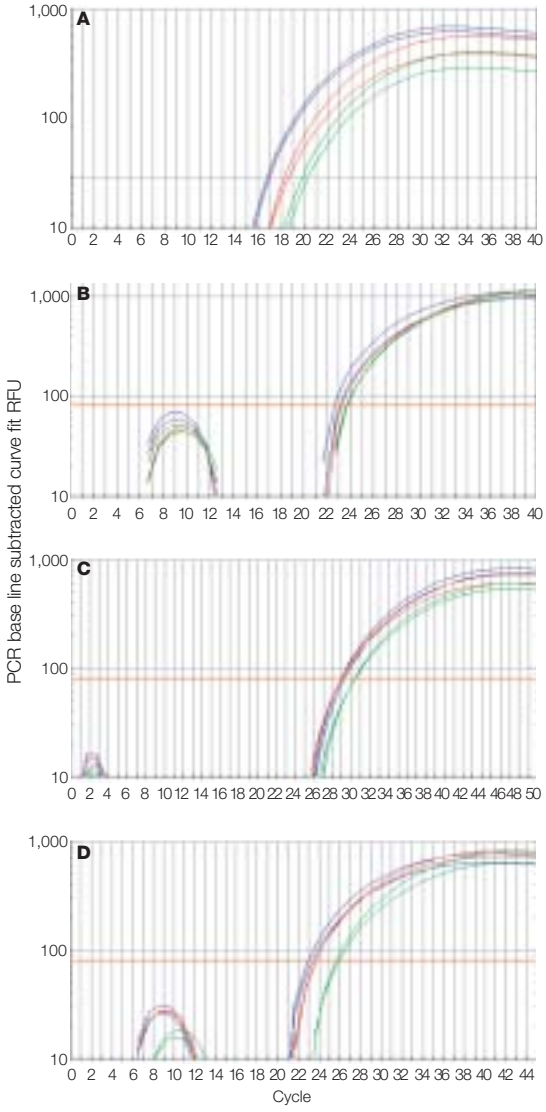


Figure. Multiplex real-time PCR of TF cDNAs. **A**, GAPDH; **B**, EBF; **C**, E2A; **D**, PU.1. Blue, red, and green traces represent cDNA derived from bone marrow IgM$^-$B220$^+$ cells, bone marrow Mac-1$^+$ cells, and spleen IgM–B220$^+$ cells, respectively. Threshold and C_T values were automatically calculated for each sample. TF probes were titrated and standard curves were generated. The slopes and corresponding efficiency for these reactions were –3.51 (93%), –3.35 (98%), –3.22 (~100%), and –3.50 (93%) for GAPDH, EBF, E2A, and PU.1 cDNA, respectively.

Rapid, Reproducible Real-Time Quantitative RT-PCR Using the iCycler iQ™ Real-Time PCR Detection System and iQ™ Supermix

Jessica N Ebright[1] and Catherine Bowes Rickman[1, 2], [1]Departments of Ophthalmology and [2]Cell Biology, Duke University Medical Center, Durham, NC 27710 USA; Correspondence: Catherine Bowes Rickman, PhD, Department of Ophthalmology, Duke University Medical Center, Box 3802, Durham, NC 27710, Phone (919) 668-0648, Fax (919) 684-3687, E-mail: bowes007@duke.edu

Introduction

We are interested in identifying genes that are differentially expressed within the central (macular) region of the human retina. Expression profiles of thousands of genes from this small, highly specialized region of the central nervous system were obtained by comparative screening of human cDNA microarrays with human macula- and mid-peripheral retina (periphery)-derived RNAs. In order to validate the macula-enriched expression of genes identified by our array analysis, we must rely on a PCR-based method of quantitation. Real-time RT-PCR quantitates the initial amount of a template with more specificity, sensitivity, and reproducibility than any other method. There are many factors that contribute to the consistent performance of a real-time quantitative RT-PCR assay, and many aspects that must be optimized when putting this powerful technology to work in a new experimental system.

Methods

Total RNA was isolated from 4 mm trephine punches of neural retina from two areas, the macula and the mid-periphery, using Trizol reagent (Invitrogen) with glycogen added as a carrier as described by Bracete et al. (1999) with the following modification: 0.9 ml Trizol reagent plus 13.5 µl glycogen (20 mg/ml, Roche Molecular Biochemicals) was added to flash-frozen tissue in a 1 ml microcentrifuge tube and vigorously homogenized for 30 sec using an Ultraturrax T8 homogenizer (Ika Laboratories). Total RNA was DNase-treated using DNA-*free* reagent (Ambion), and RNA yields were determined by fluorescence at 530 nm using RiboGreen RNA quantitation reagent (Molecular Probes) as described by the manufacturer. First-strand cDNAs were synthesized from equal amounts of total RNA (1 µg/reaction) using oligo(dT) primers and SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions.

Gene-specific primers (GSPs) were designed to anneal near the 3' end of two mRNA transcripts and to generate PCR products 75–300 base pairs long. Three GSP pairs amplify different overlapping regions of a single transcript that is enriched in the macula, while one GSP pair detects a human housekeeping gene transcript, β-actin (*ACTB*), which is constitutively expressed in the neural retina. The amplified regions spanned exon-exon junctions when possible. All primers were purchased from Proligo. RT-PCR was performed using the GSP pairs in reactions amplifying across a gradient of annealing temperatures to identify optimal reaction conditions for real-time RT-PCR, and PCR product lengths were verified on a 4.5% Super AcrylAgarose gel (DNA Technologies). Real-time quantitative RT-PCR was performed using an iCycler iQ system (Bio-Rad). The rate of accumulation of amplified DNA was measured by continuous monitoring of SYBR Green I (Molecular Probes) fluorescence. Melt curves of the reaction products were generated, and fluorescence data were collected at a temperature above the melting temperature of nonspecific products (Morrison et al. 1998).

Specifically, quantitative real-time RT-PCR on the iCycler iQ was performed in duplicate or triplicate on 1 µl of template cDNA per 20 µl reaction. Mix A reactions consisted of PCR buffer (16.6 mM $(NH_4)_2SO_4$, 67 mM Tris, pH 8.8, 6.7 mM $MgCl_2$, 10 mM β-mercaptoethanol; Loging et al. 2000), 1 mM dNTPs (Invitrogen), 0.5 U of Platinum *Taq* DNA polymerase (Invitrogen), 10 nM fluorescein calibration dye (Bio-Rad), 1 µl of a 1:1,500 dilution of 10,000x SYBR Green I stock, 500 nM of each GSP, and 1 µl of cDNA. iQ supermix reactions consisted of iQ supermix (Bio-Rad) at a final concentration of 1x, 10 nM fluorescein calibration dye, 1 µl of a 1:1,500 dilution of 10,000x SYBR Green I stock, 500 nM of each GSP, and 1 µl of cDNA. To control for pipetting losses, 19 µl of each 20 µl reaction was amplified in a 96-well thin-wall PCR plate (Bio-Rad) using the following PCR parameters: 95°C for 2 min followed by 50 cycles of 95°C for 15 sec, 60°C for 15 sec, and 72°C for 15 sec. Melt-curve analysis was performed immediately following amplification by increasing the temperature in 0.4°C increments starting at 65°C for 85 cycles of 10 sec each. The presence of a single PCR product was verified both by the presence of a single melting temperature peak representing a specific product (vs. a nonspecific

primer-dimer peak) using iCycler iQ analysis software and by detection of a single band of the expected size on a 4.5% Super AcrylAgarose gel.

Real-time RT-PCR was performed in duplicate or triplicate reactions. Each GSP pair was used with each reaction mix on each of the two different cDNA templates (derived from macula or periphery). Real-time RT-PCR reactions for detection of the endogenous control gene, *ACTB*, were always run in parallel for each cDNA template in each experimental run as a reference for accuracy of sample dilution (even if not shown in figure).

Results and Discussion

Experiment 1: Performance Over Time of Mix A Protocol Optimized for Real-Time RT-PCR

Reactions were carried out using the GSPs that amplified a 128 bp fragment of the macula-enriched transcript of interest. The amplification curve for the macula-derived sample crossed a threshold of 100 relative fluorescence units (RFU) after 21.5 cycles, and the periphery-derived sample crossed this threshold 1.7 cycles later at 23.2 cycles. These results confirmed that the transcript of interest was, indeed, enriched in the macula compared to the rest of the retina (Figure 1A). When the experiment was repeated one month later using the same reaction components, only the *ACTB*-derived PCR products were generated; none of the 128 bp target was detected (data not shown). The same set of real-time RT-PCR reactions was prepared again with previously unopened aliquots of each reagent stored at –20°C in a constant-temperature freezer. Again, only the *ACTB*-derived transcripts were amplified (Figure 1B). Traces for late amplifications (C_T > 34) of the 128 bp primer set represent primer-dimers and not specific product, as determined by melt-curve and gel analysis (not shown). These results showed that failure to amplify was not due to freeze-thaw induced deterioration of the stock reagents over time and suggested that some component of the stock reagents was unstable over time, even at –20°C.

