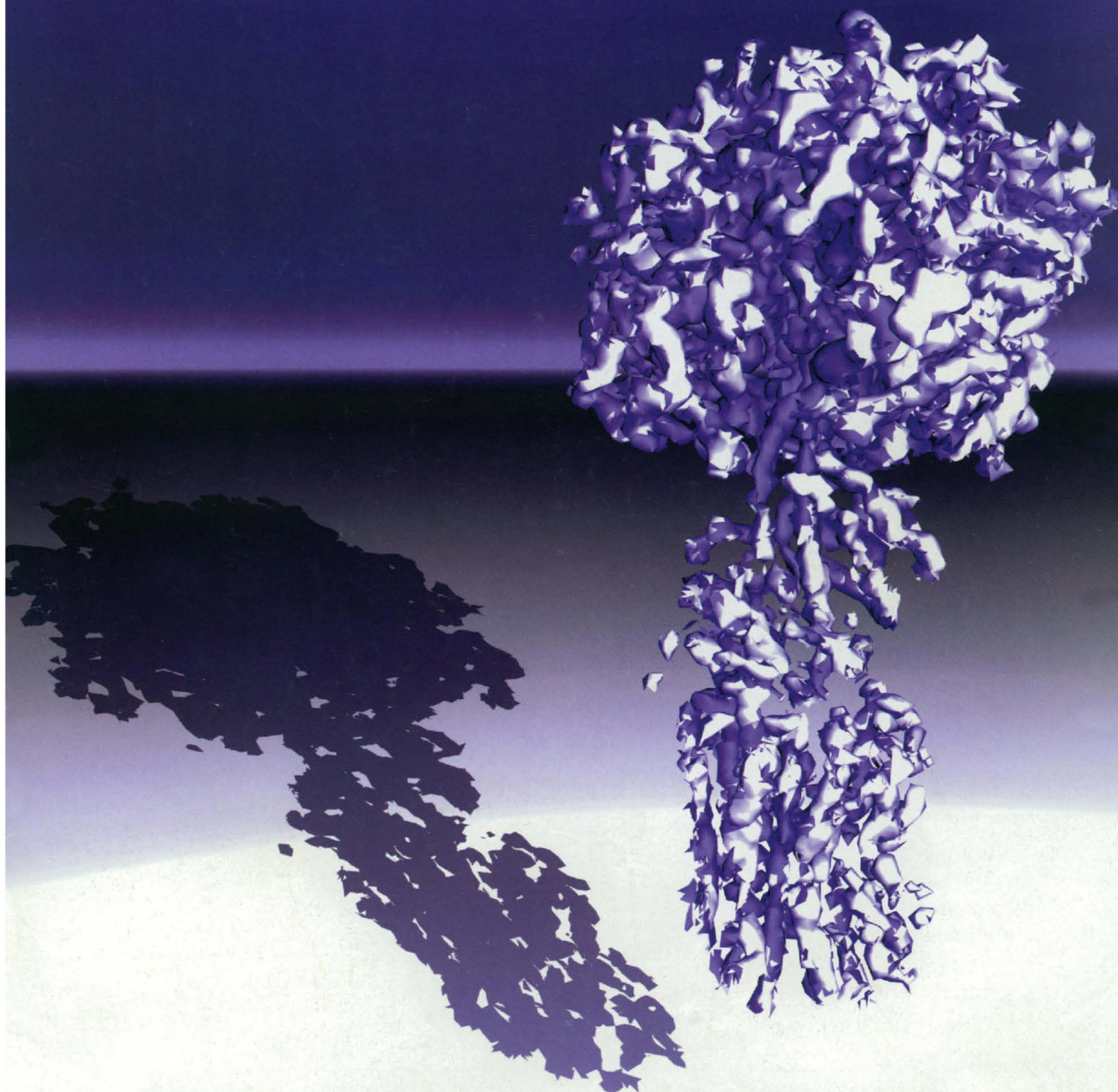


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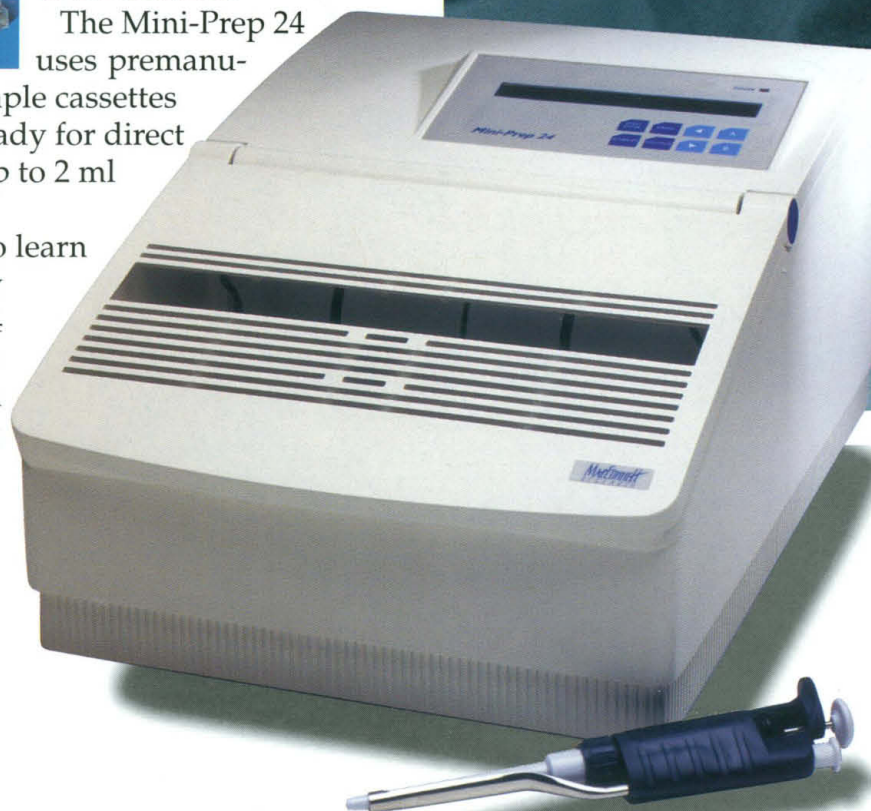
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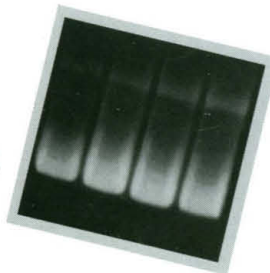
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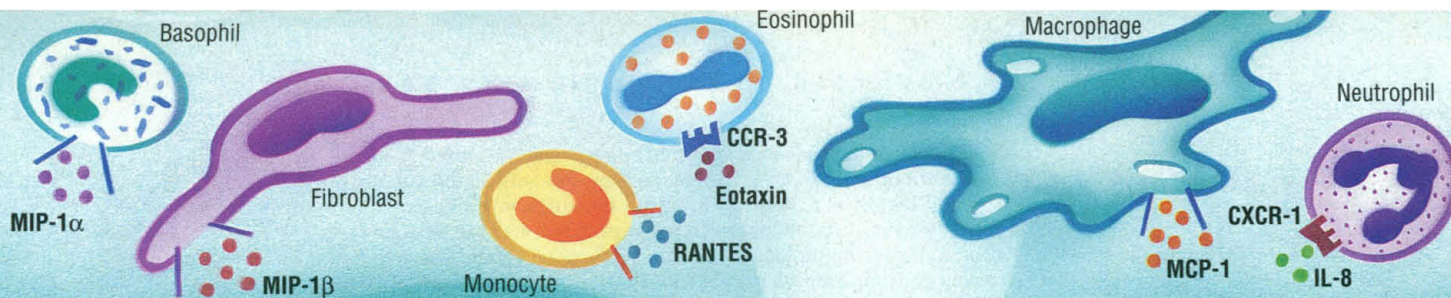
