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SCIENCE

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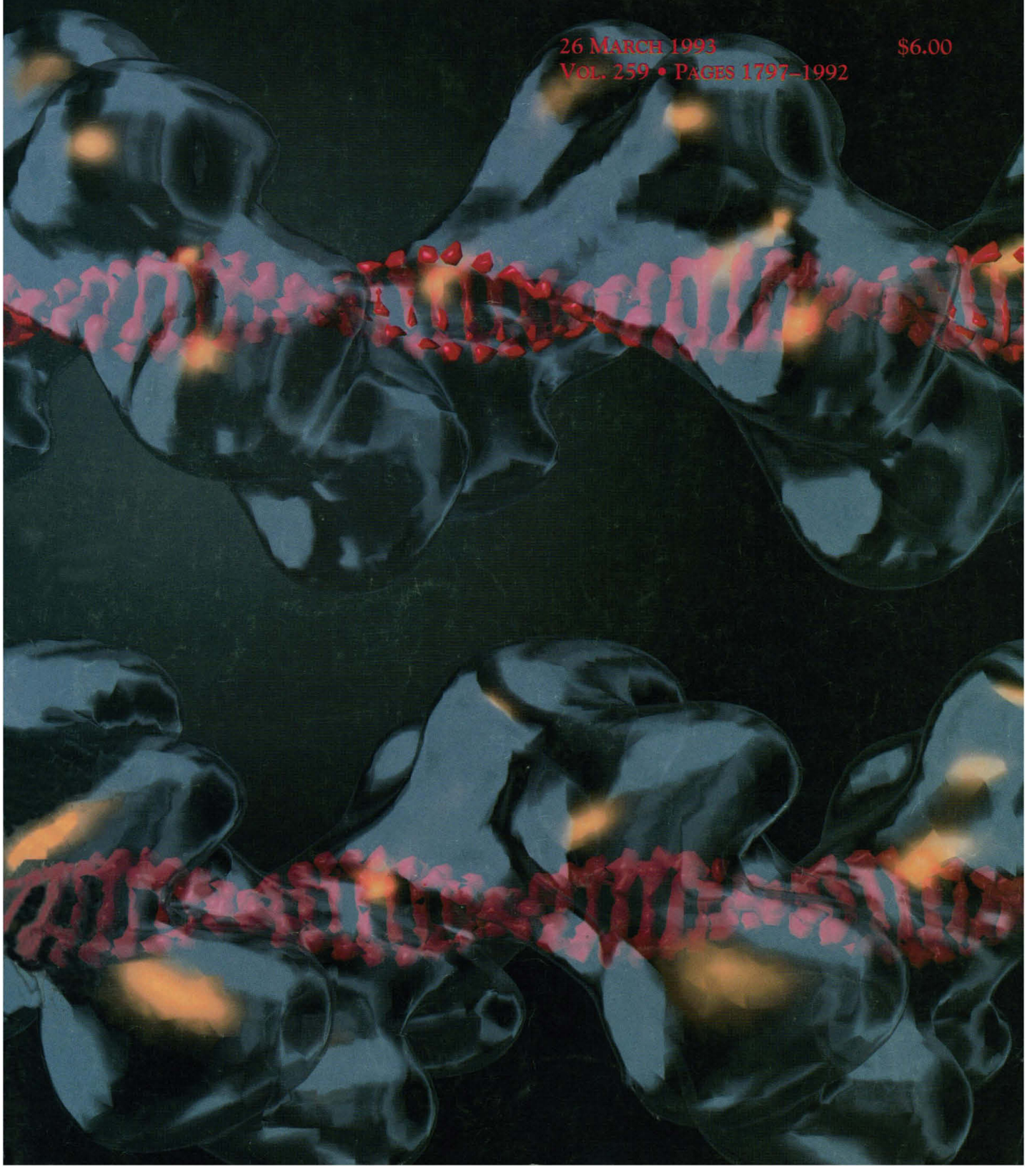

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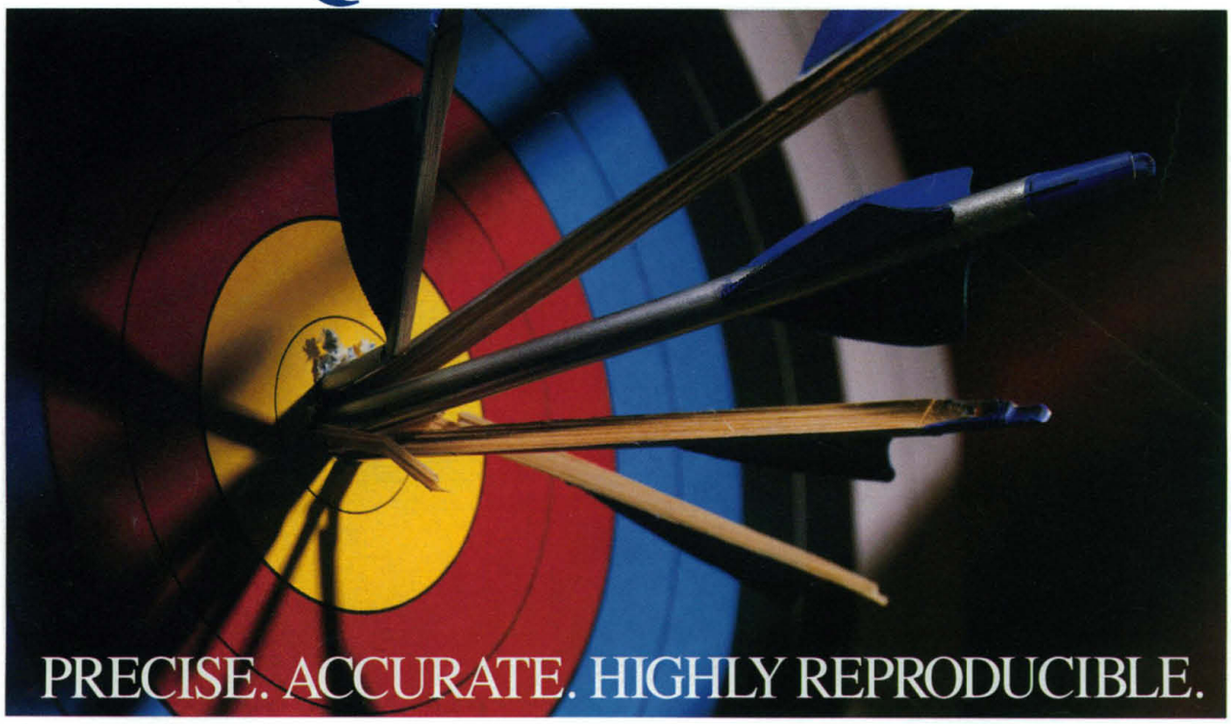

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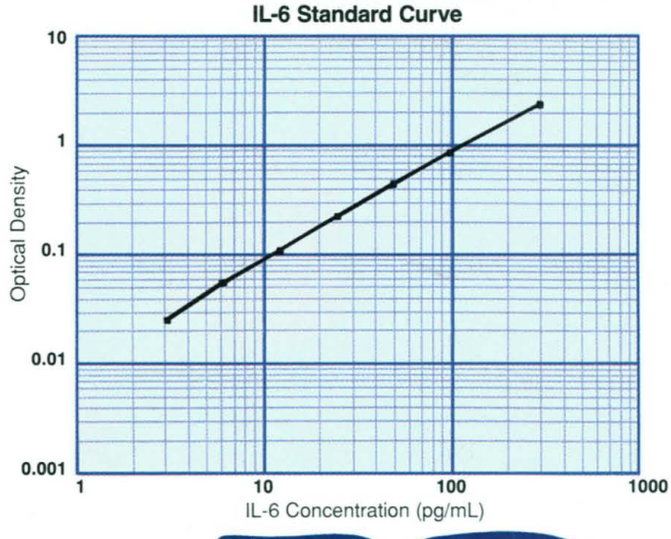

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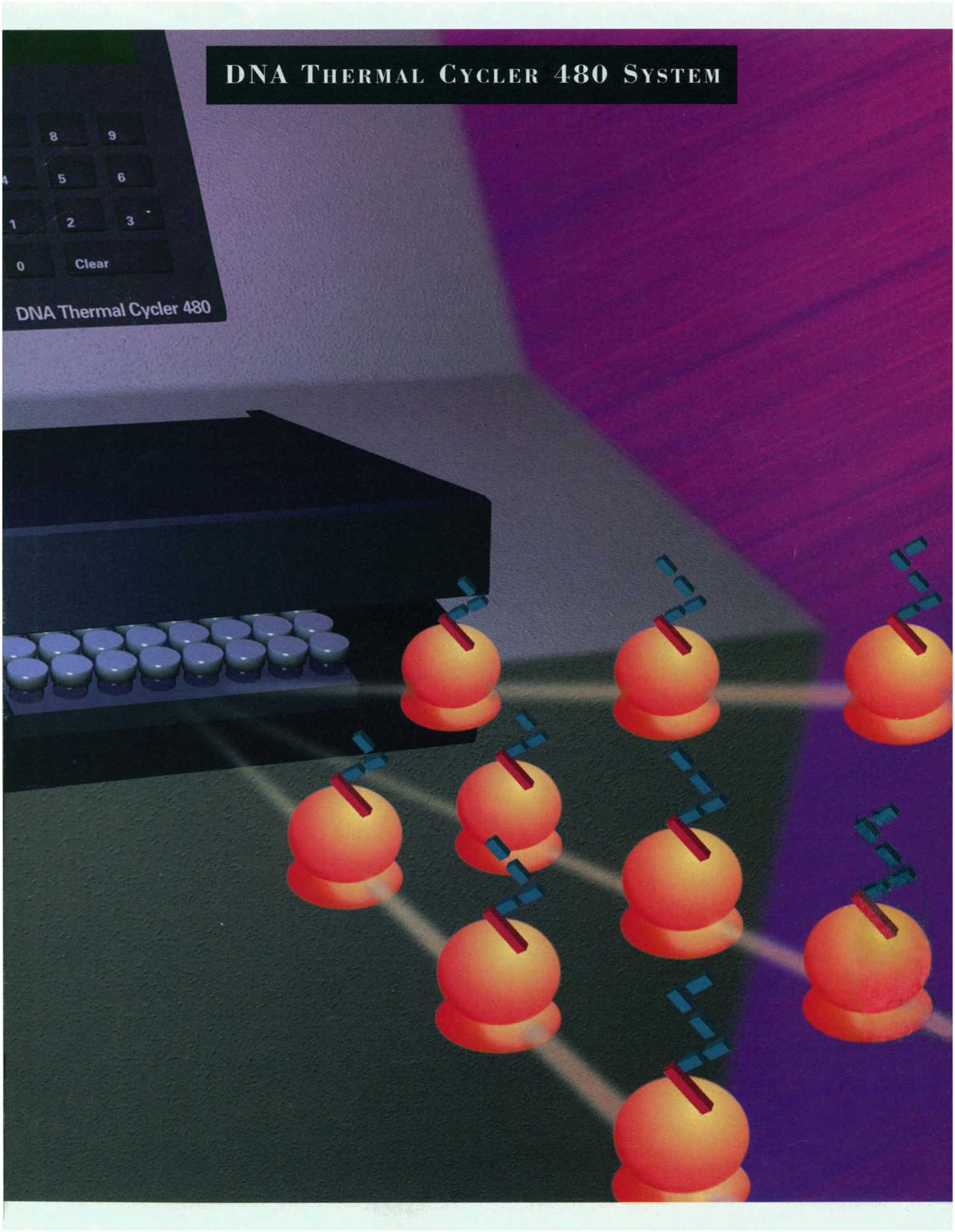
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Perkin-Elmer PCR reagents are developed and manufactured by Roche Molecular Systems, Inc., Branchburg, New Jersey, U.S.A.