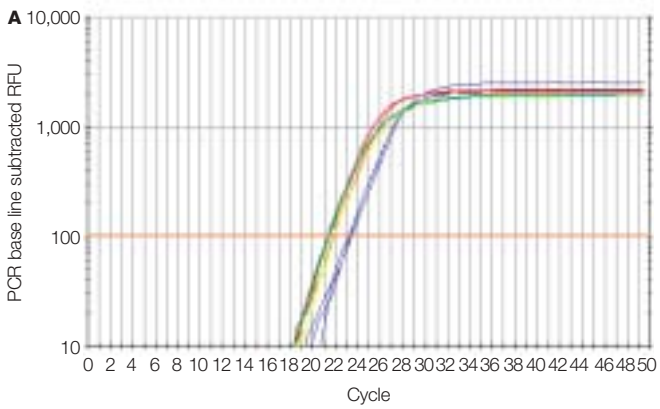
Experiment 2: Comparison of Reactions Based on iQ Supermix vs. Mix A

Use of iQ supermix rescued the assay, resulting in accumulation of the macula-derived 128 bp products crossing the threshold of 100 RFU after 19.7 cycles (Figure 2) — almost 2 cycles earlier than in the mix A-based reactions with macula cDNAs for template (Figure 1). In the iQ supermix reactions containing periphery-derived cDNAs, the 128 bp product crossed this threshold value at 21.2 cycles (Figure 2), 1.5 cycles later than the macula reactions with the iQ supermix and almost 2 cycles earlier than with the mix A periphery reactions in experiment 1 (Figure 1). Equivalent mix A reactions run at the same time failed to amplify (Figure 2). As shown in the following experiment, the reproducibility of these results as well as the stability of the iQ supermix reagents held up over time.

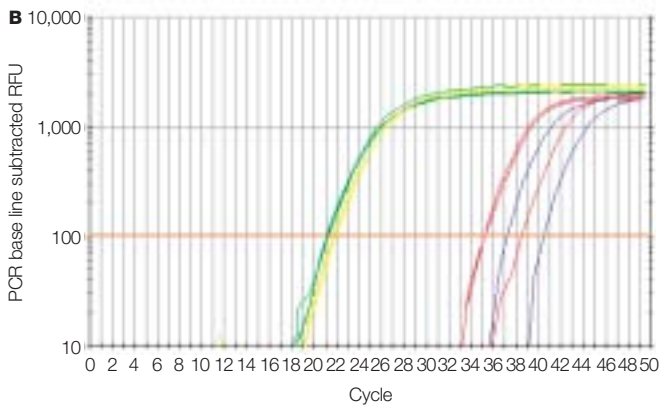
Experiment 3: Performance of iQ Supermix Reactions Over Time

Real-time RT-PCR was performed as described for experiment 2, except that the iQ supermix (2x) stock used in these reactions had been stored for 4 months at –20°C. The 128 bp segment of the macula-

Fig. 1. Real-time amplification of a 128 bp fragment of a macula-enriched target transcript and a 94 bp fragment of a constitutively expressed housekeeping gene (ACTB); mix A reactions containing either macula-derived or periphery-derived cDNA templates. **A**, traces representing amplification of the first generation of mix A reactions; **B**, traces representing amplification of the same target templates in mix A reactions prepared from reagent stocks that had been stored at –20°C for 1 month and freshly thawed. C_T, threshold cycle.



Trace	Identifier	C_T
	128 bp/macula	21.4
	128 bp/macula	21.2
	128 bp/macula	21.8
	128 bp/periphery	23.2
	128 bp/periphery	23.0
	128 bp/periphery	23.4
	ACTB/macula	21.4
	ACTB/macula	21.2
	ACTB/periphery	21.8
	ACTB/periphery	21.8



Trace	Identifier	C_T
	128 bp/macula	35
	128 bp/macula	38.5
	128 bp/macula	35.1
	128 bp/periphery	N/A
	128 bp/periphery	40.4
	128 bp/periphery	37.1
	ACTB/macula	21.0
	ACTB/macula	21.1
	ACTB/macula	21.2
	ACTB/periphery	21.6
	ACTB/periphery	21.8
	ACTB/periphery	21.7

enriched transcript was again successfully amplified. The amplification curve for the macula sample crossed the threshold of 100 RFU after 20.1 cycles, while the periphery sample crossed the threshold 1.7 cycles later at 21.8 (Figure 3). The iQ supermix is therefore more stable over time than mix A.

Experiment 4: Amplification of a Specific Transcript Using Three Different Pairs of GSPs With iQ Supermix vs. Mix A

In order to test whether primer design can affect the reproducibility of amplification curves obtained for a specific transcript in a specific tissue, real-time RT-PCR was performed using three pairs of primers designed to amplify different regions of the same target transcript. The three GSP pairs generate 128 bp, 100 bp, and 99 bp products. Duplicate reactions using each primer pair with each reaction mix (A or iQ supermix) were run for each template. The performance of the iQ supermix reactions was quite consistent for each primer pair (Figure 4A), whereas the performance of the mix A-based reactions varied for each GSP (Figure 4B).

In the iQ supermix reactions, the average C_T for all six traces representing the amplification of the macula sample with three different primer pairs was 19.9 ± 0.3 cycles. The average C_T for the periphery-derived sample was 21.4 ± 0.2 cycles, resulting in an average of a 1.5 cycle difference between the two regions of human neural retina (Figure 4A). This differential expression profile for the macula-enriched gene transcript was the same as that obtained in the two previous experiments with iQ supermix reactions (Figures 2 and 3).

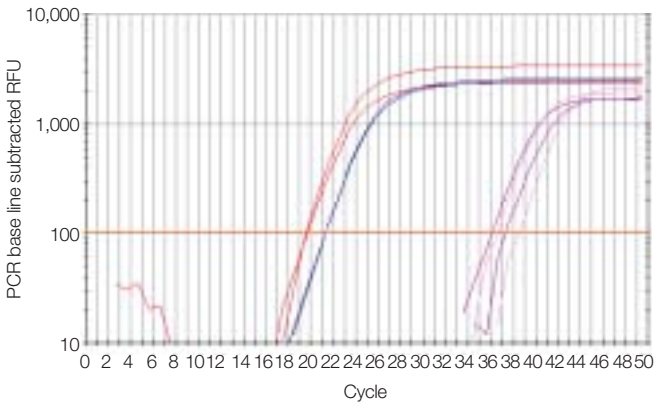
The traces representing the accumulation of PCR products in the mix A-based reactions varied with each GSP (Figure 4B), in contrast to the traces for the iQ supermix reactions (Figure 4A). In the mix A-based reactions, the 128 bp fragment was not amplified at all, whereas the products generated by the other two primer pairs were amplified at different rates. The amplification curves for the 100 bp fragment crossed threshold fluorescence at 25.4 cycles in the macula reactions and 25.8 in the periphery, while the curves for the 99 bp fragment from closer to the 3' end of the target mRNA crossed the threshold at 21.5 cycles in the macula reactions and 23.1 in the periphery. The average C_T values for the two GSP pairs that resulted in the expected-sized PCR products were then 23.4 ± 1.9 for the macula and 24.4 ± 1.3 for the periphery, for an average 1.0 cycle difference (Figure 4B). Clearly, the real-time RT-PCR results generated using the iQ supermix were more reliable and reproducible.

Conclusions

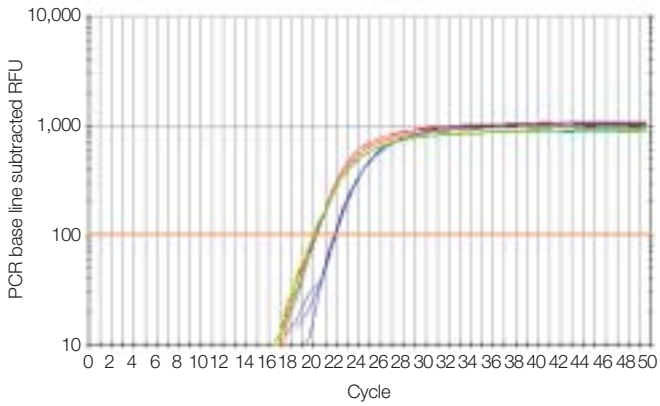
Although quantitative real-time RT-PCR is a powerful, sensitive, and reproducible method to quantitate differences in mRNA expression, many aspects of the reactions (i.e., primer design, annealing temperatures, and master mix reagents) must be optimized to put this powerful technology to work successfully in a new experimental system. Real-time RT-PCR is especially sensitive to product length, where longer length products and low- to medium-abundance transcripts cannot be amplified in reactions containing unstable reagents. While Bio-Rad's bulletin 2593 (Boeckman et al. 2001) for

Fig. 2. Amplification of a 128 bp macula-enriched target in mix A and iQ supermix reactions using cDNA templates derived from two areas of neural retina.

Fig. 3. Amplification of a 128 bp macula-enriched target alongside the constitutively expressed *ACTB* with two cDNA templates using iQ supermix after 4 months of storage at –20°C.