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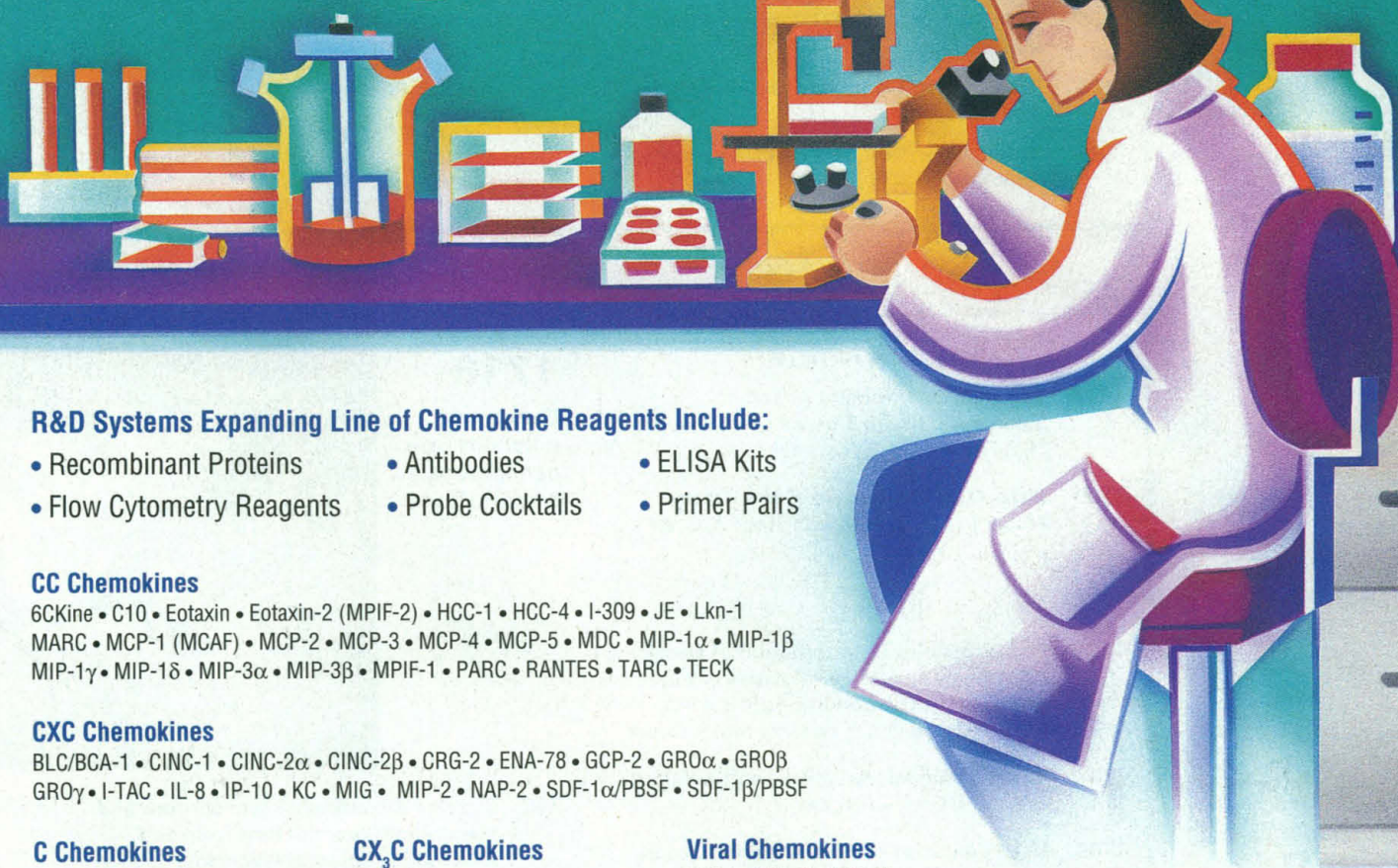
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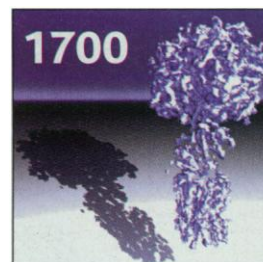
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COVER Life depends on a supply of energy, which is stored in adenosine triphosphate (ATP). The image shows details of the submicroscopic biological ATP-synthesizing machine (at 5 Å resolution), found in life-forms from microbes to humans. It provides the first glimpse of the rotating molecular motor (the lower cylinder), which uses energy from food to propel ATP synthesis in the spherical head. [Image: D. Stock, A. G. W. Leslie, J. E. Walker]