Roche

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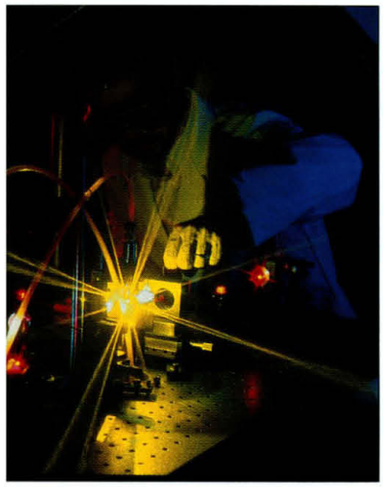


1816
Washington focuses on technology

NEWS & COMMENT

RESEARCH NEWS

POLICY FORUM

PERSPECTIVE

ARTICLES

RESEARCH ARTICLE

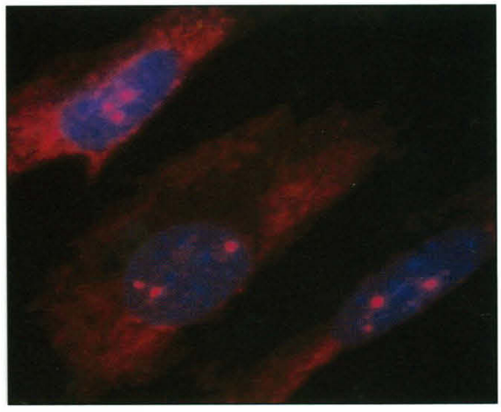
DEPARTMENTS

COVER

Transparent surface models of the helical nucleoprotein filaments formed by the yeast RAD51 protein (top) and the bacterial RecA protein (bottom). Within RAD51 filaments, DNA (red) is extended and untwisted in a manner similar to its extension and untwisting in RecA filaments. The homology of these filaments suggests that the structures for recombination in eukaryotes are similar to those in prokaryotes. See page 1896. [Computer graphic: Edward H. Egelman]

REPORTS

1867
Star dance



1904
Expression of YAC DNA transmitted through the germ line to generate transgenic mice

◩ Indicates accompanying feature

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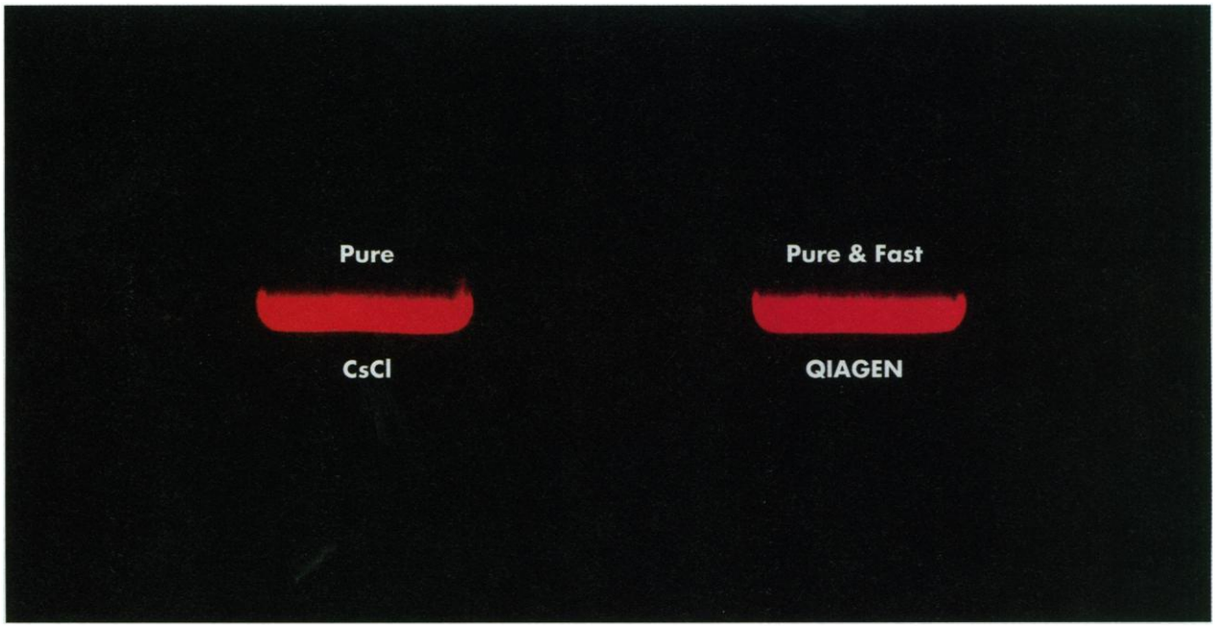

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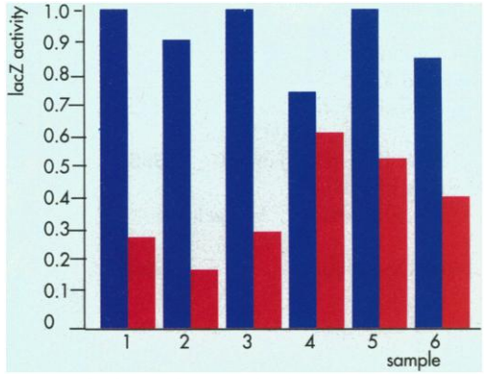

QIAGEN purified DNA for transfection: *NIH 3T3 cells were transfected with 1, 2 and 3 µg of the plasmid pRSVlacZ according to the standard calcium phosphate method. Plasmid DNAs used for transfection were prepared with QIAGEN Plasmid Mega Kit (blue bar) or by CsCl gradient centrifugation (red bar). The transfection efficiencies were determined by measurement of ß-galactosidase activity. Samples 1 and 2: 1 µg DNA: samples 3 and 4: 2 µg DNA; samples 5 and 6: 3 µg DNA. Data kindly provided by Frauke Ehlert, Institute for Molecular Biology and Tumor Research, Marburg, Germany.*

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edited by PHIL SZUROMI

Hot galaxies

Elliptical galaxies are elliptical not because they are rotating, but because the stars in them have distinctly anisotropic velocity distributions. As stellar systems, they are "hot," their shapes maintained against their own gravity by random motions characterized by three different velocity dispersions along their principal axes. Merritt (p. 1867) explains how *N*-body simulations have been used to calculate stellar orbits in a triaxial gravitational potential and show how those orbits can be populated so as to produce a mass distribution that leads to the presumed potential. Observational studies allied with these models have allowed astronomers to test for the presence of dark matter and massive black holes in elliptical galaxies.

Free-standing buckyfilms

Solid fullerenes are held together by van der Waals forces. Eom *et al.* (p. 1887) have found that these forces, usually considered among the weaker interactions, are strong enough to create free-standing films of fullerene molecules. The films were fabricated by micromachining a silicon wafer after thermal deposition of a C_{60} layer. The layer was uniformly etched down to 2000 angstroms in a CF_4 plasma. Measurements of the mechanical properties of the films suggest possible applications, including sensors and filters.

Tracing water in Mars

The amount of water originally contained in Mars and its degassing history are uncertain. McSween and Harvey (p. 1890) searched for clues of the amount of water in Mars' interior in the only direct samples of Martian rocks available—the so-called SNC meteorites, which are believed to be igneous rocks crystallized from a Martian magma and then ejected into space by a large asteroid impact. Analysis of melt inclusions frozen in crystals suggest that the water content of progenitor magmas was about 1.4 percent by weight. Degassing of this amount from all of the igneous rocks observed on Mars could yield a global ocean about 200 meters deep, which yields a volume that is still somewhat less than that required to account for the large flood structures on Mars.

Tracing ground water in the crust

Three ground-water systems of different ages and origins mix on a regional scale in the midcontinental United States. Musgrove and Banner (p. 1877) use chemical analyses to trace the origins of the waters and to characterize the mixing. Interestingly, two of the fluids are saline but acquired their salinity in distinct ways. Ground water originating in the Colorado Front Range and flowing to eastern Kansas becomes saline by mixing with brines leaching from halite-bearing rocks above the primary aquifers. Near northern Oklahoma, these waters mix with brines that may have originated from trapped Paleozoic seawater. Finally, these waters mix with dilute meteoric waters flowing west from the Ozark Dome in southern Missouri.

RecA relatives

In the presence of adenosine triphosphate, RecA polymerizes on DNA in prokaryotes to form a helical nucleoprotein filament in which double-stranded DNA is stretched and unwound. Within the filament, RecA catalyzes recognition, pairing, and strand-exchange between two homologous DNA molecules. Ogawa *et al.* (p. 1896) show that a yeast protein, RAD51, which has 30 percent sequence homology with RecA, can polymerize on DNA and form nucleoprotein filaments like those formed by RecA. The similarity of these types of filaments suggests that RAD51 may function like RecA. Story *et al.* (p. 1892) discuss the structural similarity of recombination proteins from yeast and bacteriophage to RecA.

Getting large genes into stem cells

Introducing large segments of DNA into the germ line cells of mice can be useful for functional studies of large genes and for identifying genes within the segment through their ability to complement lost genetic functions in mutant strains. Strauss *et al.* (p. 1904) used yeast artificial chromosomes to introduce a purified 150-kilobase DNA segment comprising the gene for α_1 (I) collagen into the germ line of a mouse. Modifications of existing technology resulted in significantly higher transfection efficiencies for transfer of lipid-bound DNA into embryonic stem (ES) cells. These cells were introduced into host-mouse blastocytes to generate founder chimeras for transgenic mouse strains. Fluorescence in situ hybridization and ribonuclease protection analysis confirmed that the intact transgene was present at one chromosomal location in each ES clone and was expressed at levels similar to that of the endogenous collagen gene.

Neuron model

Neurons manage to produce stable electrical signals despite changes in cell shape, replacement of ion channels, and extracellular conditions. LeMasson *et al.* (p. 1915) have developed a mathematical model for neuronal conductances in which the activity of the cell, as represented by the concentration of intracellular calcium, helps control the maximum conductances of ion currents. For example, a perturbation in extracellular potassium concentration, which induces continuous firing of potassium channels, increases intracellular calcium and restores burst firing of the channels by slowly decreasing inward currents and increasing outward currents. Such processes can also allow the neuron to maintain its pattern of electrical activity within a network. The model also allows the neuron to change its state in response to presynaptic stimulation.

Autoregulated factors

The transcription factor NF-κB is involved in gene activation in the immune, acute phase, and inflammatory responses. NF-κB is maintained in the cytoplasm in an inactive form when coupled to an inhibitory protein, IκB. When T cells are activated by stimuli such as mitogens, NF-κB is released from IκB and is translocated to the nucleus. Sun *et al.* (p. 1912) have identified an autoregulatory pathway in which NF-κB controls the expression of the IκB gene.

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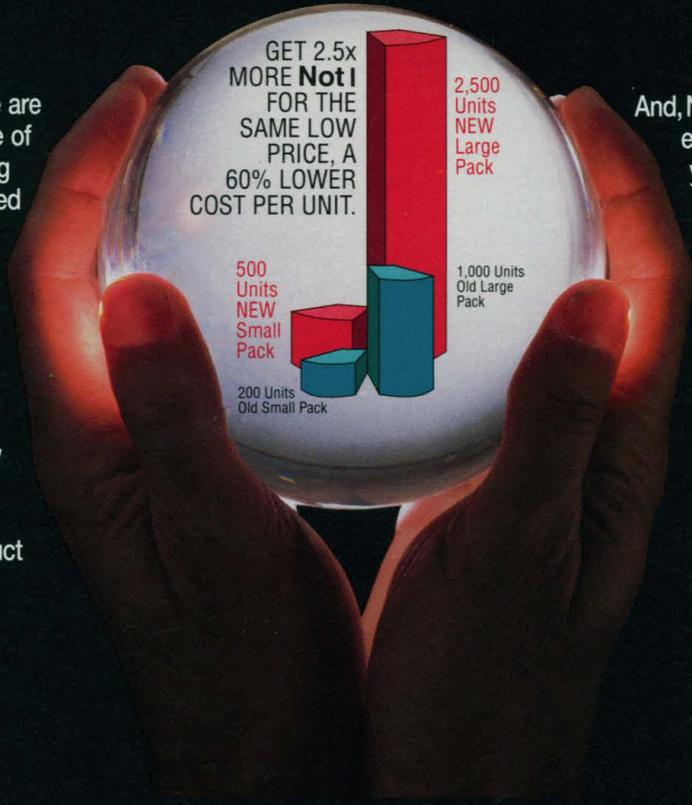

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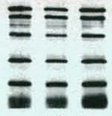

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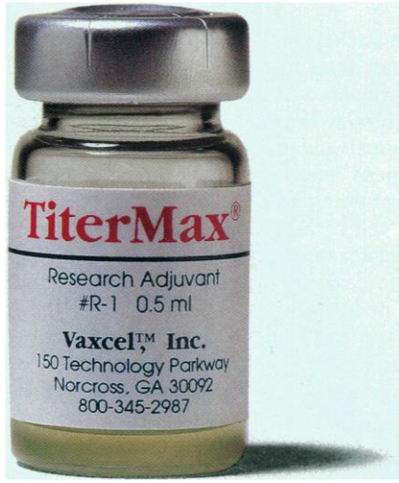

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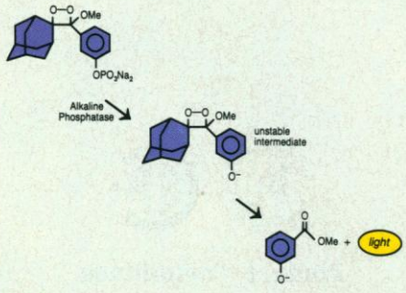
Scientists reluctant to incorporate nonradioactive labeling and detection into their research techniques have historically accused nonradioactive products of lacking sufficient levels of sensitivity. However, recent technological advancements — chemiluminescent detection, to be exact — have allowed select products to meet or exceed the sensitivity levels achievable with ^{32}P labeling and detection.