Trace	Identifier	C_T
	128 bp/macula (iQ supermix)	19.7
	128 bp/macula (iQ supermix)	19.6
	128 bp/macula (Mix A)	36.6
	128 bp/macula (Mix A)	38.6
	128 bp/periphery (iQ supermix)	21.2
	128 bp/periphery (iQ supermix)	21.2
	128 bp/periphery (Mix A)	36.0
	128 bp/periphery (Mix A)	37.0



Trace	Identifier	C_T
	128 bp/macula	20.2
	128 bp/macula	20.0
	128 bp/macula	20.0
	128 bp/periphery	21.7
	128 bp/periphery	21.9
	128 bp/periphery	21.8
	ACTB/macula	20.0
	ACTB/macula	20.1
	ACTB/periphery	20.0
	ACTB/periphery	19.6

the iCycler thermal cycler recommends amplification of PCR products only within the narrow range of 75–150 bp, we have consistently been able to amplify products in excess of 300 bp using iQ supermix (data not shown). Replicate C_T values for amplification of the control gene *ACTB* showed less variation between replicates and between experiments with either reaction mix (Figures 1 and 3). This was not simply due to the relative abundance of *ACTB* transcripts in the samples since the amount of the macula-enriched target gene was the same as *ACTB* in the macula. Instead, these results suggest that sequence-related secondary structure or transcript stability of the target gene could affect outcomes in the mix A reactions but were not a factor in the iQ supermix reactions. Finally, the iQ supermix reactions were not only more robust but also were extremely reproducible: macula-enriched target transcript C_T in the macula-derived samples was 19.9 ± 0.2 cycles (n = 11), and 21.4 ± 0.3 cycles (n = 11) in the periphery-derived samples (compare Figures 2, 3, and 4A). Here we have demonstrated that the use of iQ supermix to optimize reaction conditions allows the best consistency and reproducibility from experiment to experiment.

Acknowledgements

We are greatly indebted to Drs Gregory J Riggins and Kathy Boon, Duke University Medical Center, Durham, NC, for their helpful expert advice. Supported in part by NEI R01 EY 11286, NEI P30 EY 05722 and a Career Development Award from Research to Prevent Blindness (CBR).

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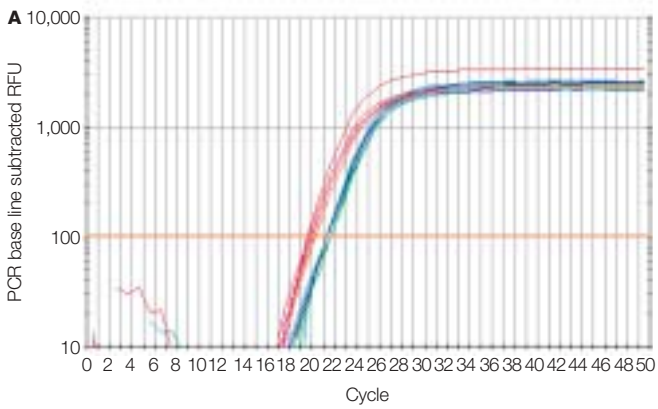
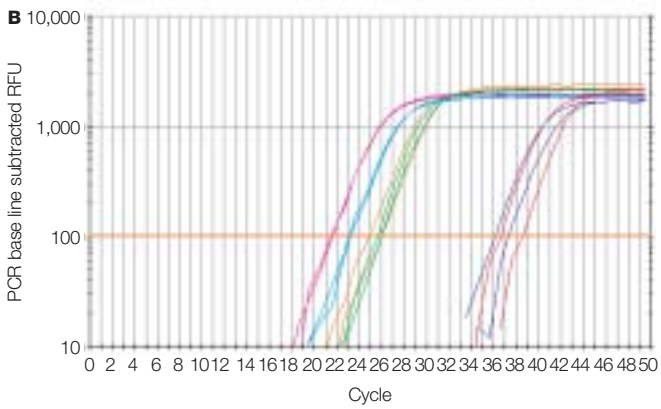
Fig. 4. Amplification of three different products from a macula-enriched transcript with two different cDNA templates using two mix conditions. **A**, traces representing the amplification of all three products using iQ supermix; **B**, traces representing the amplification of all three products using mix A.



Trace	Identifier	C_T
	128 bp/macula	19.7
	128 bp/macula	19.6
	100 bp/macula	20.1
	100 bp/macula	20.2
	99 bp/macula	19.9
	99 bp/macula	19.8
	128 bp/periphery	21.2
	128 bp/periphery	21.2
	100 bp/periphery	21.6
	100 bp/periphery	21.6
	99 bp/periphery	21.3
	99 bp/periphery	21.2



Trace	Identifier	C_T
	128 bp/macula	36.6
	128 bp/macula	38.6
	100 bp/macula	24.8
	100 bp/macula	25.9
	99 bp/macula	21.6
	99 bp/macula	21.4
	128 bp/periphery	36.0
	128 bp/periphery	37.2
	100 bp/periphery	25.6
	100 bp/periphery	25.9
	99 bp/periphery	23.2
	99 bp/periphery	23.0

Separation Reproducibility With the BioLogic™ Chromatography Systems

Kevin Thornton, PhD, Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

In chromatographic separations, sample component elution times are often used both for identification and to program automated chromatography steps like fraction collection. Therefore, the ability to obtain reproducible chromatographic separations is essential. In order to achieve reproducible sample runs, a chromatography system must maintain a precise flow rate, inject a consistent sample volume at a precise time, and detect sample components in a consistent manner. Additionally, the column used must maintain its function and flow characteristics over time. In this report we provide data that shows the highly reproducible separations that can be obtained with both BioLogic DuoFlow™ and BioLogic LP™ systems. These results were obtained on a medium-pressure BioLogic DuoFlow QuadTec™ basic system and a low-pressure BioLogic LP™ system equipped with a BioFrac™ fraction collector.

Methods

A test mixture that contained the proteins horse skeletal muscle myoglobin, chicken egg white conalbumin, and soybean trypsin inhibitor type II (all obtained from Sigma) in a ratio of 2:5:5 was dissolved in an appropriate volume of 20 mM Tris buffer, pH 8.1 (buffer A), filtered through a 0.22 µm syringe filter, and kept on ice until injected onto a column.

The test mixture was purified in consecutive anion exchange runs using an UNO™ Q1 column or an Econo-Pac® High Q cartridge connected to the DuoFlow or BioLogic LP system, respectively. Proteins were eluted with a linear gradient of 0–50% buffer B (buffer A + 1 M NaCl). All buffers were filtered and degassed prior to use. For each run on the DuoFlow system, a 50 µl sample loop was overfilled with 150 µl of the protein sample using an Econo™ gradient pump. For the BioLogic LP system, 1 ml of sample was loaded though port C of the BioLogic LP buffer selection valve. The UV detector and conductivity monitor supplied with each system were used to monitor the UV signal and conductivity, respectively. Protocol details are summarized in Tables 1 and 2 and the system components used are listed in Table 3.

Results

The BioLogic DuoFlow and BioLogic LP systems were used to successfully purify a protein test mixture with excellent reproducibility. A total of 80 ion exchange runs were performed on the BioLogic DuoFlow QuadTec system as a queue of alternating anion exchange and equilibration runs (160 runs total). As shown in Figure 1, the 80 separations were virtually indistinguishable. The observed protein retention times (Table 4) demonstrate consistent sample introduction and pump performance. The standard deviation associated with the gradient slope, 4.88 ± 0.01 mS/cm/min, indicates excellent pump and mixer performance, which is critical for discrete protein resolution. These results confirm that the BioLogic DuoFlow chromatography system provides high run-to-run reproducibility.

The BioLogic LP system (left) allows creative solutions for low-pressure applications. The BioLogic DuoFlow systems (right) provide analytical to preparative separation capabilities.

Table 1. Separation protocol on the BioLogic DuoFlow system.

Description	Buffer Composition	Volume, Flow Rate
Anion exchange chromatography		
1. Isocratic flow	Buffer A 100%	0.4 ml at 2.0 ml/min
2. Zero baseline for QuadTec		
3. Isocratic flow	Buffer A 100%	1.0 ml at 2.0 ml/min
4. Load/inject with auxiliary pump fill		0.2 ml at 1.0 ml/min
5. Isocratic flow	Buffer A 100%	2.0 ml at 2.0 ml/min
6. Linear gradient	Buffer A 100 to 50% Buffer B 0 to 50%	20.0 ml at 2.0 ml/min
7. Isocratic flow	Buffer A 50% Buffer B 50%	5.0 ml at 2.0 ml/min
Column equilibration		
1. Isocratic flow	Buffer B 100%	4.0 ml at 2.0 ml/min
2. Isocratic flow	Buffer A 100%	2.0 ml at 2.0 ml/min
3. Hold until conductivity <2.7 mS/cm	Buffer A 100%	2.0 ml/min
4. Hold until <0.1 AU at 280 nm	Buffer A 100%	2.0 ml/min
5. Isocratic flow	Buffer A 100%	4.0 ml at 2.0 ml/min
6. Zero baseline for QuadTec		
7. Isocratic flow	Buffer A 100%	1.0 ml at 2.0 ml/min

Table 2. Anion exchange chromatography protocol on the BioLogic LP system.