1662

GM foods: Science, stakes, and risks

NEWS OF THE WEEK

- 1654 **BUDGET FINALE: NIH Gets \$17.9 Billion in Another Record Year**
- 1655 **BIOTECH PATENTS: Genentech, UC Settle Suit for \$200 Million**
- 1655 **GEOSCIENCE FACILITIES: NSF Proposes Marriage of Rocks and Waves**
- 1658 **DATABASES: Scientists Decry Antipiracy Bill**
- 1659 **WOMEN IN SCIENCE: EU Confronts the Gender Gap**
- 1661 **NEUROSCIENCE: Learning Visualized, on the Double**
- 1661 **NUCLEAR PHYSICS: DOE Gives Up on Brookhaven Reactor**

NEWS FOCUS

- 1662 **FOOD FIGHT**
RISKS AND BENEFITS: GM Crops in the Cross Hairs
Monarch Press Release Raises Eyebrows
Biotech Critics Watch the Watchdogs
- INDUSTRY RESPONSE: Ag Biotech Moves to Mollify Its Critics**
- 1668 **ART AND SCIENCE: Duchamp and Poincaré Renew an Old Acquaintance**
- 1670 **REEMERGING DISEASES: Stalking a Killer in Russia's Prisons**
- 1671 **CHINESE ACADEMY OF SCIENCES: CAS President 'Engineers' Major Reform of Institutes**

DEPARTMENTS

NETWATCH
1643

THIS WEEK IN SCIENCE
1645

SCIENCESCOPE
1657

RANDOM SAMPLES
1675

CONTACT SCIENCE
1677

NEW PRODUCTS
1751

AAAS NEWS AND NOTES
1753

GORDON RESEARCH CONFERENCE
1756

RESEARCH

RESEARCH ARTICLES

- ▼1697 **The Male Determinant of Self-Incompatibility in *Brassica*** C. R. Schopfer, M. E. Nasrallah, J. B. Nasrallah
- ▼1700 **Molecular Architecture of the Rotary Motor in ATP Synthase** D. Stock, A. G. W. Leslie, J. E. Walker

REPORTS

- 1705 **First-Principles Determination of Elastic Anisotropy and Wave Velocities of MgO at Lower Mantle Conditions** B. B. Karki, R. M. Wentzcovitch, S. de Gironcoli, S. Baroni
- 1707 **Core Rotational Dynamics and Geological Events** M. Greff-Lefftz and H. Legros
- 1709 **Eight Centuries of North Atlantic Ocean Atmosphere Variability** D. E. Black, L. C. Peterson, J. T. Overpeck, A. Kaplan, M. N. Evans, M. Kashgarian
- 1713 **van der Waals Interactions in the Cl + HD Reaction** D. Skouteris, D. E. Manolopoulos, W. Bian, H.-J. Werner, L.-H. Lai, K. Liu

1716

Converting polymers into nanoporous ceramic films



- 1716 **Ordered Bicontinuous Nanoporous and Nanorelief Ceramic Films from Self Assembling Polymer Precursors** V. Z.-H. Chan, J. Hoffman, V. Y. Lee, H. Iatrou, A. Avgeropoulos, N. Hadjichristidis, R. D. Miller, E. L. Thomas
- 1719 **Single-Bond Formation and Characterization with a Scanning Tunneling Microscope** H. J. Lee and W. Ho



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SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 1999 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$110 (\$62 allocated to subscription). Domestic institutional subscription (51 issues): \$325; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$90. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

SCIENCE'S COMPASS

EDITORIAL

- 1677 **Biological Warfare**

LETTERS

- 1679 **A Barely Tested Hypothesis** D. W. Hogg.
Cutting Back the Calories G. S. Gerhard.
Response R. Weindruch and T. A. Prolla. **Philanthropy with Modest Means** E. Bond, M. Peck, K. Hede. **The Bible Code** M. Bar-Hillel. **Corrections and Clarifications**

POLICY FORUM

- 1683 **SCIENTIFIC PUBLICATIONS: The Challenge for Chinese Scientific Journals** S. Ren, P. Liang, G. Zu

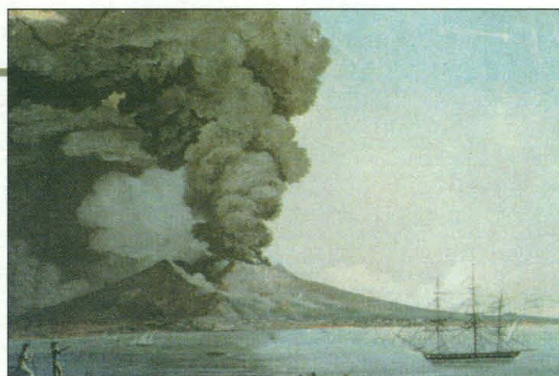
BOOKS ET AL.

- 1684 **ECOLOGY: *Ecological Assembly Rules*** *Perspectives, advances, retreats* E. Weiher and P. Keddy, Eds., reviewed by N. J. Gotelli

- 1684 **Browsings**

PERSPECTIVES

- 1685 **VOLCANISM: Will Vesuvius Erupt? Three Million People Need to Know** G. Heiken



1685

Harbinger
of future
eruptions?