New information identifies ideal techniques for nonradioactive labeling and detection.

Those products, part of the Genius™ System with Lumi-Phos™ 530 from Boehringer Mannheim, guarantee detection of 0.1 pg of target nucleic acid in less than one hour. In addition, they allow detection of single-copy genes in human DNA in less than 30 minutes when used in Southern blotting techniques.



Molecular structure of the active ingredient in Lumi-Phos 530

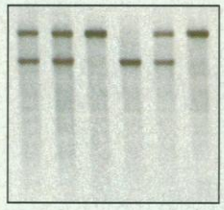
The Genius products rely on the very same procedures already used for radioactive labeling, yet provide results not just hours earlier, but *days* earlier.

Results can be recorded on X-ray film and, because the chemiluminescence lasts more than 48 hours, multiple exposures are possible. Those films can be easily scanned and quantified by densitometry.

Southern blotting: single-copy gene detection in 30 minutes

The accepted wait for accurate single-copy gene detection using ^{32}P labeled probes has always been one to three days. Now identical results can be achieved in 30 minutes with the Genius System and its chemiluminescent substrate.

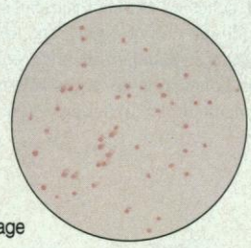
In addition, the probes are stable, allowing researchers to reuse them multiple times without relabeling. Furthermore, Southern blots developed with the Genius System can be repeatedly stripped and reprobed using the same procedures as the older radioactive method.



Human genomic Southern blot (RFLP) showing chemiluminescent detection of single-copy genes following a 2.5 minute X-ray film exposure

Library screening: positive colonies visible in one hour

Using probes labeled with the Genius System, researchers are detecting positive colonies and plaques within one hour versus the overnight exposures required with radioactive techniques.

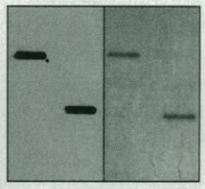


Colorimetric detection of recombinant lambda gt 10 phage

Because the Genius System can use DNA, RNA or oligonucleotide probes, many research needs can be met with a single system. Probe labeling utilizes the same techniques currently employed to prepare radioactive probes, and multiple filters can be processed simultaneously.

Dot blots: guaranteed results in under one hour

With exposure times reduced from overnight to 30-60 minutes, critical answers are relayed quickly, helping research progress as soon as possible.



Comparison of slot blot hybridizations using Genius-labeled oligonucleotide probe (left panel, 60 minute film exposure) and ^{32}P-labeled probe (right panel, overnight film exposure)

Performance is guaranteed as the Genius System products are function tested in dot blot (also referred to as slot blot) assays.

The unique digoxigenin-labeled probes are stable for at least one year and can be reused multiple times, offering savings in terms of the time and money normally spent relabeling decayed radioactive probes. By using the same probe, experimental variables are reduced and reproducibility between experiments is provided.

0.1 pg DNA Guaranteed

Technique-specific information and bibliography available upon request

To receive additional information about the Genius System products, their applications and specifications, or to receive a bibliography of publication references, call Boehringer Mannheim at **800-428-5433**.

If you would like to speak directly with a specialist about your individual research needs, call **800-262-4911**.

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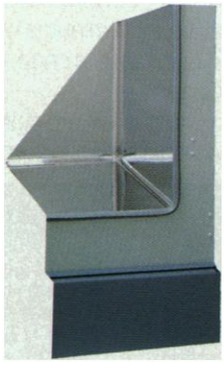
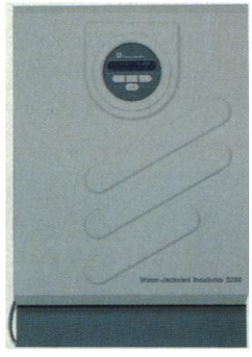


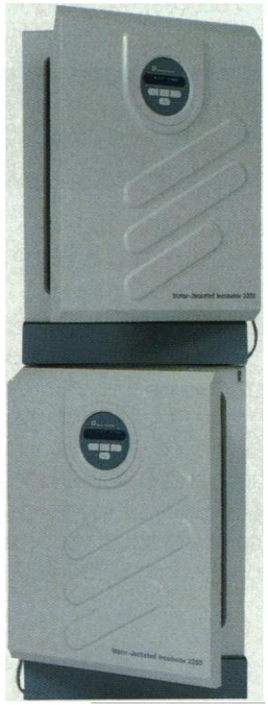
Cleanability

A crevice-free, stainless steel interior with mirrored finish features 100 percent 1/2" radius coved corners. No harsh finish to harbor contaminants. Construction of the chamber promotes aseptic culturing conditions.



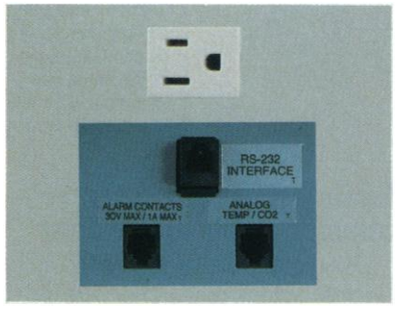
Precision Control

A door-mounted control panel features a microcomputer with vacuum fluorescent alpha/numeric display. Programming is easy with the touch key pad. Self-prompting messages allow you to control and monitor the critical performance parameters of the incubator. Proportional and integral (PI) algorithms combine with fuzzy logic characteristics to provide a stable culturing environment. A patented interactive dual temperature probe system provides precise balance of chamber air for ultimate temperature control and uniformity.



Functional Design

The 3250 water jacketed incubator gives you the flexibility to address any lab space requirements. Set on a countertop or stack them for maximum utilization of space. The incubator door swing can be reversed to accommodate lab layout or for side-by-side operation.



Interface Protection

Remote monitoring of temperature and CO_2 can be achieved through remote alarm contacts, millivolt output signal and optional RS232 interface. Standard electrical outlet is provided for connection to accessory items, such as temperature recorder, gas guard, etc .

Product Compliance

U.L. Listed and C.S.A. Certified, every 3250 incubator is manufactured to conform with the intense guidelines of these testing facilities. Copies of U.L. and C.S.A. documentation are available from Forma for compliance verification.

Direct sales representation in the USA.
International sales and service through authorized distributors in over 70 countries.

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Fax: 1-614-373-6770 Telex:29-8205
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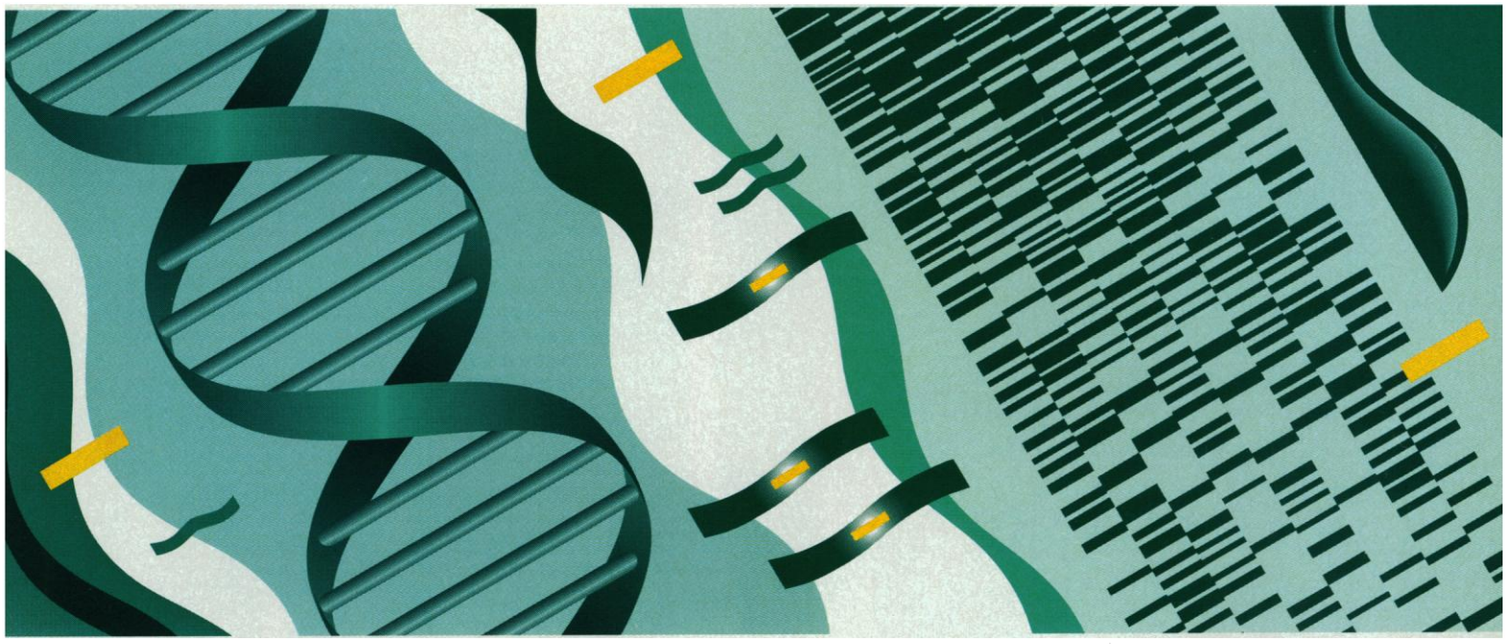
SEQUENASE* IMAGES™ NON-ISOTOPIC SEQUENCING



New Sequenase* Images™ Non-Isotopic DNA Sequencing System

For fast, non-radioactive detection and reliable results.

- **Features Sequenase Version 2.0 T7 DNA Polymerase**
- **A simple blotting procedure using Sequenase Images membrane**
- **Hard-copy results on standard X-ray film**
- **Reactions to results in a single 8-hour day**
- **Compatible with Mn Buffer,** dITP, Sequence Extending Mix and Glycerol-Tolerant Gel Buffer†**

The Sequenase Images System (Product No. 71350) includes the Sequenase Images Non-Isotopic DNA Sequencing Kit (Product No. 71352) and the Sequenase Images Detection Kit (Product No. 71355)

Features of the Images Product Line include:

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- **Gene Images Kit**
  Product No. 75275
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  Detection of Northern blots
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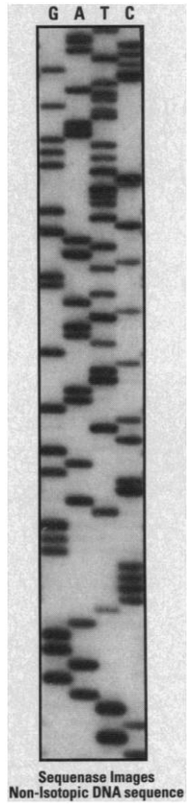
International: 216-765-5000.
Telex: 980718. Fax: 216-464-5075.



Sequenase Images
Non-Isotopic DNA sequence

*Sequenase is a registered trademark of United States Biochemical Corporation. This reagent (kit) contains materials covered by or suitable for use under one or more U.S. Patent Nos.: 4,795,699; 4,946,786; 4,942,130; 4,962,020; 4,994,372, 5,145,776 and 5,173,411. Patents pending in the U.S. and other countries.
**Mn Buffer - Purchase includes a nonexclusive sublicense solely for use with Sequenase® T7 DNA Polymerase. No other license is granted expressly, impliedly, or by estoppel.
†Glycerol Tolerant Gel Buffer - Pat. pending.

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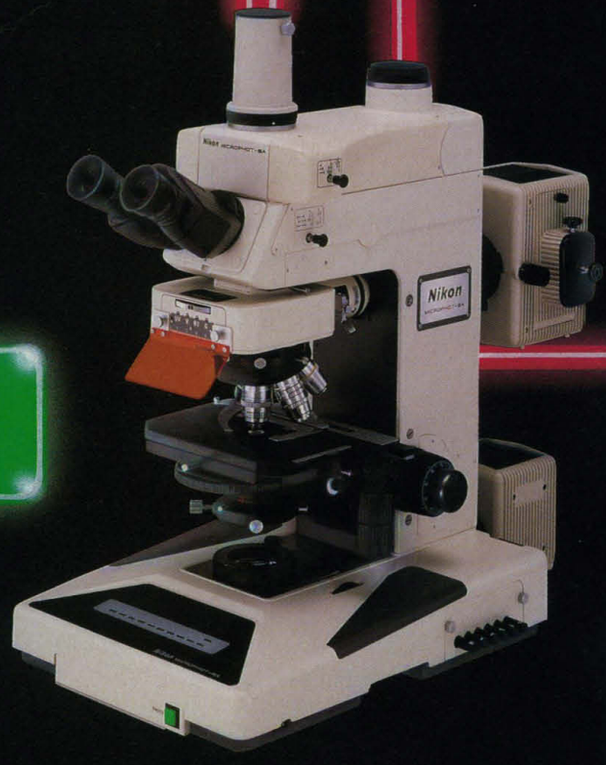
The system:
Analytical microscopy workstation

The instrument:
Nikon Microphot-SA

High Resolution Video Camera

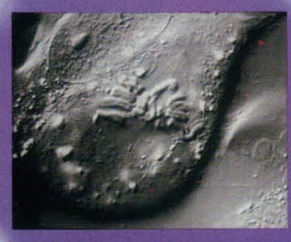
Automatic 35mm Camera with Spotmeter

Image Processing & Analysis



Host Computer

Microspectro-photometer



The advanced Microphot-SA research microscope is designed to be the heart of your analytical workstation, today and tomorrow.