	Buffer Composition	Volume, Flow Rate
1.	Buffer A	1 ml at 1.5 ml/min
2.	Sample	1 ml at 1.5 ml/min
3.	Buffer A	5 ml at 1.5 ml/min
4.	Linear gradient of 0–50% buffer B	20.0 ml at 1.5 ml/min
5.	Buffer B	5.0 ml at 1.5 ml/min
6.	Buffer A	15 ml at 1.5 ml/min

Table 3. Comparison of system components used to test reproducibility.

	BioLogic DuoFlow	BioLogic LP
Column	UNO Q1 column	Econo-Pac High Q cartridge
Protein load	300 µg/50 µl	900 µg/1 ml
Loading method	Sample loop	Buffer selection valve
UV detector	BioLogic QuadTec	BioLogic LP optics module

On the BioLogic LP, 25 consecutive separations were performed using its Multirun feature. As shown in Figure 2, the separations were highly reproducible and exhibited extremely consistent protein elution times (Table 4). Gradient performance was also highly reproducible with a small standard deviation (Figure 2); the observed average gradient slope was 3.59 ± 0.05 mS/cm/min. These results show that the BioLogic LP gives the consistent mixing and reliable pump performance required from a low-pressure chromatography system.

Table 4. Retention times for test proteins separated on Bio-Rad chromatography systems. Shown are average retention times ± SD for 80 consecutive runs for the BioLogic DuoFlow and 25 for the BioLogic LP system.

	BioLogic DuoFlow	BioLogic LP
Myoglobin	1.48 ± 0.02 min	3.67 ± 0.02 min
Conalbumin	4.54 ± 0.01 min	10.92 ± 0.04 min
Soybean trypsin inhibitor	7.38 ± 0.01 min	15.10 ± 0.05 min

Conclusions

These results demonstrate that the BioLogic DuoFlow and BioLogic LP chromatography systems routinely deliver highly reproducible results. This high level of reproducibility is critical for obtaining consistent sample purity and for automating laboratory chromatography processes. Whether your separation requirements are analytical or preparative, the BioLogic DuoFlow and BioLogic LP chromatography systems provide high-performance, reliable results.

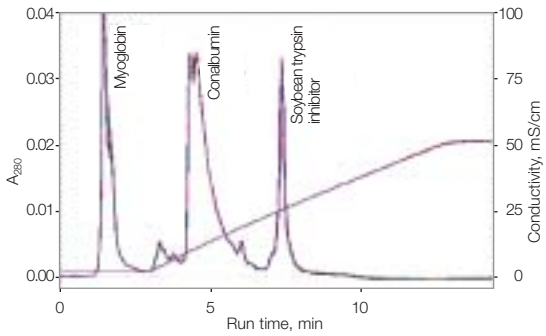


Fig. 1. Reproducibility of a BioLogic DuoFlow QuadTec system. Shown is an overlay of 80 runs. Red line, conductivity trace.

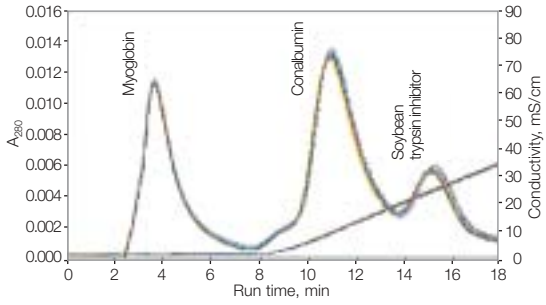


Fig. 2. Reproducibility of a BioLogic LP system. Shown is an overlay of 25 consecutive separations using the multi-run feature.

Sample Preparation Solutions for Cellular Protein Fractionation

T Slyker,[1] B Gillece-Castro,[2] M Nguyen,[1] L Sapp,[2] MG Brubacher,[1] and WB Strong,[1] [1]Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA USA and [2]Waters Corporation, 100 Cummings Center Suite 407N, Beverly, MA USA

Introduction

Two-dimensional (2-D) gel electrophoresis is a widely used, proven method for proteome analysis. The quality and value of the information obtained from a 2-D electrophoresis experiment is highly dependent upon the initial sample preparation. In order to identify the most complete array of cellular proteins it is often necessary to reduce the complexity of the protein sample. A strategy for reduction in sample complexity is especially important when analysis of low-abundance and membrane proteins is the goal. Ideally the method(s) employed should be simple, reproducible, general to a wide variety of cell types, and result in a low conductivity protein sample that is free of substances that interfere with 2-D electrophoresis. With these aims in mind, we will present several solutions for convenient and efficient extraction of cellular proteins into discrete, more easily manageable fractions that are enriched in certain classes of proteins such as cytosolic, nuclear, membrane and signaling. The majority of these procedures are intended to provide tools that simplify the preparation of membrane proteins that are generally considered difficult to isolate.

These protocols address two key issues in applying 2-D gels to proteomics analysis of complex samples. One issue is how to handle both the enormous chemical diversity and the wide dynamic range of proteins in a biological sample so that low-abundance and housekeeping proteins can be monitored and identified. We have developed a number of protocols to quickly prepare highly enriched protein fractions based on protein solubility. One protocol separates a complex sample into enriched fractions of nuclear and cytoplasmic proteins. A second protocol enriches for membrane-associated signaling proteins. Other protocols enrich for membrane proteins. All protocols enhance the capability to monitor and identify proteins of biological interest.

The other issue is how to solubilize these hydrophobic proteins so they can be separated in 2-D gels. A number of new detergents and more stringent chaotropic agents have been incorporated into a complete protein solubilization buffer (PSB), which efficiently solubilizes hydrophobic proteins for

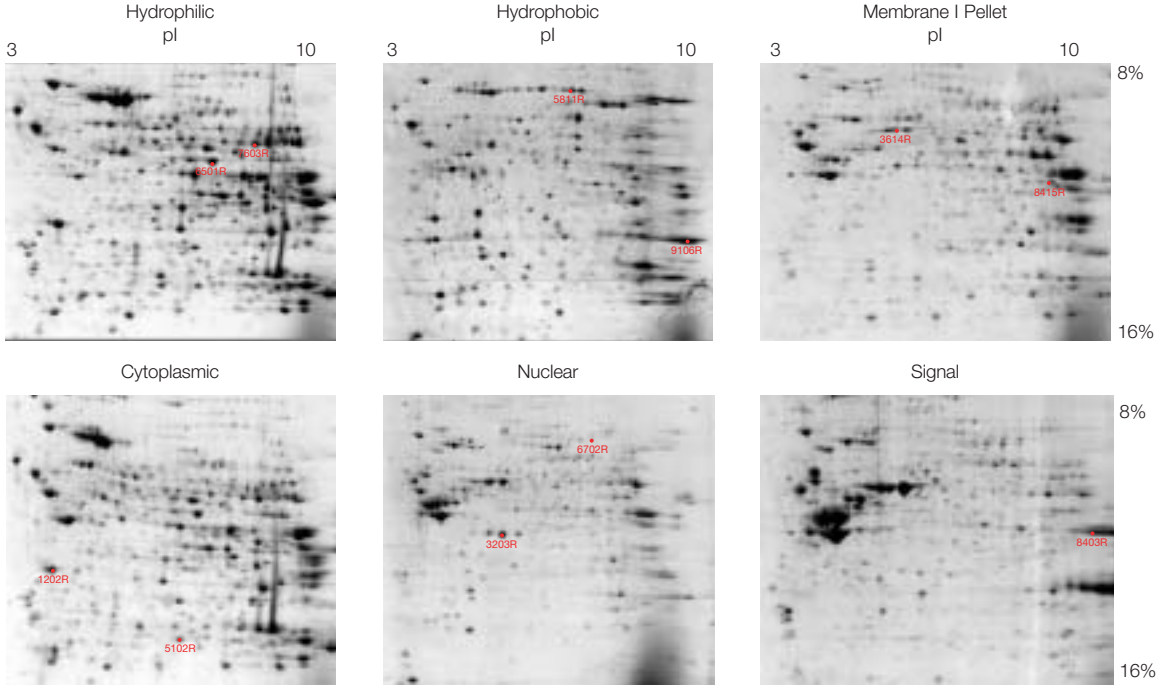


Fig. 1. Mouse liver samples fractionated using three sample preparation protocols. Enriched samples from each protocol were run on 2-D gels. Each gel is identified by the fractionation procedure used to generate the sample. The gels labeled "Hydrophillic", "Hydrophobic", and "Membrane I Pellet" were of samples generated using the ReadyPrep protein extraction kit (membrane I). The gels labeled "Cytoplasmic" and "Nuclear" were of samples generated using the ReadyPrep protein extraction kit (cytoplasmic/nuclear). Proteins from both phases were recovered for further analysis. The gel labeled "Signal" was of a sample generated from the ReadyPrep protein extraction kit (signal). PDQuest identification numbers and a red triangle on the gel images indicate spots that were cut for MS analysis. The protein identifications for these spots are listed in the table, and a sample mass spectrometry identification is shown in Figure 2.

immediate application to a 2-D gel. Using both the simplified enrichment and enhanced solubilization protocols, we significantly expanded the capabilities of applying 2-D gels for proteome analysis of complex cellular extracts. 2-D gel electrophoresis and MALDI peptide mass fingerprinting data will be presented to illustrate the effective application of these techniques to improved sample prefractionation and protein identification.