- ▼ 1687 **PROTEIN STRUCTURE: Molecular Rotary Motors** R. H. Fillingame
 1700
 1722
- 1689 **COSMOLOGY: Cosmic Explosions in an Accelerating Universe** M. Livio
- ▼ 1690 **PLANT BIOLOGY: No Stigma Attached to Male Rejection** H. Dickinson
 1697
 1729
- REVIEW**
- 1692 **COGNITIVE PSYCHOLOGY: Interacting Minds—A Biological Basis** C. D. Frith and U. Frith

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- ▼ 1722 **Mechanical Rotation of the c Subunit Oligomer in ATP Synthase (F_0F_1): Direct Observation** Y. Sambongi, Y. Iko, M. Tanabe, H. Omote, A. Iwamoto-Kihara, I. Ueda, T. Yanagida, Y. Wada, M. Futai

- 1724 **Visualization of Dioxygen Bound to Copper During Enzyme Catalysis** C. M. Wilmot, J. Hajdu, M. J. McPherson, P. F. Knowles, S. E. V. Phillips

- ▼ 1729 **A Breakdown of *Brassica* Self-Incompatibility in ARC1 Antisense Transgenic Plants** S. L. Stone, M. Arnoldo, D. R. Goring

- 1732 **Arthritis Provoked by Linked T and B Cell Recognition of a Glycolytic Enzyme** I. Matsumoto, A. Staub, C. Benoist, D. Mathis

- 1735 **Proapoptotic Bcl-2 Relative Bim Required for Certain Apoptotic Responses, Leukocyte Homeostasis, and to Preclude Autoimmunity** P. Bouillet, D. Metcalf, D. C. S. Huang, D. M. Tarlinton, T. W. H. Kay, F. Köntgen, J. M. Adams, A. Strasser

- 1738 **Differentiation Stage-Specific Inhibition of the Raf-MEK-ERK Pathway by Akt** C. Rommel, B. A. Clarke, S. Zimmermann, L. Nuñez, R. Rossman, K. Reid, K. Moelling, G. D. Yancopoulos, D. J. Glass

- 1741 **Phosphorylation and Regulation of Raf by Akt (Protein Kinase B)** S. Zimmermann and K. Moelling

- 1745 **Building Neural Representations of Habits** M. S. Jog, Y. Kubota, C. I. Connolly, V. Hillegaart, A. M. Graybiel

TECHNICAL COMMENTS

On the Distribution and Abundance of Species R. D. Maddux and K. Athreya.

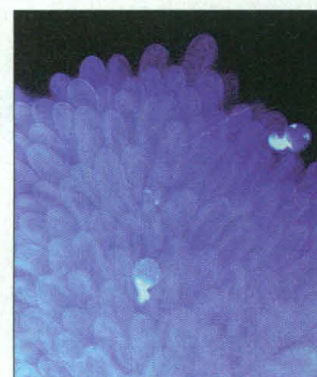
Response J. Harte, A. Kinzig, J. Green

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Polymorphism in the IL-13 Promoter

T. C. T. M. van der Pouw Kraan, L. A. Aarden, C. L. Verweij. **Response** K. M. Gillespie, K. L. Anderson, P. W. Mathieson

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1697

Regulation of self-
incompatibility

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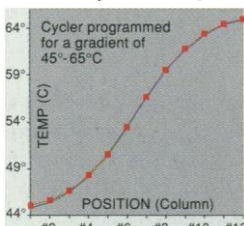


Gradient Calculator Especially Useful

Easy to Transfer "Golden" Parameters to Actual Protocols

Most researchers would agree that gradient cyclers are great in concept—but their utility is significantly compromised if an optimized protocol does not transfer well to normal, non-gradient operation. This "Achilles heel" of gradient cyclers can often be traced to imprecise knowledge of either incubation time or incubation temperature during the gradient step. Whatever technology is used, there will always be lags—often not well known—before samples reach the new temperature.

MJ has long had an excellent reputation for delivering time/temperature control with precision, so extra efforts were expended to address these issues. Thus time control includes "dynamic ramping" (see below), while temperature control incorporates a new software feature called the "gradient calculator".



Reported temps (dots) vs. independently acquired thermal data in 4 cyclers (lines).

This calculator is so precise and accurate that it reports the temperatures in individual columns to within $\pm 0.4^\circ\text{C}$ of the NIST standard, making transfer of values to normal operation very reproducible. Just look above how reported temperatures from the gradient calculator superpose *almost perfectly* with independent NIST-traceable data from 4 different cyclers.