Capable of being run from a host computer, it is uniquely adapted to complex multi-imaging requirements. For instance, perform simultaneous real-time image processing and microspectrophotometry. Or put world famous Nikon CF® optics to work for both high resolution video microscopy and photomicrography, in all techniques including DIC and fluorescence.

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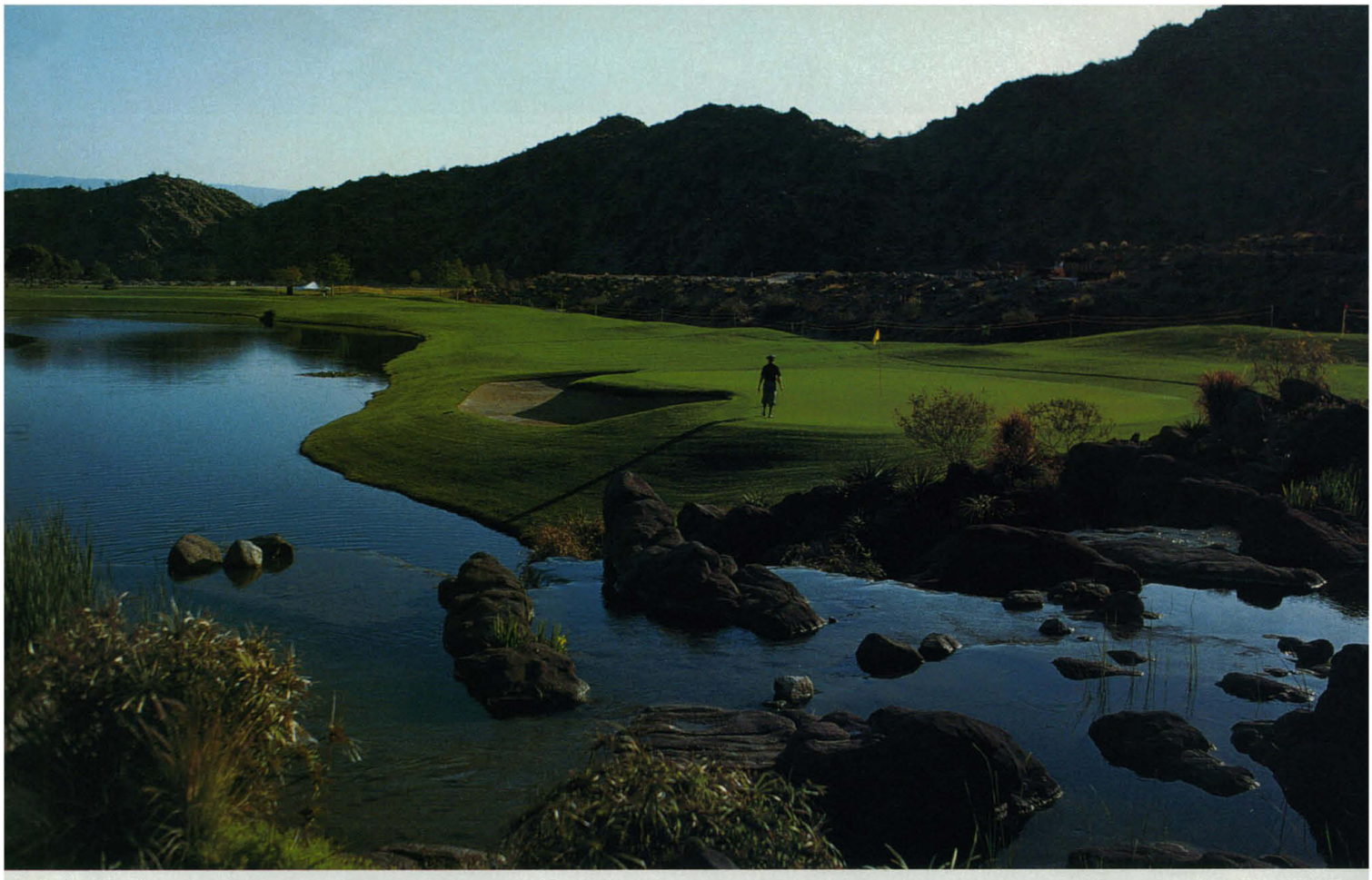
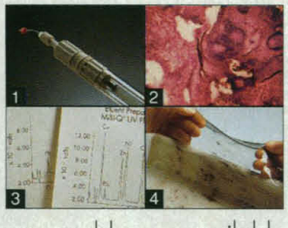


There Are Places You Expect To Find Water Hazards. Your Lab Shouldn't Be One Of Them.

Water hazards on a golf course can cost you a couple of strokes. In a lab, however, water hazards in the form of ions, pyrogens and organics can cost you months of research work.

That's why it's critical for you to have a water purification system that removes contamination instead of one that contributes to it. If you don't, you could end up like one of these facilities:



1. A lab doing protein purification with HPLC found that endotoxins in its water system contaminated proteins.

2. Organics in another facility's water system kept mammalian cells from growing in defined medium.

3. A semiconductor plant testing for copper had its chromatographic results ruined by contamination in their water system.

4. In yet another lab, phosphates being used to clean the water system got into the water used for polyacrylamide gels, rendering them useless.

In each of these cases, when the lab switched to a Milli-Q® Plus Water Purification System, all their problems disappeared.

That's because the Milli-Q Plus system delivers the highest quality water available: 1.5 liters per minute at 18 megohm and < 10ppb TOC (with RO pretreatment).

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And unlike other systems, changing our module is easy. Instead of separate bowls, our QPAK™ purification module has an all-in-one design that takes hardly any time to change out and prevents you from ever having to come in contact with wetted surfaces.

Also, because we don't use separate bowls, the system has virtually no hold-up volume. This results in faster flush-ups, along with reduced extractables and bacterial growth.

To further prevent water degradation, the system even recirculates water automatically every hour for approximately five minutes.

So beware of less expensive water purification systems that claim to do as good a job as the Milli-Q Plus system: They may prove hazardous to your work.

For additional information about the Milli-Q Plus system, including a brochure, technical bulletins and data sheet, call 1-800-225-1380.

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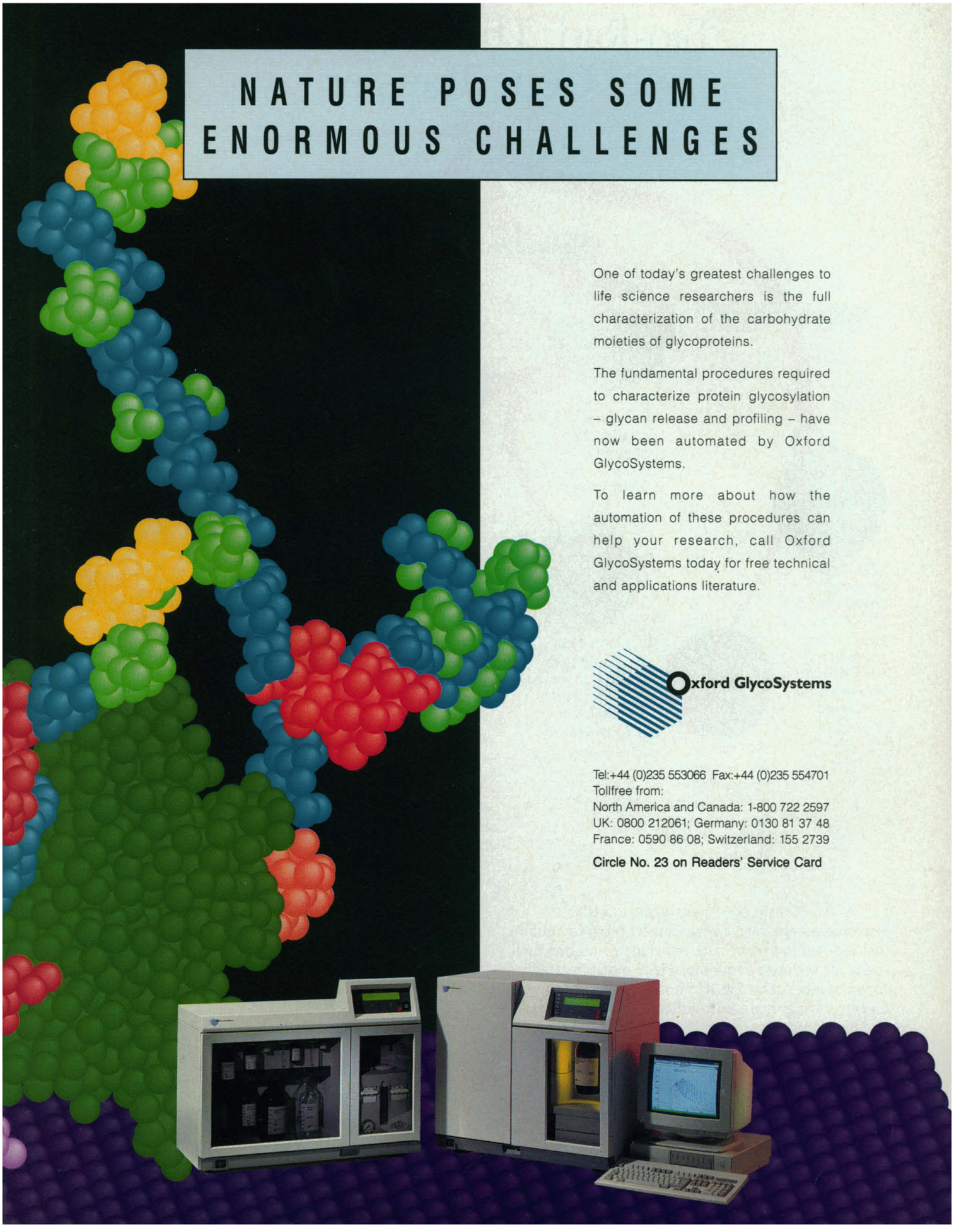


Bio-Rad: The Evolution of Gene Transfer

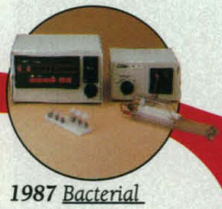
Earlier methods of Gene Transfer

Electroporation

Particle Delivery

Support Data

1986 *The Gene Pulser® system brings affordable electroporation to biochemists and molecular biologists.*



1987 *Bacterial electroporation efficiencies increased dramatically with the introduction of the Pulse Controller and 0.2 cm cuvettes.*

10^{10}

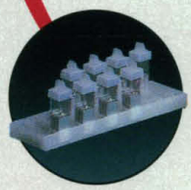
1988 *Bio-Rad pioneers 10^{10} transformants/µg: E. coli protocol optimized.*

1991 *Bio-Rad introduces DuPont's Biolistic® PDS-1000/He particle delivery system to the world market for plant and animal applications.*

1989 *Bio-Rad guarantees high efficiencies with the introduction of convenient Electro-Competent® cells.*

1993 *Particle delivery system expands to accommodate live animal work including gene therapy and genetic immunization.**

1991 *Bio-Rad introduces the E. coli Pulser™ unit —the first dedicated pulser for efficient, economical library construction.*



1990 *Prokaryotic applications and efficiencies boosted by new 0.1 cm cuvettes.*

1986-2010 *The users club binder —yours free— continually keeps you up to date on the latest gene transfer protocols for plant and mammalian cells, bacteria, yeast and other fungi, etc.*

At Bio-Rad we don't have a gene transfer instrument—we have a gene transfer program. One that Bio-Rad has pioneered, researched, and provided to the life science community. Plant and animal cells, bacteria, other cells: Bio-Rad not only gives you the tools to efficiently transform them, but also supports you throughout your research with unparalleled support, free telephone consultation, and unlimited access to published protocols and yet unpublished data. We have been dedicated to gene transfer since 1986, and we will continue to further your success with high quality electroporation and particle delivery products.

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* Tang, D., DeVit, M. and Johnston, S.A., *Nature*, **356**, 152-154 (1992). Biolistic is a registered trademark of the DuPont Company.