Methods

Sample Preparation

Mouse liver tissue samples were processed with three different sample preparation kits to extract and enrich specific subclasses of proteins. The kits used were the ReadyPrep protein extraction kit (membrane I), the ReadyPrep protein extraction kit (cytoplasmic/nuclear), and the ReadyPrep protein extraction kit (signal). The ReadyPrep protein extraction kit (membrane I) employs a temperature-dependent phase partitioning in Triton X-114 detergent to partition the sample into hydrophobic and hydrophilic protein fractions (Bordier 1981, Santoni et al. 2000). The ReadyPrep protein extraction kit (cytoplasmic/nuclear) isolates the nucleus from the cytoplasm, then uses a strongly chaotropic extraction buffer (Dignam et al. 1983, Zerivitz and Akusjarvi 1989). ReadyPrep protein extraction kit (signal) takes advantage of the limited solubility of plasma membrane microdomains in nonionic detergents at 4°C to yield a protein pellet that is enriched in membrane-associated signaling proteins (Simons and Ikonen 1997, Brown and Rose 1992, Parton and Simons 1995, Anderson et al. 1992). Instructions for each kit were followed exactly. Protease inhibitors were added to the starting buffers immediately prior to use to prevent proteolysis during extraction. Following extraction, if required, protein samples were processed with the ReadyPrep 2-D cleanup kit to remove salts and detergents and to create a low-conductivity sample suitable for isoelectric focusing (IEF). This cleanup kit was also employed, if required, to concentrate proteins from dilute samples. Prior to IEF all samples were solubilized in PSB, to which 50 mM DTT, 2 mM TBP, and 0.2% Bio-Lyte® ampholytes (3–10) were added, and applied to 17 cm, pH 3–10 NL, ReadyStrip™ IPG strips. Approximately 455 µg of protein was loaded to each strip.

2-D Electrophoresis

The IPG strips were focused using a PROTEAN® IEF cell for ~60,000 V-hr at a final focusing voltage of 10,000 V. The cell was set for rapid voltage ramping. Focused IPG strips were loaded onto 8–16% SDS-PAGE gels following equilibration for 10 min with DTT containing buffer followed by 10 min with iodoacetamide containing buffer. Following electrophoresis, gels were fixed for 30 min, stained with Bio-Safe™ Coomassie stain for 1 hr, and then destained for at least 2 hr before scanning with a GS-800™ densitometer.

Protein Identification

2-D gel images were processed for analysis with PDQuest™ 2-D analysis software and spots of interest were cut from the gels using the ProteomeWorks™ spot cutter. Peptide mass fingerprint data was obtained from excised 2-D gel spots using the Mass*PREP* station robotics system for protein digestion and MALDI target spotting. Gel spots were destained, reduced, alkylated, dried, digested with modified trypsin (Promega) while heated at 37°C for 5 hr, and extracted with an acidic solution. Peptides were then automatically spotted onto MALDI target plates mixed with a matrix solution of 2 mg/ml α-cyano-4-hydroxycinnamic acid. MALDI mass spectra were acquired on a M@LDI-LR instrument in reflector mode. A nitrogen laser (λ = 337 nm, Laser Science) was pulsed at 20 Hz. Ions were accelerated to 15 kV after a time-lag focusing pulse of 2,750 V at 500 ns. Data were collected from the sample well and a near point lock mass (ACTH clip 18–39, Sigma). The MALDI mass spectra were processed to identify monoisotopic peaks using the Micromass algorithm MaxEntLite. The search engine used to identify peptide mass fingerprint data was ProteinLynx Global *SERVER* 2.0 software.

Table. Protein identifications from enriched fractions of mouse liver samples.

Gel Name	SSP #	Protein Name	Gene Name	Subcellular Location*
Cytoplasmic	1202R	Senescence marker protein 30	SMP30	Cytoplasm
Cytoplasmic	5102R	Antioxidant protein 2	AOP2	Cytoplasm
Nuclear	3203R	Retinoic acid receptor α	RARA	Nuclear receptor
Nuclear	6702R	Lamin A	LMNA	Nuclear structural protein
Signal	8403R	Argininosuccinate synthase	ASS1	Plasma membrane caveolae
Hydrophilic	6501R	α-Enolase	ENO1	Cytoplasm
Hydrophilic	7603R	Glutamate dehydrogenase precursor	GLUD	Mitochondrial matrix
Hydrophobic	5811R	Succinate dehydrogenase flavoprotein subunit	SDHA	Inner mitochondrial membrane
Hydrophobic	9106R	Voltage-dependent anion channel protein 1	VDAC1	Outer mitochondrial membrane
Membrane I pellet	8415R	Acyl-CoA dehydrogenase, medium chain	ACADM	Mitochondrial matrix
Membrane I pellet	3614R	Formiminotransferase-cyclodeaminase	FTCD	Golgi

* Based on information in SWISS-PROT protein sequence database.

Results

Three separate sample preparation protocols were used to fractionate and enrich mouse liver protein samples prior to 2-D electrophoresis. Although the proteins for each gel were prepared from the same starting material, the final gel results following fractionation provide completely different views of the mouse liver proteome (Figure 1). Proteins that were unique to the fractionated sample were selected for identification (see Figure 2 for an example). The table shows selected proteins from these gels that are associated with the cellular structures enriched for in the sample separation protocols.

Conclusions

Applying specialized sample preparation solutions for the fractionation of cellular proteins from complex protein mixtures provides a simple and rapid method for enrichment of both low-abundance and membrane-associated proteins prior to sample analysis by 2-D electrophoresis. Understanding the cell biology of membrane proteins is especially important in the search for new drug targets, as ~50% of the known drug targets are membrane proteins. We have shown that application of three new kits for protein fractionation can provide a simple way to differentiate the proteome of a complex sample into a number of manageable fractions. Key features of these kits include:

- Rapid separations — Extraction from a crude sample to an enriched, gel-ready sample takes <3 hr
- Reproducible results — Each kit is based on proven chemistries and provides detailed instructions for application
- Increased protein solubilization — The PSB solution has been optimized to enhance solubilization of membrane-associated proteins for 2-D separation
- Improved 2-D results — The ReadyPrep 2-D cleanup kit provides quantitative recovery of samples with elimination of detergent and salt contaminants

Applying these sample prep kits to enrich proteins from complex biological samples should enhance the quality of the data that is obtained with any 2-D proteomics program.

Fig. 2. ProteinLynx Global *SERVER* 2.0 results screen identifying lamin A protein from the mouse liver nuclear fraction.

References

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Santoni V et al., Membrane proteomics: use of additive main effects with multiplicative interaction model to classify plasma membrane proteins according to their solubility and electrophoretic properties, Electrophoresis 21, 3329–3344 (2000)

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Zerivitz K and Akusjarvi G, An improved nuclear extract preparation method. Gene Anal Tech 6, 101–109 (1989)

For a listing of the kits used in these experiments, refer to the What's New section of this issue (page 15).

The XenoWorks™ System for Gene Transfer by Microinjection

Gene Transfer and Bio-Rad

Gene transfer is an important step in many gene expression studies. Once a gene has been characterized, sequenced, etc., it often needs to be studied in vivo, expressing its product in a true biological environment. A large number of methods are used to introduce genes into cells, and Bio-Rad has been at the forefront of this field since introducing the first commercially available electroporator in 1986.

Microinjection, the use of pressure to control the flow of material through a fine glass micropipet, is a particularly powerful gene transfer technique since the material can be viewed through a microscope as it is physically introduced into the target cell. This ability to target a specific cell is one of microinjection's most important features, but it is not the only one. By lowering the pressure within the micropipet, it is also possible to extract material from a cell and even exchange cellular contents and organelles between cells, as described below.

A typical microinjection workstation will include three basic components, shown in Figure 1: a microscope (to view the injection process), a positioning device for the micropipet (a micromanipulator), and a pressure control device (the microinjector).

Precise Positioning

Until the late 1980s, suspended cell injection used either mechanical positioning devices to control the micropipet, or hydraulic micromanipulators, using water, oil, or air-filled tubes to transfer the movement of a joystick to the device holding the micropipet. These devices had responsive movement and smooth action, and were reasonably reliable, but suffered from a very limited range of travel. In most cases an additional coarse positioning system was required to place the micropipet tip in the microscope's field of view before injection could begin.

From the very start of the product's development, Bio-Rad's intent was to create a micromanipulator that is as smooth, responsive, and intuitive to use as the micromanipulators that had been introduced before, yet provides the long travel range (up to 27 mm) possible with the use of stepper motors. The XenoWorks micromanipulator uses precision stepper motors to move the micropipet around the working area, actuated by the movement of an ergonomic inverted joystick. Because the motor's resolution is 40 nm, successive steps of the motor can be strung together, resulting in linear movement that is extremely smooth and particularly responsive. The key to the smooth and responsive movement is the embedded software, designed to operate the device so that it is almost impossible to tell that there is an electronic linkage, not a mechanical one, between the joystick and tip of the micropipet. The use of electronic motors offers a number of additional benefits: It is possible to program the micromanipulator to return to preset coordinates at the single press of a key on the joystick interface — useful to ensure that the micropipet tip is never accidentally lost from the field of view. It is also possible to set different ranges of movement depending on the precision of the injection procedure. Placing the system in coarse mode results in travel of 12 mm for one complete swing of the joystick. At the smallest setting, one joystick swing provides just 100 µm of travel for particularly fine manipulations.