Thus time control includes "dynamic ramping" (see below), while temperature control incorporates a new software feature called the "gradient calculator".

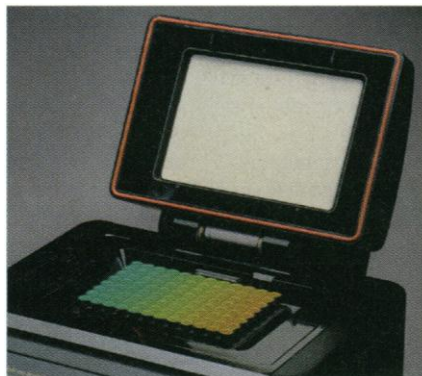
Precision Control of Time as well as Temp

"Dynamic Ramping" Incubates Each Sample for Same Period

In some gradient cyclers, the gradients develop gradually. When cooling to an annealing gradient, for example, the highest temperature stabilizes long before the lowest one does. This means that the time spent at incubation is different at each temperature—thus two critical parameters are being varied at the same time.

Not so with MJ cyclers. Careful engineering has led to "dynamic ramping" where each column of wells ramps at a different rate, for ramp rates are much less critical. The results are consistent incubation times column-to-column, with only temperature varying among samples.

NEW GRADIENT HELPS OPTIMIZE ANNEALING AND DENATURATION



DNA Engine™, with the thermal gradient shown in artificial colors from data collected by an IR camera.

Optimized Denaturations Surprisingly Important

It is well known in the biological community that DNA amplification reactions should have optimized annealing temperatures for best results. Denaturation is quite important as well—but only the savvy optimize this step.

Too bad. MJ's scientific staff finds that denaturation often leads to problems. Use of a lower denaturation temperature, such as $90^\circ\text{--}92^\circ\text{C}$, is generally recommended whenever possible. Not only does it preserve enzymatic activity for later cycles, it also reduces breakdown of fluorescent dyes in cycle sequencing. On the other hand, higher temperatures, such as $95^\circ\text{--}96^\circ\text{C}$, may be required for GC-rich templates from organisms such as *Mycobacteria*.

ALL EXISTING DNA ENGINES & TETRADS CAN BE UPGRADED

Standard Feature on New Thermal Cyclers

WALTHAM, Mass. — MJ RESEARCH is pleased to announce the introduction of an advanced gradient feature that is now standard on all DNA Engine & Tetrad thermal cyclers. This powerful new function allows precision thermal gradients as high as 24°C to be developed across 96-well blocks, at any temperature between 30° and 105°C . This greatly assists in developing robust protocols, for the optimal annealing and denaturation temperatures give strong results without lots of "ampli-schmutz" or other unwanted artifacts appearing in the gel.

Many reactions benefit from careful temperature optimization, especially sensitive ones, such as dye-terminator cycle sequencing. GC-content, length of molecule, concentration of magnesium—all these lead to differences in optimal "heat" for annealing and denaturation. This is why empirical experiments can almost always enhance even the *best* calculations for T_m .

But who wants to do a dozen runs of slightly variant protocols? Gradient cyclers make this chore much easier by allowing a dozen different incubation temperatures in a single run. The user simply selects a range of temperature, and the cycler does the rest. The optimal temperatures become obvious in the gel—with thick "meaty" bands unbracketed by artifact.

How to Get Upgrade

In a nutshell, visit the MJ website. For DNA Engines manufactured after 1/1/99, the gradient feature is a simple software upgrade that is provided free and can be installed by users. For older DNA Engines or Tetrads, a new logic board is also required, and this upgrade is available inexpensively from MJ or its distributors.

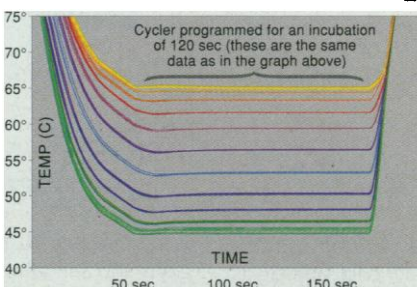
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Data from four cyclers are superposed in this graph, with each trace representing the average temperature measured in a column of wells. Note the consistency of incubation periods, the cycler-to-cycler reproducibility (each trace is made up of four separate lines), and the even spread of incubation temperatures between the programmed targets of 45° and 65°C .

PCR is covered by patents owned by Hoffmann-La Roche, Inc. & F. Hoffmann-La Roche Ltd. Users should obtain license to perform the reaction.