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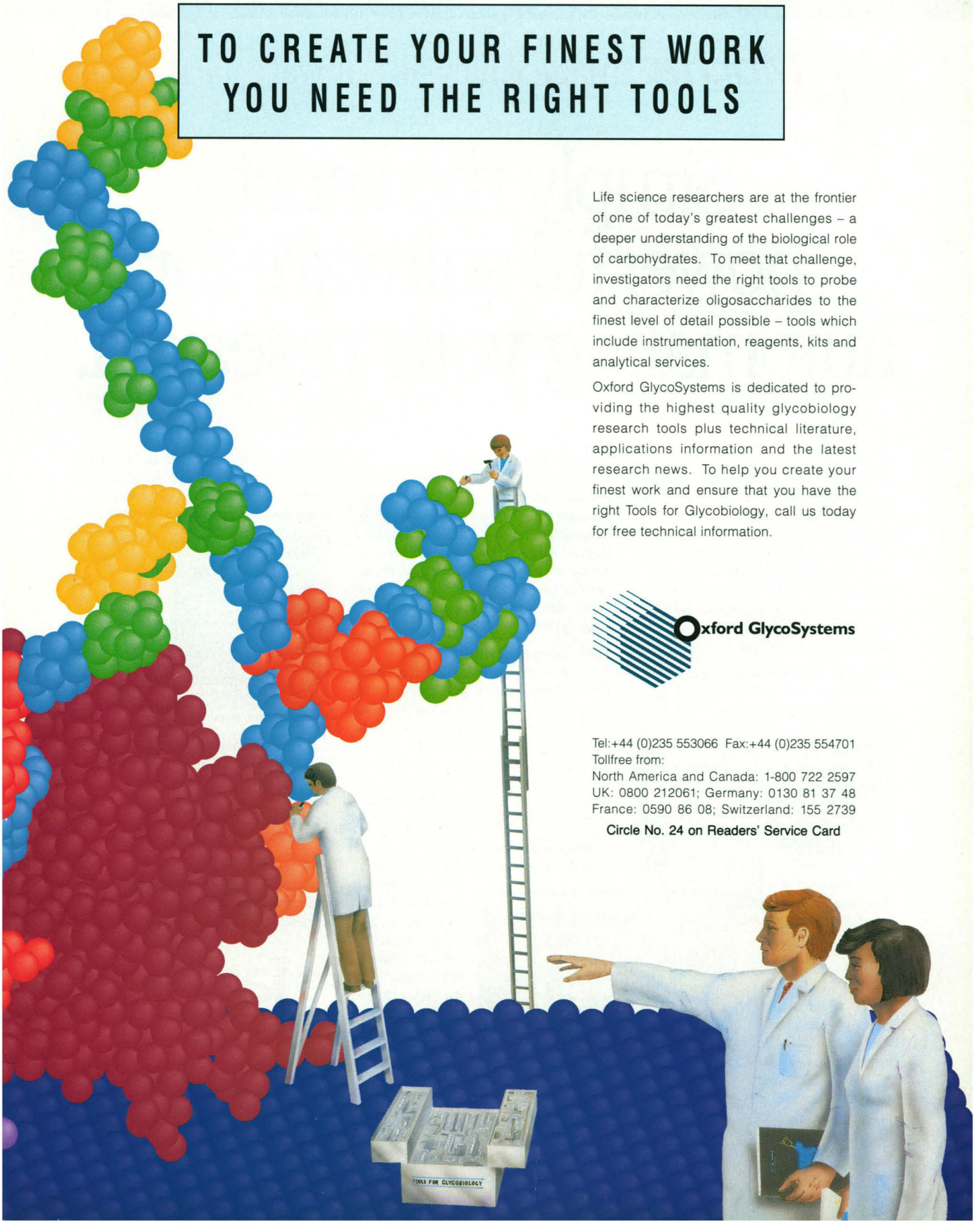
TO CREATE YOUR FINEST WORK YOU NEED THE RIGHT TOOLS



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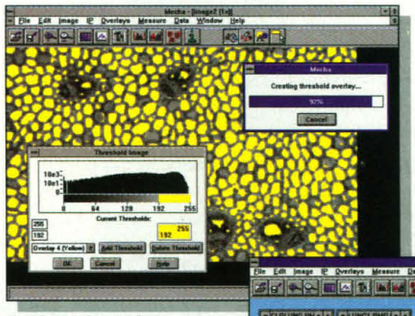

Analyze color and black and white images.

Integrated data worksheet.

Multiple Image Formats

MOCHA accepts a range of image formats so you can work with images from video cameras, scanners, or even from disk. With features such as pseudo-clearfield equalization, multiple image splicing, floodfill measurements, and a host of enhancement filters, you can process and analyze your images swiftly, with or without access to the original image source!

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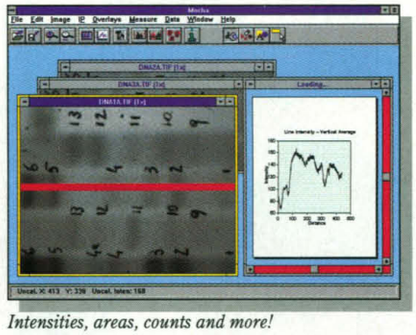
MOCHA can analyze saved or scanned color and b/w images on a standard PC (386 or better) using a single monitor, with or *without* a frame grabber. If you work with live images, MOCHA supports a variety of popular color and monochrome frame grabbers in both single and dual monitor configurations. This flexibility means you can use MOCHA for desktop image analysis (no additional hardware required), or as a workstation for capture and analysis of live video images.



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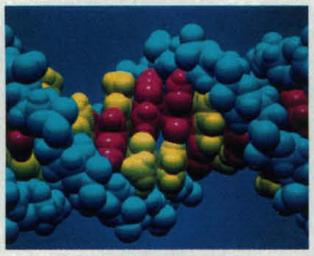
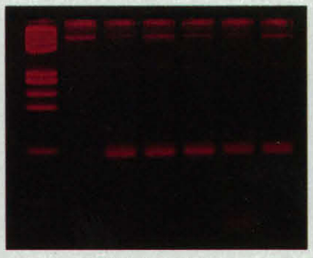
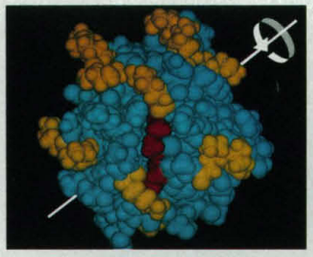
One Fluorimeter Fits All.



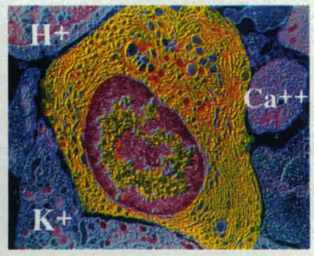
DNA Quantitation



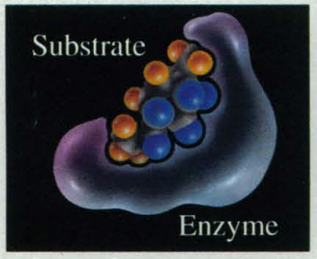
Gel Scanning



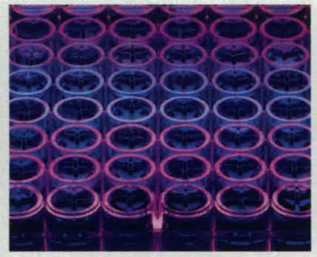
Macromolecular Anisotropy Measurements



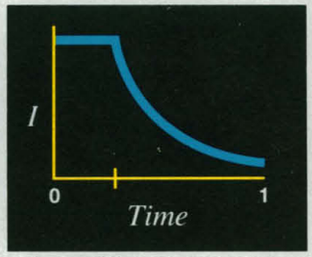
Intracellular Ion Determinations



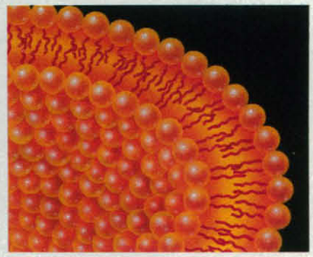
Enzyme Kinetic Analysis



Microplate Assays



Stopped-Flow Kinetic Analysis



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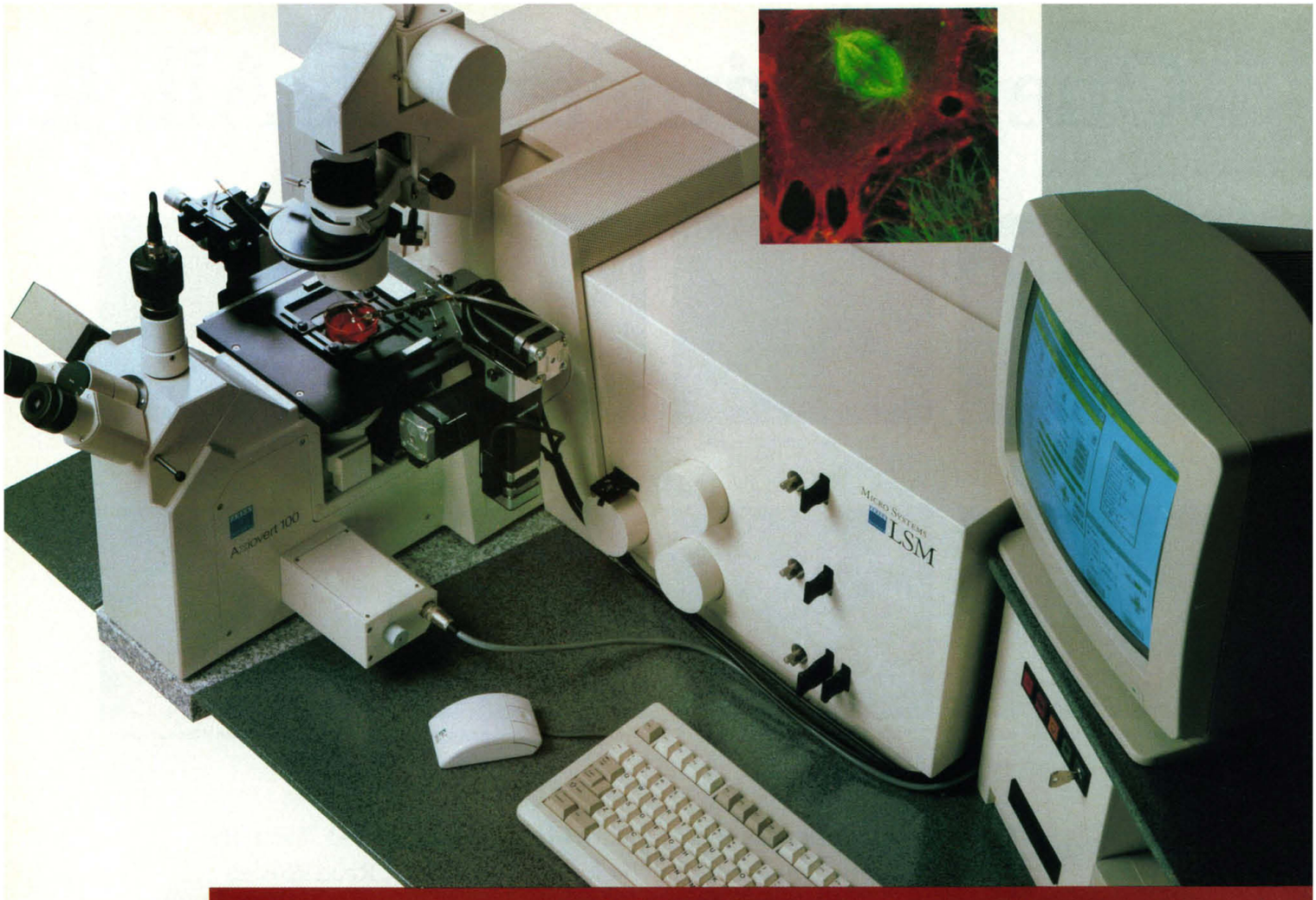

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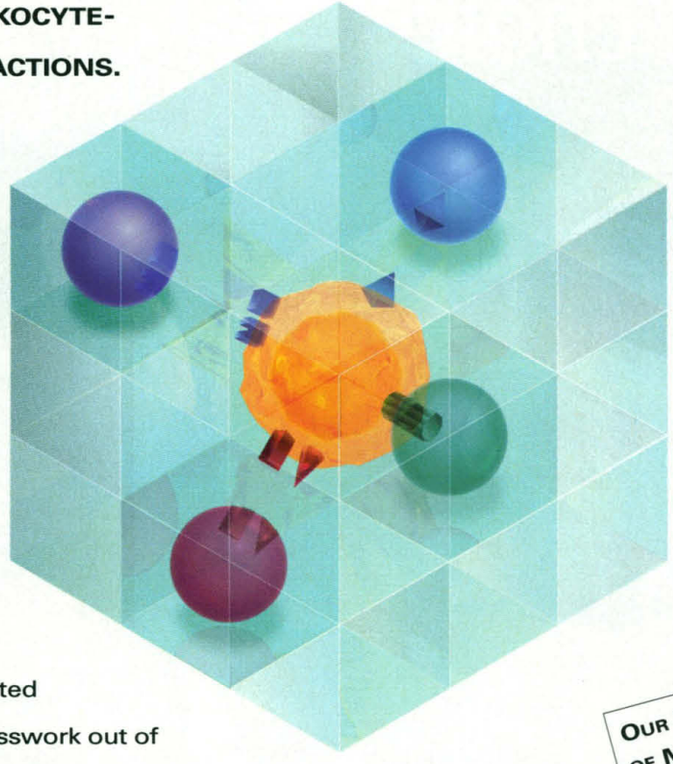