Precise Pressure Control

Bio-Rad offers two different XenoWorks microinjectors to accommodate a number of different microinjection applications.

The XenoWorks analog microinjector is designed around the need for gentle positive and negative pressure with extremely fine control over the contents of the micropipet. Examples of such

Fig. 1. Bio-Rad's XenoWorks microinjection workstation.

The XenoWorks micromanipulator joystick has been designed with great ergonomic comfort in mind. The joystick is inverted so it is more comfortable to use over extended periods than upright joysticks, and the action keys are raised and can be located and actuated without looking away from the microscope.

The XenoWorks analog microinjector: compact, stable, and versatile.

The XenoWorks digital microinjector, one device for many different pressure control requirements.

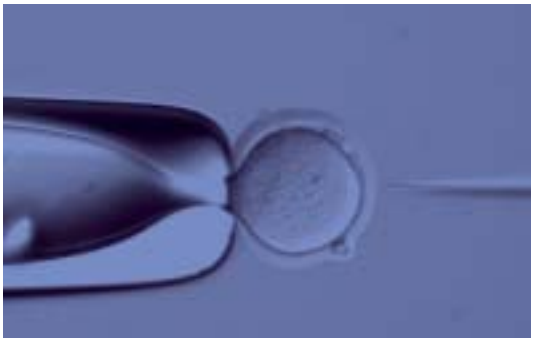


Fig. 2. Microinjection of DNA into the pronucleus of a mouse embryo to create transgenic offspring.

Fig. 3. Microinjection transfer of genetically modified embryonic stem cells into a mouse blastocyst to create gene knockout offspring.

applications include nuclear transfer, embryonic stem cell transfer, and intracytoplasmic sperm injection. The analog microinjector is built around a precision syringe (a 500 µl syringe is supplied, but other sizes can be fitted to allow customization of the device's sensitivity). In addition, it is possible to alter the length of the tubing and to use the device with different hydraulic fluids.

The XenoWorks digital microinjector is a two-channel, full-featured microinjector capable of cell holding, and of gentle aspiration and injection as well as high-pressure solution injection. It achieves precise control through the use of air only; no other hydraulic fluid is used in the system. This makes the device extremely easy to use and to maintain.

Applications

Although the XenoWorks microinjection system is ideally suited to almost every microinjection application, a number of applications particularly benefit from the XenoWorks features.

The XenoWorks digital microinjector, when used with two micromanipulators, creates an ideal workstation for the production of transgenic animals. The injection of DNA solutions into a single-celled mammalian zygote to create transgenic offspring requires precise pressure control within the injection micropipet, allowing highly reproducible volumes to be introduced into the zygote's pronucleus (Figure 2). Simultaneously, the zygote itself must be held gently but firmly in place by gentle suction using a second, holding, micropipet. The suction control within the holding pipet must be precise and extremely stable: too little will cause the zygote to be pulled from the holding pipet as the injection pipet is withdrawn, but too much may damage the zygote.

A similar array of instrumentation is used for applications such as embryo reconstruction. The transfer of genetically modified embryonic stem cells into the blastocyst of a mammalian embryo to create a gene knockout is a typical example (Figure 3), as is nuclear transfer for animal cloning. As with the transgenic animal workstation, gentle suction is applied to the holding pipet to gently grip the embryo or oocyte. The injection pipet is used to aspirate and inject the cells to be transferred.

A number of additional microinjection applications can be supported by the XenoWorks product line. For more information, contact your local Bio-Rad sales representative, request bulletin 2813, or visit us at **www.bio-rad.com/genetransfer/**

Ordering Information

Suggested System Configurations

Zygote Pronuclear DNA Microinjection	
Micromanipulator (Left)	165-2801
Micromanipulator (Right)	165-2802
2 Microscope Adaptors	165-28xx*
Digital Microinjector	165-2805/6

Embryonic Stem Cell Transfer Into Blastocysts	
Micromanipulator (Left)	165-2801
Micromanipulator (Right)	165-2802
2 Microscope Adaptors	165-28xx*
Digital Microinjector	165-2805/6
Or	
2 Analog Microinjectors	165-2808

Microscope Adaptors

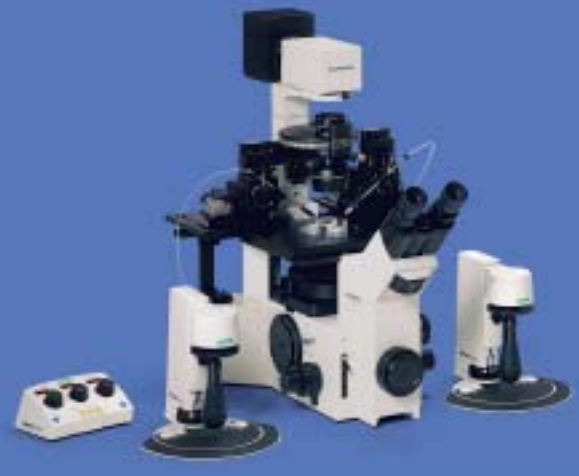
Catalog #	Description
165-2830	Microscope Adaptor for Olympus IX-50/70
165-2831	Microscope Adaptor for Olympus IX-51/71/81
165-2840	Microscope Adaptor for Nikon TMD
165-2842	Microscope Adaptor for Nikon Diaphot/ Eclipse TE 200/300
165-2843	Microscope Adaptor for Nikon TE 2000
165-2850	Microscope Adaptor for Zeiss Axiovert 100/135
165-2852	Microscope Adaptor for Zeiss Axiovert 200
165-2860	Microscope Adaptor for Leica DM IL
165-2862	Microscope Adaptor for Leica DM IRB/E/IRE2

* Select the adaptors appropriate for your microscope.

genetransfer

Winning Performance

With its advanced controls, the new XenoWorks™ system from Bio-Rad makes successful microinjection a breeze.



Whether you're delivering DNA into a pronucleus or transferring stem cells to a blastocyst, the XenoWorks microinjection system helps you sail through your work.

You'll feel the difference the moment you take the controls. The ergonomic design with height-adjustable joystick, micromanipulator position memories, and variable movement radius makes the XenoWorks system faster, easier, and more comfortable to guide than other microinjection instruments.

- Air injection system provides exceptional stability and reproducibility
- Intuitive interface (no hierarchical menus or programming)
- Mechanically stable micromanipulator for piezo-drilling applications

For more information, contact your Bio-Rad representative or set course for **www.bio-rad.com/genetransfer/**

Microinjection Lipofection Biolistics Electroporation

Visit us on the Web at **discover.bio-rad.com**
Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

BIO-RAD

Selecting Competent *E. coli* Cells for Transformation

Bacterial transformation is a routine procedure in molecular biology laboratories. It is a fundamental step in the construction of complex recombinant DNA molecules, in making cDNA and genomic libraries, and in screening large collections of plasmid clones. Because it is so routine, researchers seek transformation techniques that are quick, easy, and reliable. Bio-Rad has recently introduced new lines of competent *E. coli* cells for your transformation needs.

Bacterial Transformation

Transformation, the introduction of recombinant DNA into host bacterial cells, is an effective way to propagate and express that DNA. The most commonly used hosts are genetically modified *E. coli* strains. While these strains have genetic characteristics that support cloning and gene expression, such strains do not naturally take up DNA. *E. coli* and other hosts can be made "competent" to take up DNA either by a combined chemical/heat shock treatment or by electroporation.

In chemical transformation, a host *E. coli* strain is suspended in a solution containing divalent cations, typically Ca^{2+}, and briefly exposed to an elevated temperature (heat shock). (The cations are thought to decrease electrostatic repulsion between DNA molecules and the bacterial cell surface.) During the heat shock, pores open in the host cell membrane, allowing passage of DNA. When the cells are quickly chilled, the pores close, allowing the cells to retain the introduced DNA. Chemical transformation is generally economical and easy, requiring only basic laboratory equipment and reagents.

Electroporation or electrotransformation is a method in which host cells in a suspension with DNA are transformed by the passage of a short, controlled pulse of electricity. Like chemical transformation, electroporation causes pores to open in the host cell membrane, allowing passage of DNA. At the termination of the electrical pulse, the pores close, allowing the cell to retain the introduced DNA. Electroporation requires the use of an electroporator, such as Bio-Rad's MicroPulser™, Gene Pulser®, or Gene Pulser Xcell™ systems. Electroporation routinely yields higher numbers of transformed cells but requires specialized instruments.

For either method, best success is achieved with host cells that have been pretreated to ensure optimum DNA uptake and survival under either chemical transformation or electroporation conditions. Such cells are called chemi-competent and electro-competent, respectively.

Matching Transformation Method and Efficiency to Application

A high transformation efficiency (expressed as number of transformants obtained per µg of input DNA) is more critical for some applications than for others, so the application should guide the choice of host cell preparation and transformation method. For example, the highest transformation efficiency possible should be sought for library construction or any experiment where the amount of DNA available for transformation is limited. Electrotransformation is most suitable for these demanding applications. For routine applications such as subcloning, where the availability of input DNA is not a limiting factor and fewer transformants need to be recovered, the lower transformation efficiencies of chemical transformation give acceptable results.