POLLEN PREFERENCES

Self-incompatibility in certain plants allows a plant to distinguish its own pollen from among that of other plants of the same species, and preferentially react to nonself pollen in order to limit inbreeding (see the Perspective by Dickinson). Stone *et al.* (p. 1729) have analyzed the activities of ARC1, which shows a phosphorylation-dependent interaction with a kinase located in the stigmatic plasma membrane. Their *in vivo* studies show that ARC1 is a key regulator of self-incompatibility from the female side. Schopfer *et al.* (p. 1697) have now identified a critical component from the pollen side—a polymorphic protein expressed in the pollen that interacts with the stigmatic proteins to determine whether the outcome will be fertile.

CRANKING OUT ATP

Although the common form of energy used in cells is adenosine triphosphate (ATP), energy is stored both in chemical forms such as fat or starch and in electrochemical forms such as ion gradients across cellular membranes. Fat and starch are metabolized to produce small amounts of ATP and large amounts of energy in the form of a gradient of protons, which is then converted into ATP by an enzyme known as ATP synthase. Stock *et al.* (p. 1700; see the cover) present the crystal structure of the membrane-bound portion (F_0) of the synthase and the axle that connects it to the ATP-synthesizing part (F_1). F_0 transforms the energetically downhill movement of protons into rotation of an internal axle (the γ subunit of F_1) as shown by Sambongi *et al.* (p. 1722). These workings of a nanogenerator are discussed in a Perspective by Fillingame.

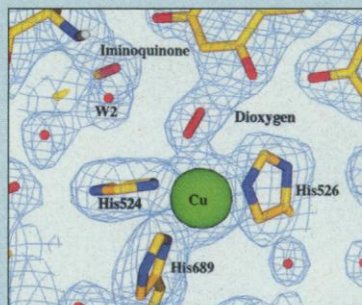
THE SUN, THE MOON, AND THE PLUMES

There have been several episodes of large-scale volcanism recorded on Earth's surface, and many of these events are thought to have been initiated by the upwelling of a major thermal anomaly (a plume) from the core-mantle boundary. Greff-Lefftz and Legros (p. 1707) have modeled a possible connection between episodes of volcanism and the initiation of a plume by the interplay of the gravitational forces between Earth, the moon, and the sun. They show that fluid outer-core oscillations set up by the lunar-solar tides and core rotation resonated with the

solar tidal waves and that these resonances, which could initiate a plume, occurred at about the same time as three episodes of volcanism in the Precambrian.

OXYGEN IN ACTION

Despite the importance of oxygen as an electron acceptor in many enzymatic reactions, the mechanism of oxygen activation by redox enzymes is not well understood. Wilmot *et al.* (p. 1724) determined x-ray structures



of freeze-trapped intermediates related to the oxidative half-reaction of copper amine oxidase. They also determined the oxidation state of the quinone cofactor for each structure by using single-crystal spectrophotometry. Their studies reveal the binding site for dioxygen and the hydrogen peroxide product and show the proton transfer pathways to the oxygen. The product aldehyde inhibits enzyme reoxidation in the crystal, and thus the release of the aldehyde is rate-determining.

THE WINDS OF CHANGE

The subdecadal variability of climate in the region of the tropical North Atlantic Ocean during the past century is thought to be well understood, but longer term variations that occur over decades to centuries are more difficult to resolve because instrumental records do not extend far enough into the past and most existing sedimentary records do not have sufficient temporal resolution. Black *et al.* (p. 1709) present a 825-year-long record from a southern Caribbean sediment core that reveals decadal and century-scale trade wind variations which indicates that natural climate changes also occurred abruptly in the preindustrial past. These findings also highlight the link between thermohaline circulation in the Atlantic Ocean and other tropical climatic variations.

NANOPOROUS CERAMIC FILMS VIA POLYMERS

Block copolymers, in which segments (blocks) of different chemical composition are connected, can phase-separate at the nanometer scale and form intricate structures. Chan *et al.* (p. 1716) show that copolymers containing two hydrocarbon blocks separated by a block made from a silicon-containing polymer can form an open nanoporous network with a double gyroid morphology. A single-step process that uses ozone and ultraviolet light converts the silicon-containing block into a ceramic framework and removes the remaining hydrocarbon material to create nanoporous ceramic films.