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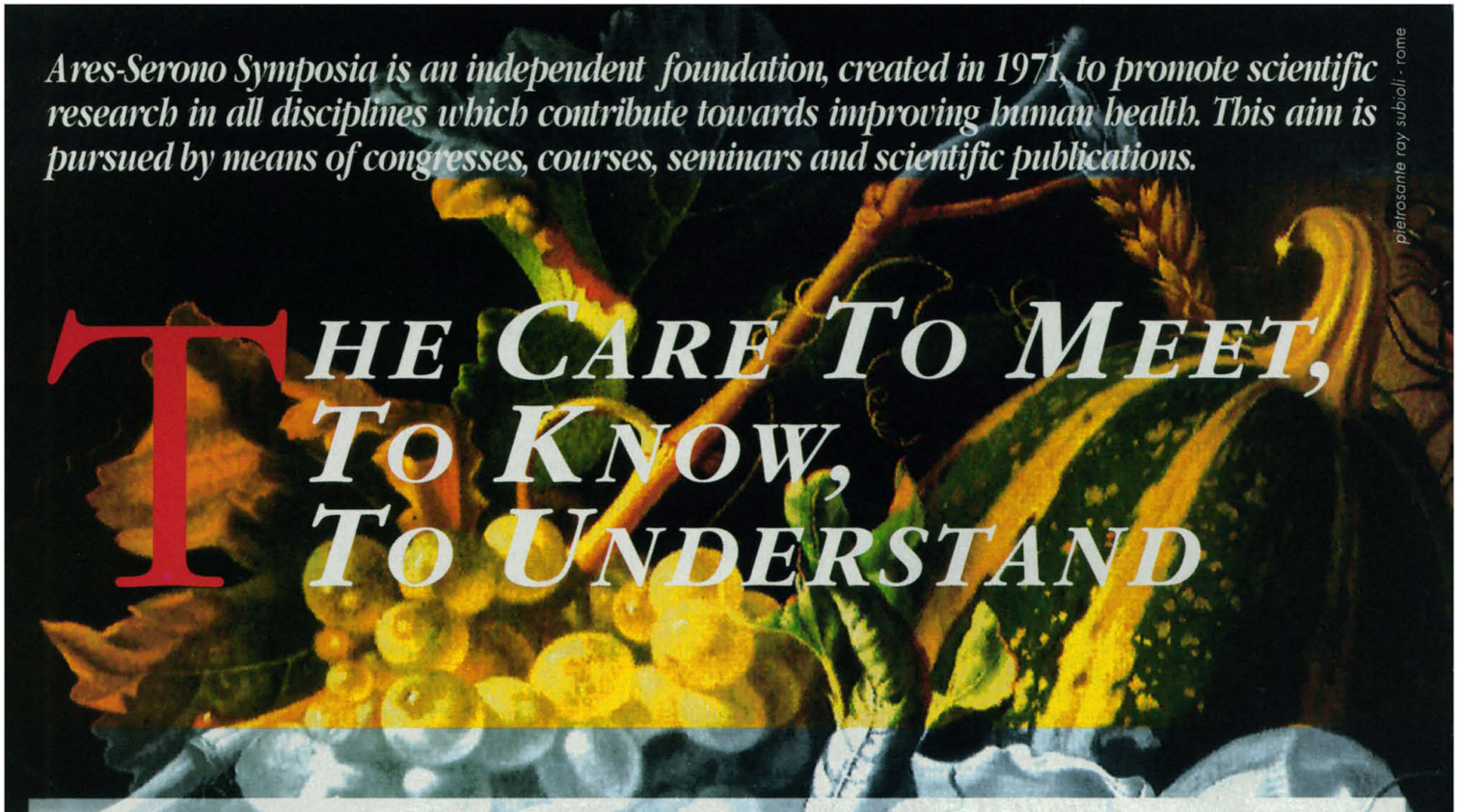

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J. Navarro (USA)
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D. Corda, S. Garattini and A. Luini (I)
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M.L. Aubert and P.C. Sizonenko (CH)
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S.A. Aaronson (USA) and R. Verna (I)
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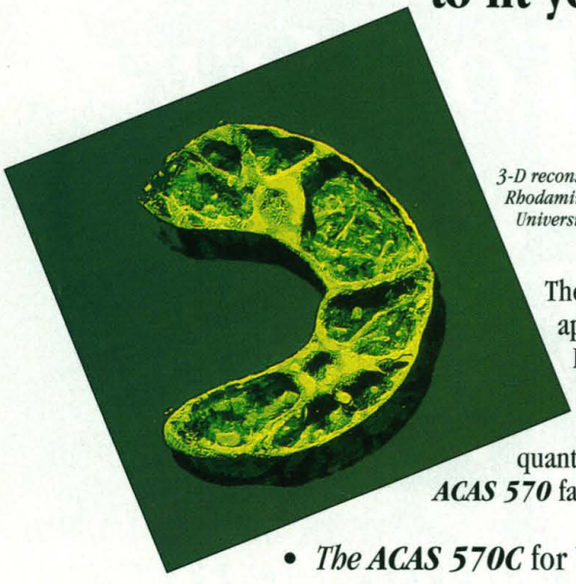

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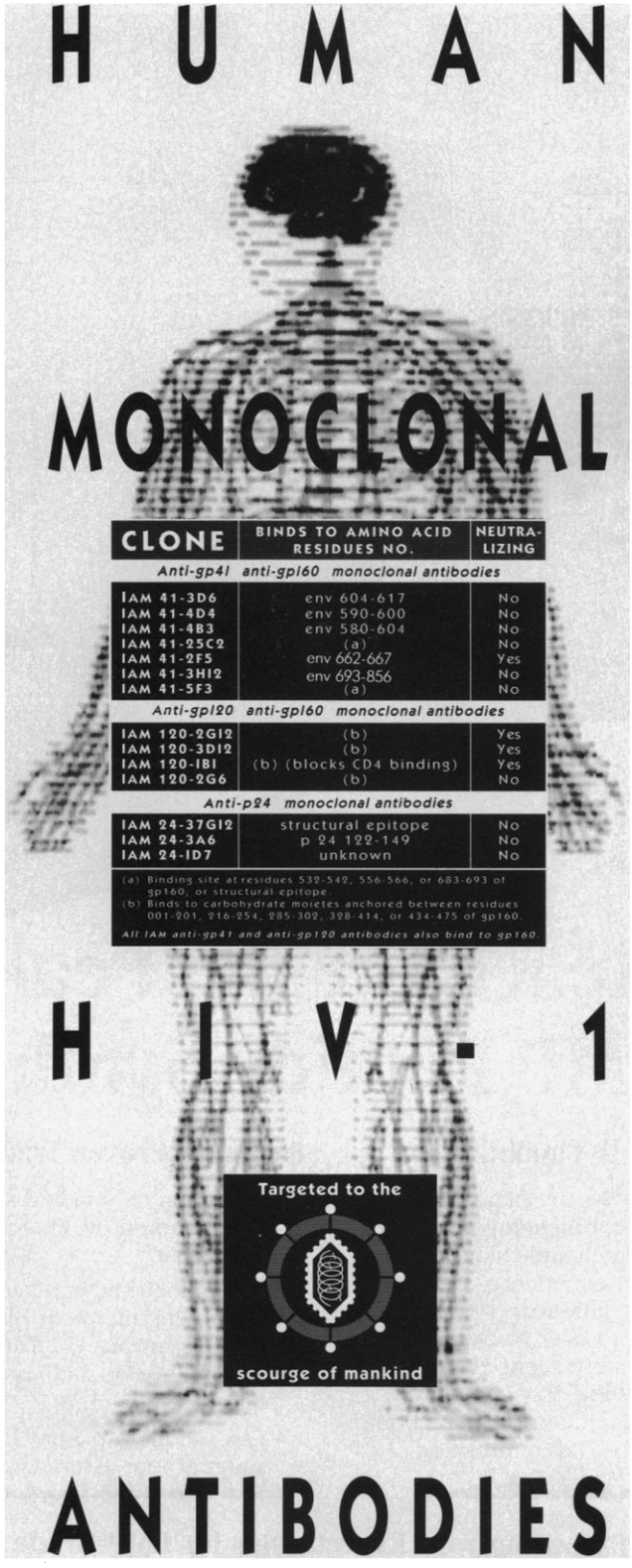

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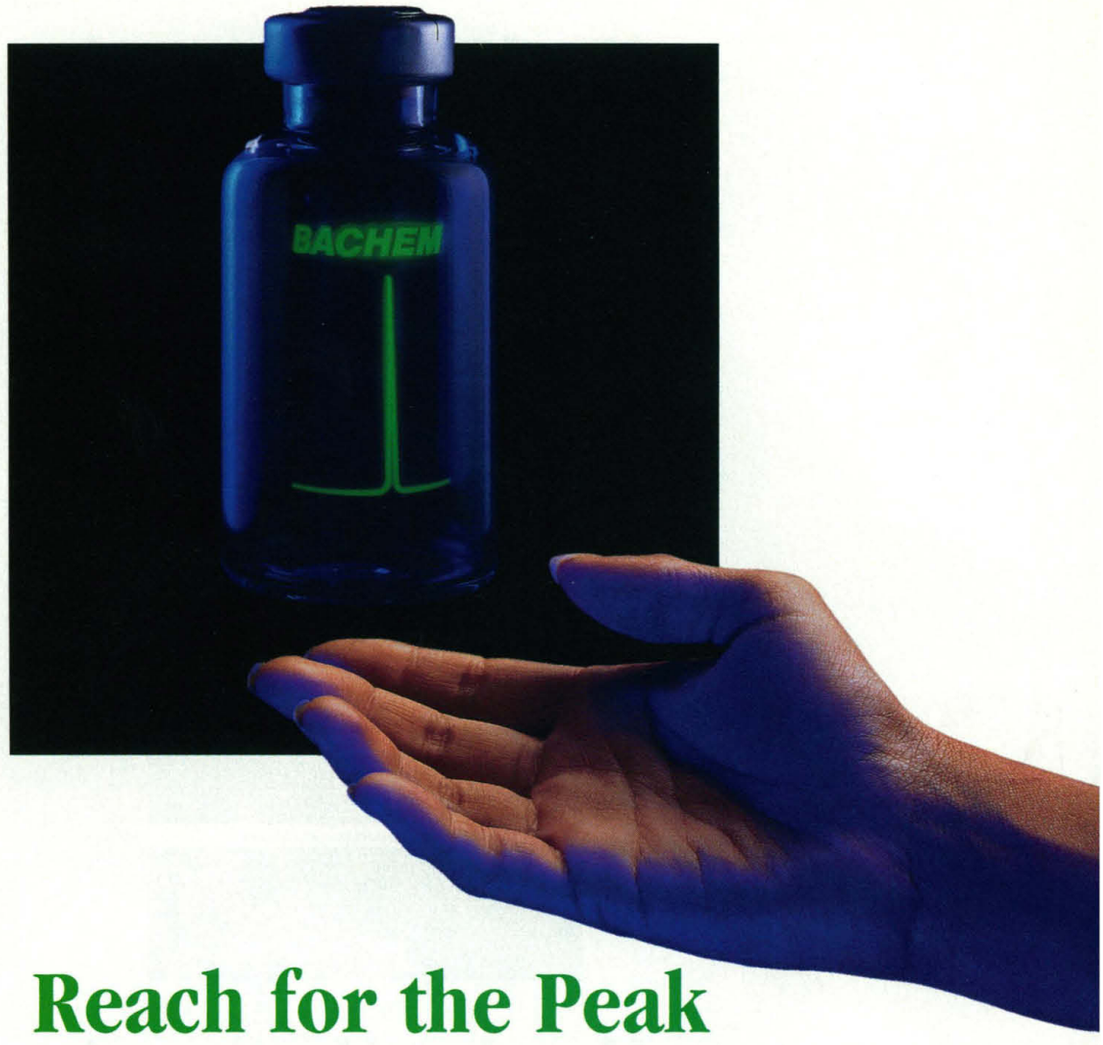

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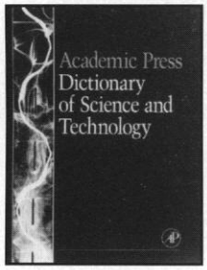