Some Important Host Features

The ability to transform with exogenous DNA is not the only criterion for choosing a host strain. Various genetic markers have been incorporated into laboratory *E. coli* strains in order to enhance the retention, stability, and expression of the transforming DNA in the host.

Restriction system mutations — Mutations in the host restriction system (*mcr*A, *mcr*BC, and *mrr* genes) allow cloning of methylated DNA. Most eukaryotic DNA is methylated, and would be targeted for degradation by bacterial restriction systems, so restriction system mutations enable the generation of representative genomic libraries. Methylation-dependent restriction system mutations are important in plasmid rescue protocols, where plasmid DNA is retrieved after integration into genomic DNA. Other restriction mutations such as *hsdR17* (r_k^-, m_k^+) prevent cleavage of DNA by endogenous bacterial restriction endonucleases, allowing, for example, cloning of PCR products.

***recA, endA,* and *deoR* mutations** — The *end*A mutation eliminates nonspecific endonuclease activity, allowing production of high-quality, high-yield plasmid minipreps. The *rec*A mutation reduces recombination events, useful in cloning unstable sequences containing repeats. The *deo*R mutation enables the maintenance of large plasmids. These considerations are particularly important for library construction, where cloning full-length sequences

and the highest possible representation of input DNA are important.

F' episome — The presence of an F' episome makes cells susceptible to infection by bacteriophage M13, permitting the synthesis of single-stranded DNA. This feature is useful, for example, to generate templates for sequencing or to create subtractive cDNA libraries. An F' strain that also has the *supE* marker is useful for phage display applications.

Blue/white screening — The *lacZΔM15* marker allows blue/white screening when a transforming vector carries the complementing portion of the β-galactosidase gene. Many commercially available vectors are designed for blue/white screening. Transformed colonies containing cloned inserts are identifiable when they are grown on medium containing the chromogenic substrate X-gal.

***lacI*^{q} gene** — The *lac* repressor, encoded by the *lacI* gene, regulates *lac*, *tac*, and *trc* promoters. The *lacI*^{q} mutation overexpresses the repressor, suppressing transcription of cloned inserts until the *lac* promoter is induced by addition of IPTG. This feature can be important if expression of an insert is undesirable or toxic under certain conditions.

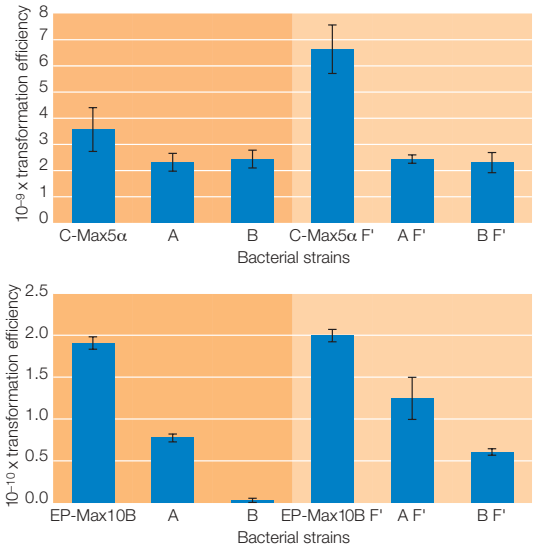
EP-Max™ 10B Electro-Competent Cells

Bio-Rad's new EP-Max10B and EP-Max10B F' electro-competent cells are recommended for cloning applications that demand the highest transformation efficiencies, such as generation of cDNA and genomic libraries, or transformations with DNA that is scarce or limited. The high transformation efficiencies possible with saturating amounts of DNA (>1 x 10^8 using 250 ng DNA) make EP-Max10B cells ideal for library construction. The use of saturating amounts of DNA maximizes library size while reducing the number of transformants required for a representative library.

C-Max™5α Chemi-Competent Cells

New C-Max5α cells provide the best performance of any comparable host cell preparation for routine cloning applications where high efficiencies are not as critical. C-Max5α chemi-competent cells provide transformation efficiencies of >1 x 10^9 with 50 pg of DNA and >1 x 10^6 using saturating levels of DNA (25 ng).

For more information, request bulletin 2870. For ordering information, see page 5.



Comparison of transformation efficiency of Bio-Rad's chemi-competent (top panel) and electro-competent (bottom panel) cells. A and B are comparable strains from the top two suppliers of competent cells. All transformations were performed according to the manufacturer's recommended protocol, using pUC19 DNA.

Host cell selection guide.

	EP-Max10B	EP-Max10B F'	C-Max5α	C-Max5α F'
Transformation efficiency, cfu*/µg pUC19 DNA	>1 x 10^{10}	>1 x 10^{10}	>1 x 10^9	>1 x 10^9
mrr, hsdRMS, mcrBC for cloning of methylated DNA	Yes	Yes	No	No
cDNA cloning	Yes	Yes	Yes	Yes
F' episome for ssDNA synthesis	No	Yes	No	Yes
lacZDM15 for blue/white screening	Yes	Yes	Yes	Yes
lacI^q for regulated expression	No	Yes	No	Yes
Phage display	No	No	No	Yes
recA for reduced recombination	Yes	Yes	Yes	Yes
endA for high quality plasmid preps	Yes	Yes	Yes	Yes
deoR for large plasmids	Yes	Yes	No	No

*Colony forming units

Genotype of Bio-Rad's competent cells.

Cell Type	Transformation Method	Genotype
EP-Max 10B	Electroporation	F$^-$ *mcrA Δ(mrr-hsdRMS-mcrBC)* ϕ80d*lacZΔM15 ΔlacX74 deoR recA1 endA1 araD139 Δ(ara, leu)7697 galU galK rpsL nupG* λ$^-$
EP-Max10B F'	Electroporation	*mcrA Δ(mrr-hsdRMS-mcrBC)* ϕ80d*lacZΔM15 ΔlacX74 deoR recA1 endA1 araD139 Δ(ara, leu)7697 galU galK rpsL nupG* λ$^-$/F'[*lacI*^{q}*ZΔM15* Tn*10* (Tet^r)]
C-Max5α	Heat shock	F$^-$ ϕ80d*lacZΔM15 Δ(lacZYA–argF)U169 recA1 endA1 hsdR17*(r_k^-, m_k^+) *phoA supE44* λ$^-$ *thi-1 gyrA96 relA1*
C-Max5α F'	Heat shock	ϕ80d*lacZΔM15 Δ(lacZYA–argF)U169 recA1 endA1 hsdR17*(r_k^-, m_k^+) *phoA supE44* λ$^-$ *thi-1 gyrA96 relA1/* F'[*lacI*^{q} Tn*10* (Tet^r)]

Multidimensional Chromatography With the BioLogic DuoFlow™ System

Multidimensional chromatography is used to perform multi-step chromatographic separations in a single automated run. This technique is particularly useful for routine separations. Generally, separation techniques that are sample independent provide the greatest benefit when included as part of a multidimensional experiment. These experiments usually involve some type of affinity purification step, such as proteins tagged with histidine, glutathione-S-transferase, or maltose binding protein. The affinity step is followed by a high-resolution step such as hydroxyapatite, size exclusion, or ion exchange chromatography. The table outlines a few common multidimensional chromatography experiments.

Experiments such as those in the table require advanced automation methods, which provide significant advantages in time savings and results to research laboratories. The BioLogic DuoFlow chromatography system is ideally suited to running multidimensional chromatography experiments due to its easily programmed software, sophisticated valving power, and high degree of setup flexibility.

The powerful queuing feature of the BioLogic software makes programming multidimensional chromatography experiments simple. Multi-dimensional experiments are written as a series of individual one-dimensional chromatography methods that are then run sequentially as part of a queued run. Using the examples in the table, an experiment would be written as an affinity method combined with either a desalting method or a size exclusion method to create a two-dimensional experiment. To create the three-dimensional experiment, the affinity method would be combined with both a desalting method and an ion exchange method.

The type and complexity of experiments that can be run will depend on the hardware installed on the DuoFlow system. The figure shows a schematic example of a multidimensional chromatography setup using the BioLogic DuoFlow Maximizer™ system. This multidimensional chromatography scheme is possible due to the capacity of the BioLogic Maximizer™ valve system for multiple valves. With this setup, two-dimensional experiments can be run on up to six samples or three-dimensional experiments on up to five samples in a single automated run. Since no user interaction is required during the run, this setup results in tremendous time savings. This is especially important where process automation is critical to research success, such as in structural biology laboratories.