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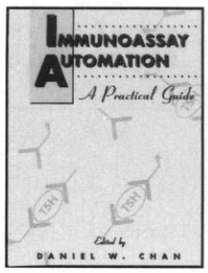

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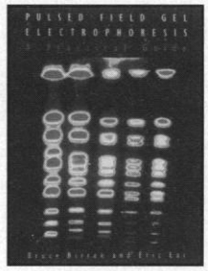

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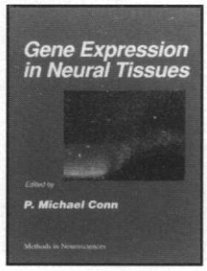

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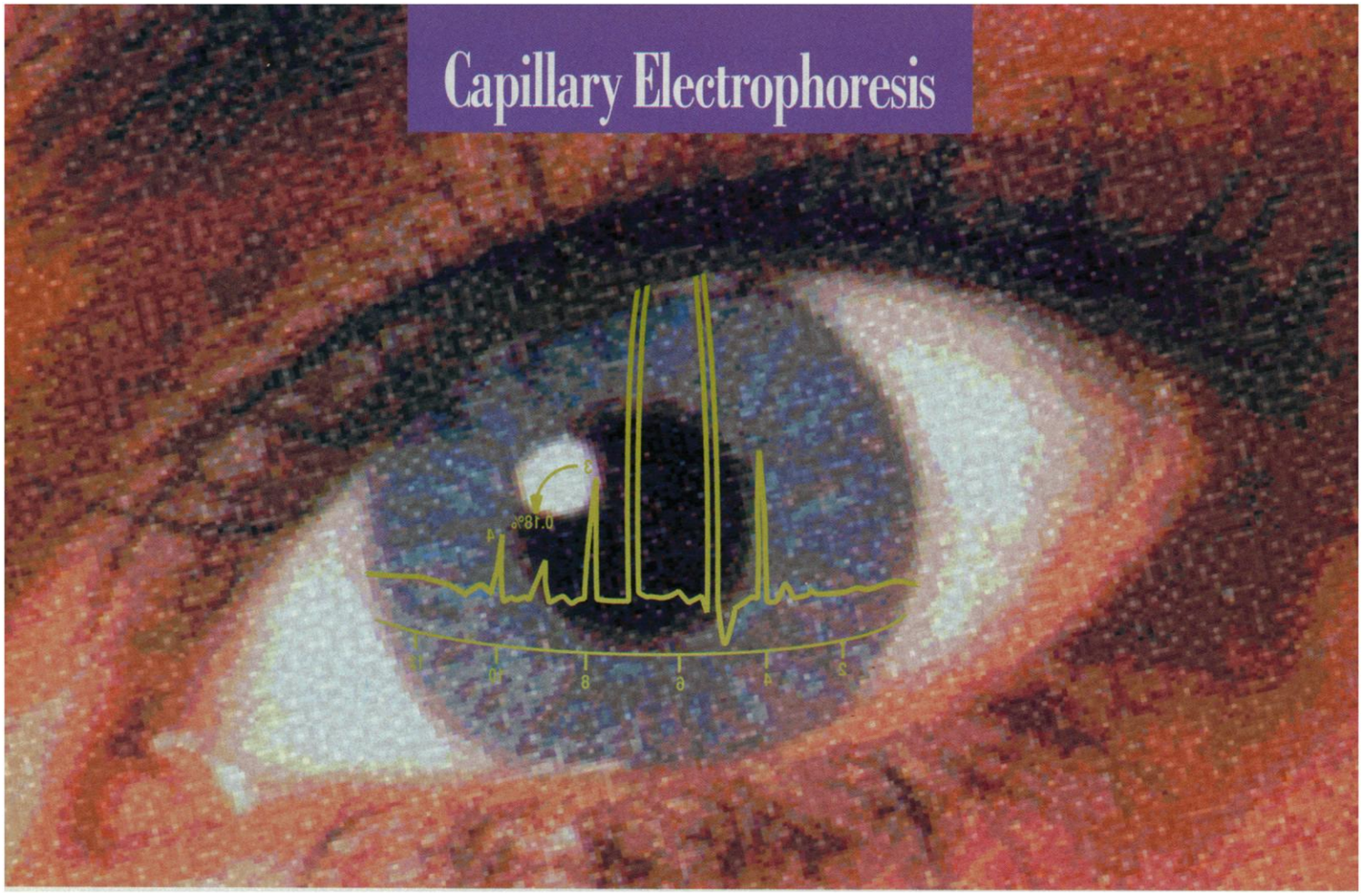

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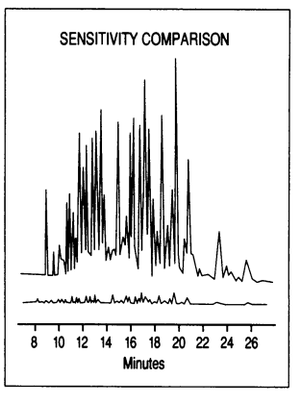

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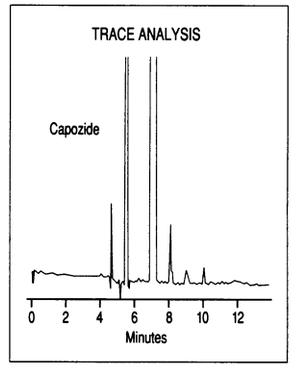

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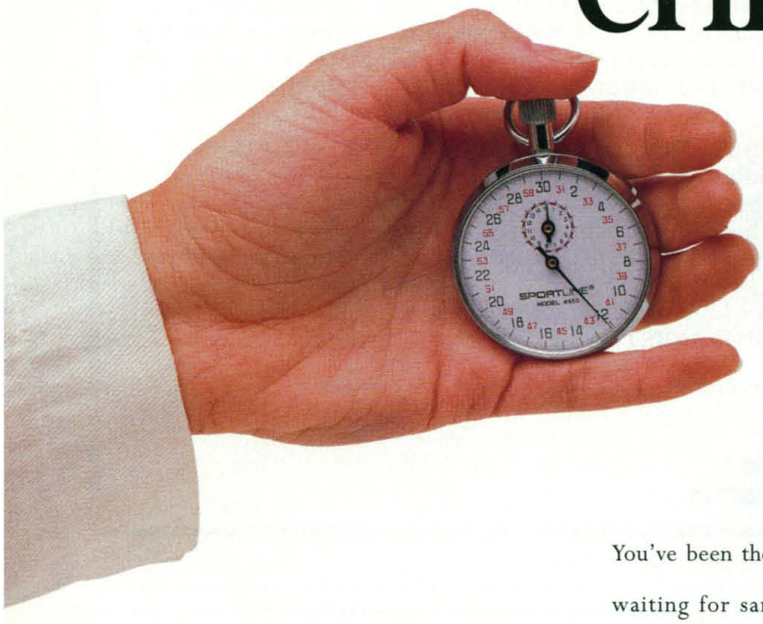

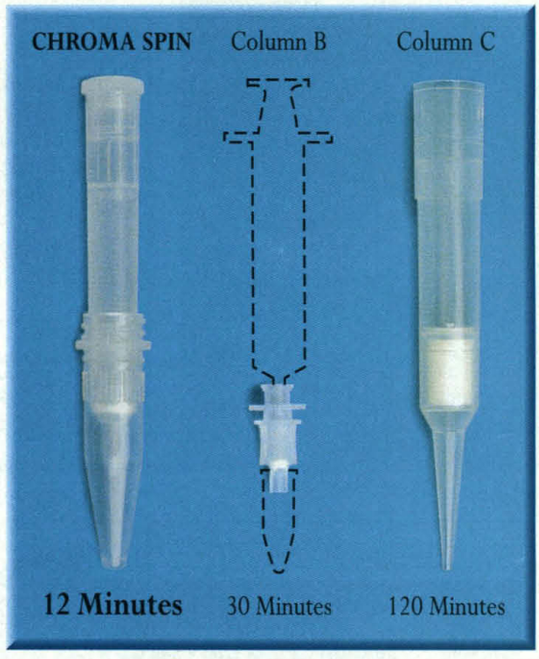

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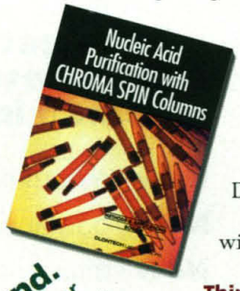

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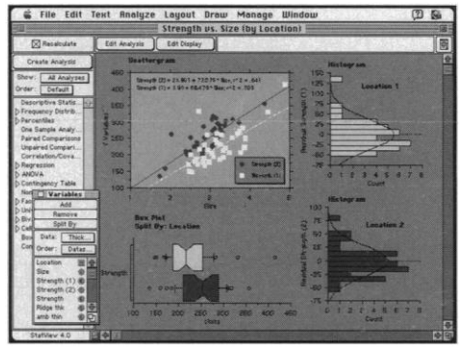

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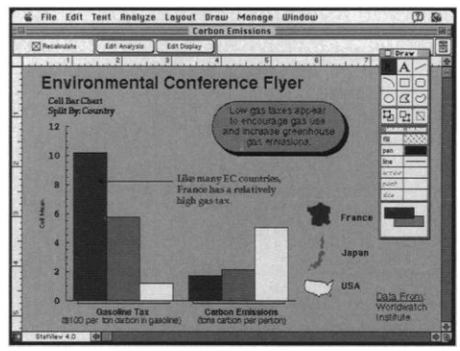

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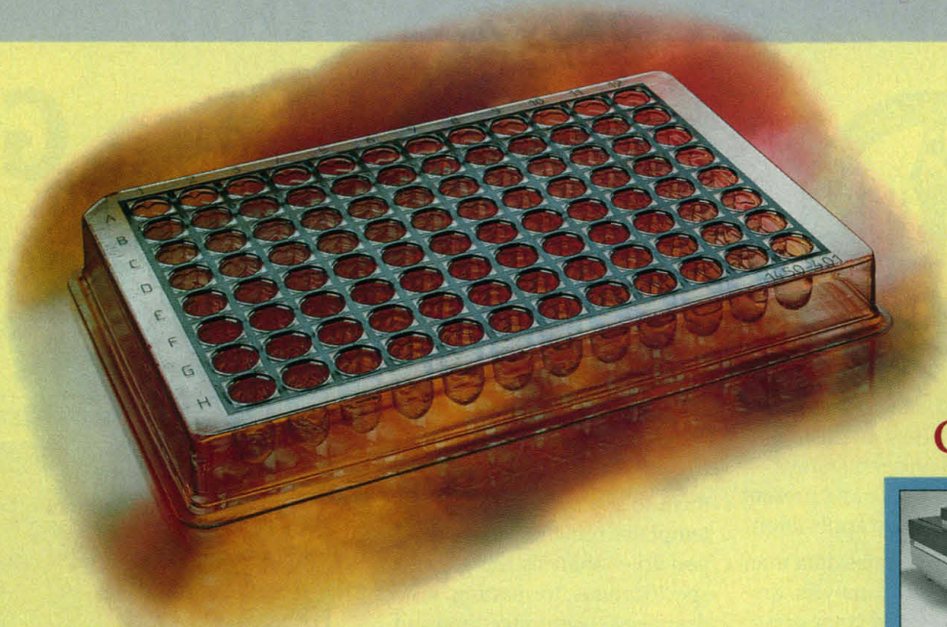

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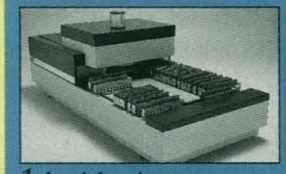

1st rack-based counters - RackGamma (LKB-Wallac, 1975), RackBeta™ (1977)

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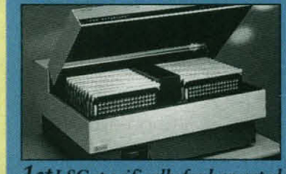

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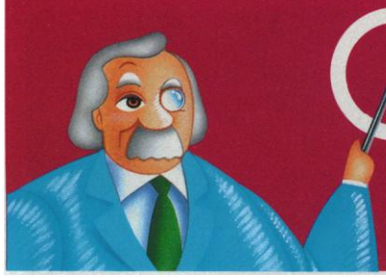


One Hour Protocol

including DNA elution/purification from agarose gels and ligation reaction, achieves subcloning or size-fractionated deletion cloning within one hour.

■By combining SUPREC™-01, SUPREC™-02 and the DNA Ligation Kit, the ONE HOUR PROTOCOL facilitates DNA recovery followed by cloning with speed and simplicity. Furthermore, the amount of DNA required for this protocol could be at the level comparable to what would generally be used for analytical electrophoresis (0.5 ~2 μg for subcloning and 5 μg for size fractionated deletion cloning).