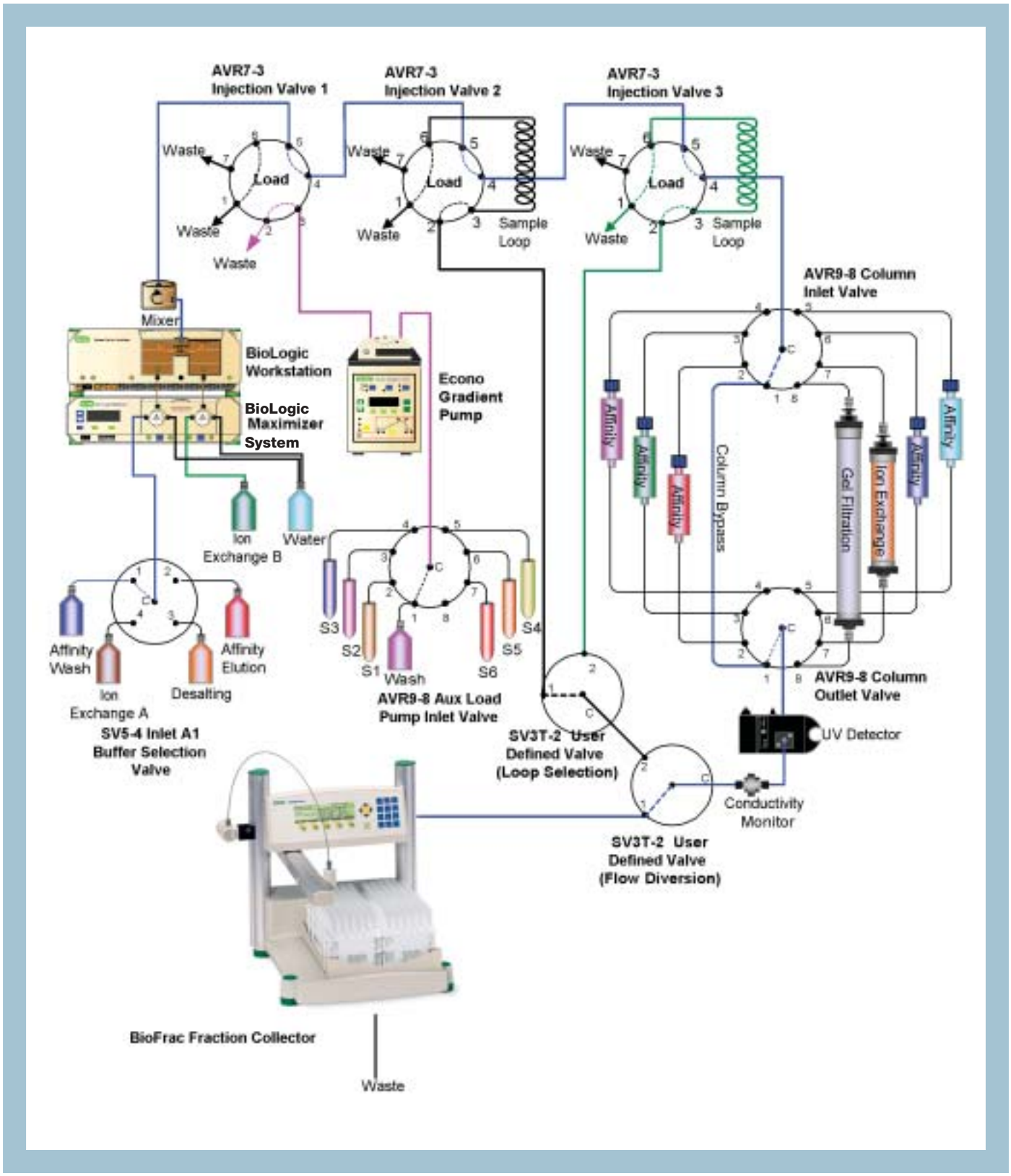
The hardware allows the user to optimize column positions and pathlengths to fit specific experimental needs. The software allows the user to assign meaningful valve names, which simplifies programming. Key components of this setup include the use of the BioLogic Maximizer valve system, an Econo™ gradient pump, an SV5-4 buffer selection valve, an AVR9-8 sample inlet valve, an AVR9-8 column switching valve, and an SVT3-2 user-defined loop selection and flow diversion valve.

Multidimensional chromatography provides many advantages to research applications, and the BioLogic DuoFlow system provides a powerful yet highly flexible solution to setting up and running these experiments.

Table. Common multidimensional chromatography experiments.

Experiment Type	Dimension 1	Dimension 2	Dimension 3
2-D	Affinity	Desalting	
2-D	Affinity	Size exclusion	
3-D	Affinity	Desalting	Ion exchange

Figure. Multidimensional chromatography with auto sample load and inject.



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Convenience Tools for Electrophoresis and Blotting

Would you like to have an extra hand to help you handle or load gels, or extra hours in the day to get more done? We may not be able to give you extra hands or time, but we do have some electrophoresis and blotting tools that will make your life in the lab a little easier. These tools will add convenience and ease to your day-to-day laboratory routines. You won't believe how useful they are until you try them.

Gel Clip

Are you nervous about handling large gels? Don't be intimidated. Large format gels are not that difficult to work with if you have the right equipment. The new gel clip is a handy tool that improves large format gel handling and, as a result, helps eliminate gel breakage. It allows gels to be manipulated and transferred from place to place around the laboratory, without ever touching the gel.

Traditionally, you lift a gel by two corners using your thumb and forefinger. This causes a nonuniform strain on the gel, which can cause it to tear. Instead, imagine having ten sets of thumbs and forefingers that spread across the entire length of the gel. So, instead of only being grasped by two tiny corners, the gel is supported along its entire edge. This provides an even distribution of the gel weight, so it can easily be lifted without tearing (Figure 1). That's exactly how the gel clip works, and it's that simple.

Gel/Cassette Assembly Tray and Roller

Proper assembly of the gel and membrane sandwich is critical to the success of any blotting experiment. Often, insufficient soaking of membranes and fiber pads, trapped bubbles, and poor gel-to-membrane contact lead to incomplete or inconsistent transfer. These problems are easily avoided using the assembly trays and rollers that accompany the Trans-Blot® Plus cell and Criterion™ blotter.

The assembly trays accommodate the gel holder cassettes, fiber pads, and filter paper as well as the transfer buffer required to completely immerse these items. Not only do these trays provide the perfect fit for gel sandwich assembly, but they also come with additional features for assembly convenience. The Criterion blotter assembly tray provides separate compartments for gel and membrane soaking as well as for easy and tidy assembly of gel blot sandwiches (Figure 2). The Trans-Blot Plus gel/cassette assembly tray comes with a lid that can be used for gel storage as well as for presoaking membranes (Figure 3).

During gel and membrane sandwich assembly, excess moisture or trapped bubbles at the gel-membrane interface may result in poor gel-to-membrane contact and subsequent failure of molecules to bind efficiently to the membrane. A roller is a useful blotting tool that removes trapped bubbles during sandwich assembly and ensures proper contact between gel and membrane. Easier to use and more sturdy than a pipet or glass tube, a 6" roller is included with purchase of the Trans-Blot Plus cell and a 2" roller is included with the Criterion blotter. Each roller may also be purchased separately for use in Southern and northern blotting.

Fig. 1. Lifting a PROTEAN Plus precast gel (25.6 x 23 cm) off the glass plates using the gel clip.

Fig. 2. Criterion blotter assembly tray. Removing bubbles from the gel/membrane sandwich using the Criterion roller.

Fig. 3. Trans-Blot Plus gel/cassette assembly tray, lid, and roller.

For more information on the Trans-Blot Plus cell, see page 19 or request bulletin 2866.

Ordering Information

Catalog #	Description
165-3414	Gel Clip, 1
170-3994	Trans-Blot Plus Gel/Cassette Assembly Tray
170-3998	Trans-Blot Plus Roller, 6" wide
170-4089	Criterion Gel/Blot Assembly Tray
165-1279	Criterion Roller, 2" wide

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- Xenoworks system CD-ROM (catalog #165-2870)

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- PowerPac HC power supply flier (bulletin 2882)
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- Whole gel eluter and mini whole gel eluter brochure (bulletin 2108)
- Model 491 prep cell and mini prep cell brochure (bulletin 1964)
- Western blotting products brochure (bulletin 2033)
- Trans-Blot Plus cell flier (bulletin 2866)
- Criterion XT precast gel flier (bulletin 2912)
- ReadyAgarose 96 Plus precast gel system product information sheet (bulletin 2929)

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- Combination of 2-D gel and liquid-phase electrophoretic separations as proteomic tools in neuroscience (bulletin 2859)

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- SEQueaky Kleen H_2O adaptor product information sheet (bulletin 2931)
- Full compatibility of the SEQueaky Kleen H_2O dye terminator removal kit with the Beckman Coulter CEQ 8000 genetic analysis system (bulletin 2939; available on the Web only)
- Extended read lengths obtained after purification of cycle-sequencing reactions with the SEQueaky Kleen H_2O dye terminator removal kit (bulletin 2949; available on the Web only)
- Comparison of PCR Kleen spin columns to traditional methods for purification of PCR products prior to sequencing (bulletin 2950; available on the Web only)

Sample Preparation, Proteomics
- Aurum ion exchange mini kits and columns product information sheet (bulletin 2928)
- ReadyPrep sample preparation kits product information sheet (bulletin 2934)

Sample Quantitation, Imaging, and Bioinformatics
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- Bio-Plex system and technology brochure (bulletin 2890)
- Bio-Plex system tools brochure (bulletin 2879)
- Bio-Plex system software brochure (bulletin 2880)
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