Products Required

1. SUPREC™-01 (Code No.9040)

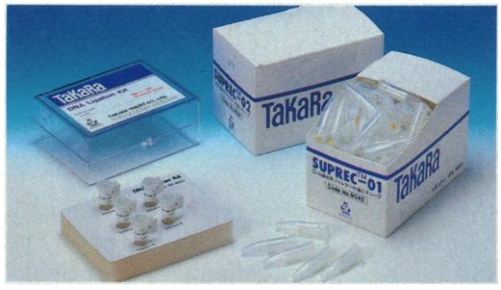
Microcentrifuge cartridge for DNA recovery from agarose gel.

2. SUPREC™-02 (Code No.9041)

Microcentrifuge cartridge for sample concentration and/or for removal of small DNA fragments.

3. DNA Ligation Kit (Code No.6021)

Two-component system containing all the reagents necessary to perform DNA ligation in 30 minutes with considerably high transformation frequency.



Please contact your local representative for details and pricing.

One Hour Protocol

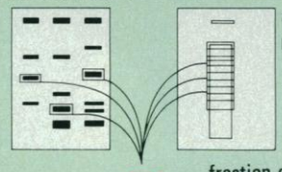
Step1

Cut 1~2 μg of DNA with restriction enzyme and separate by agarose gel electrophoresis.

Cut out the gel containing the DNA band of interest.

Treat 5 μg of plasmid DNA with Kilo-Sequence Deletion Kit (Code No.6030) and separate by electrophoresis.

Cut out the gel containing the DNA fraction of required size.



Step2 SUPREC™-01 — 5min

Put the agarose gel slice into the inner cartridge of SUPREC™-01 and centrifuge at 10,000 rpm for 5 min.

Step3 SUPREC™-02 — 3min

Transfer the filtrate into the inner cartridge of SUPREC™-02 and centrifuge at 6,000 rpm for 3 min. Add 15 μl of TE and collect DNA retained on the filter membrane by pipetting.

Step4 LIGATION — 15min

Add 80 μl of A solution and 15 μl of B solution of the Ligation Kit into the 10 μl of recovered DNA solution. Allow to stand at room temperature (16~25°C) for 15 min.

Step5 TRANSFORMATION — 31min

Add the 10 μl of Ligation solution to 100 μl of competent cells and leave in the ice bath for 15 min. After heat shock (at 42°C for 30 sec), add 890 μl of SOC culture medium and cultivate with shaking at 37°C for 15 min.

Total 54min

Step6 PLATING

Concentrate the culture medium into 200 μl and plate 10 μl and all the rest respectively.

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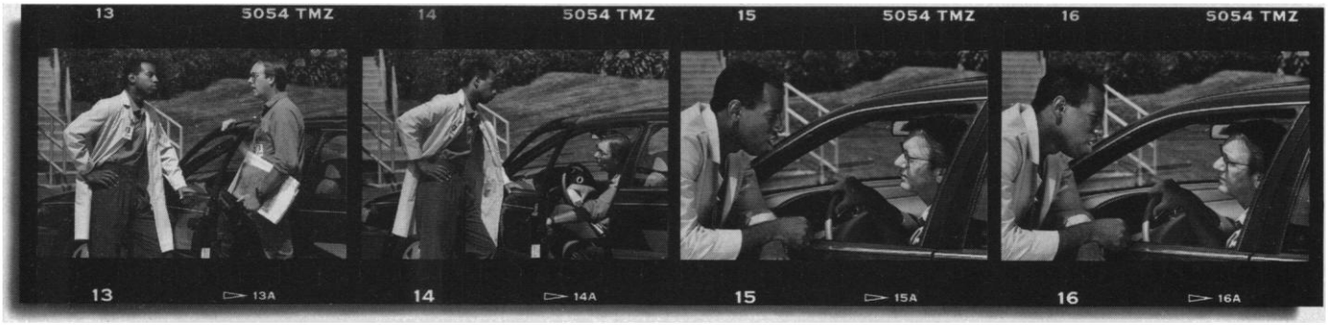

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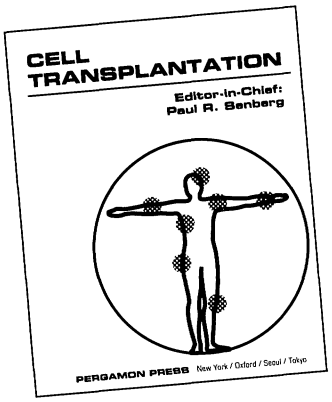

CELL TRANSPLANTATION

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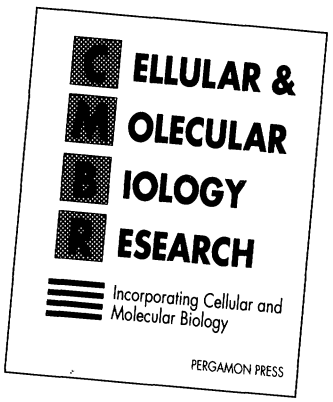

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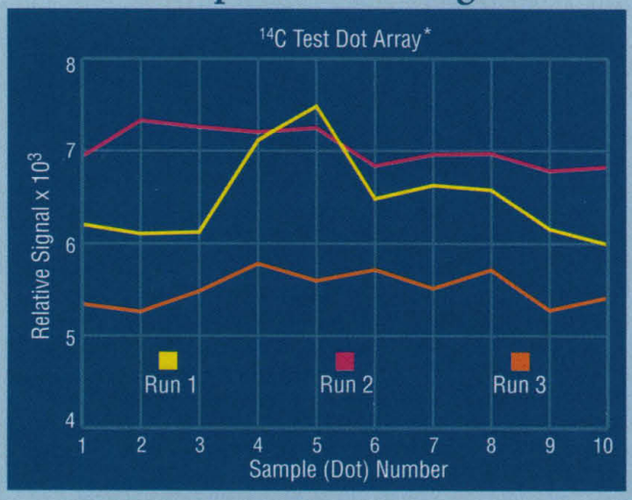

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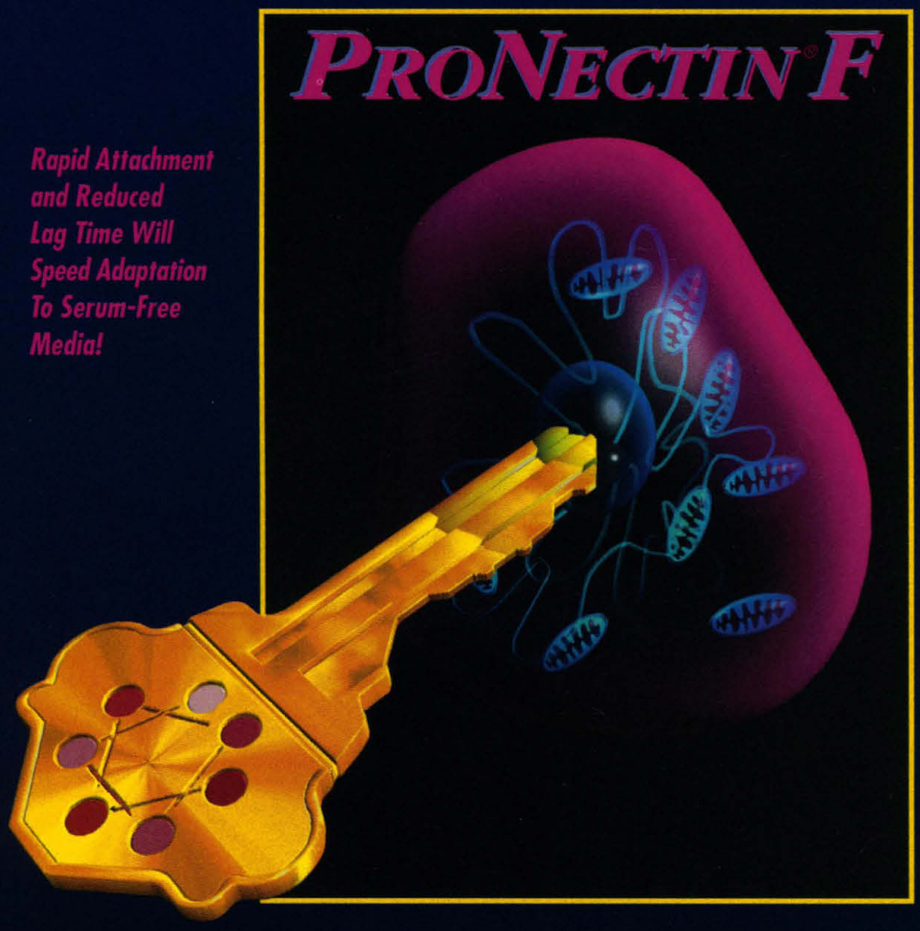

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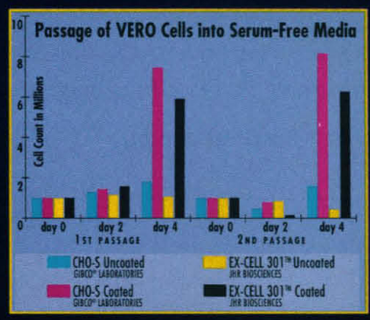

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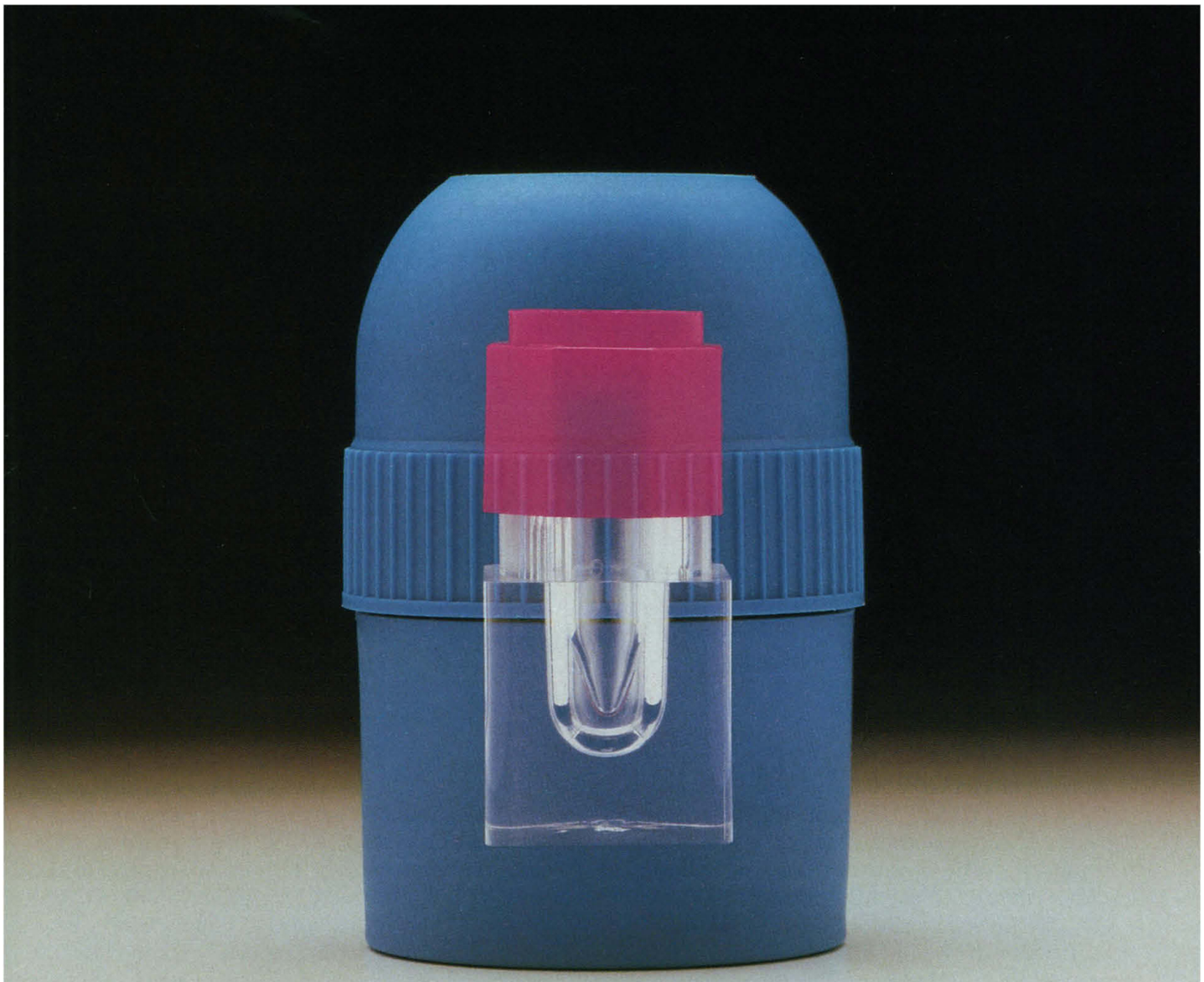

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