MOLECULAR SHUTTLE

The scanning tunneling microscope (STM) allows great control over atomic and molecular manipulation on surfaces. The microscope tip can also be used to record vibrational spectra that allow the identification and characterization of single adsorbed molecules and the bonds they form with the substrate. Lee and Ho (p. 1719) used these combined capabilities of their low-temperature STM to study the bond formation between single iron atoms on a silver surface and carbon monoxide (CO) molecules delivered with the STM tip. Complexes of iron with one or two CO molecules were formed and characterized.

JOINT ANTIGEN

Rheumatoid arthritis is an autoimmune disease in which both T cells and B cells become activated. Most current research has centered upon finding specific antigens of the joint that could be the immunogenic targets for T cell-mediated damage. In a mouse model of arthritis that seems to mimic many of the features of the human condition, Matsumoto *et al.* (p. 1732) have determined that the antigen with the arthritogenic activity for the B cells as well as the T cells is from the same protein, glucose-6-phosphate isomerase, a ubiquitously expressed enzyme. Thus, local autoimmunity need not be caused by a tissue-specific antigen.

DEATH IS ROLE WITHIN A NORMAL LIFE

Proteins of the Bcl-2 family play both roles in apoptosis, either promoting or inhibiting cell death. The smaller proteins that contain only the third Bcl-2 homology domain (BH3-only proteins, such as

CONTINUED ON PAGE 1647

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No other combination for manual cycle sequencing gives more information per gel — long reads, accurate sequence. Doubt-free results.

Above: Elimination of bands across all four lanes (BAFLs) in plasmid template.

Fig. a) Using the Thermo Sequenase Radiolabelled Terminator Cycle Sequencing Kit.

Fig. b) Compared to standard ³²S-dATP cycle sequencing where a strong BAFL is observed.

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Rediscover the chemistry

THIS WEEK IN SCIENCE

CONTINUED FROM PAGE 1645

Bim) are thought to have pro-death properties, but their role in normal apoptotic functions in vivo, such as homeostasis and immune function, has been unclear. Bouillet *et al.* (p. 1735) now report results for a gene-targeted Bim-deficient mouse which show that this BH3-only protein is critical for normal homeostasis of white blood cells, cytokine withdrawal, and calcium-induced apoptosis and also acts as a barrier against autoimmune disease. Thus, Bim is a part of the physiological apoptosis machinery.

WORKING IT OUT DOWNSTREAM

A cell's response to a particular growth factor or hormone involves a relay of signals between the cell surface and the nucleus. The small GTP-binding protein Ras is activated by numerous growth factor receptors and can regulate more than one signaling pathway in response to a single growth factor, depending on the cell type. Hence, Ras can serve as a branch point for signal propagation, and tight regulation of the pathways it stimulates is needed to ensure the proper biological outcome. Two reports describe the cross communication that occurs between two pathways that lie downstream of Ras, the Raf kinase-MEK (mitogen-activated protein kinase)-ERK (extracellular-regulated kinase) pathway and the PI3K (phosphatidylinositol 3-kinase)-Akt kinase pathway. Zimmermann and Moelling (p. 1741) demonstrate that Akt inhibited Raf activity by phosphorylating a known regulatory site on Raf. In breast cancer cells, Akt directly interact-

ed with Raf, and inhibition of Akt resulted in increased Raf activity. The authors suggest that in these cells, this mechanism may regulate growth response to insulin-like growth factor (IGF). Rommel *et al.* (p. 1738) also reveal that Akt associated with Raf and inhibited the Raf signaling pathway in differentiated muscle cells (myotubes) but not in undifferentiated precursor cells. Activation of the Akt pathway or inhibition of the Raf pathway resulted in a hypertrophic phenotype (thickening) similar to that induced by IGF on muscle cells. These studies suggest that integration of these two pathways into a particular cellular response may depend on cell type or the stage of cell differentiation.

FORCE OF HABIT

Patterns of behavior can be learned and become so routine as to occur unconsciously. Jog *et al.* (p. 1745) monitored neural activities within the basal ganglia, a portion of the brain known to be involved in the control of movement. These neural activities changed over time in rats learning a T-maze (the rats associated a tone cue with the correct direction in which to turn at a T-junction toward a food reward). As the rat's accuracy and speed improved, the ensemble of neurons appear to fire most actively at the beginning and at the end of what became a habitual behavior. The representation of entire sequences of motor commands within the basal ganglia may relate to the difficulties that Parkinson's patients experience in beginning or ending movements.

TECHNICAL COMMENT SUMMARIES

Species and Regular and Irregular Areas

The full text of these comments can be seen at www.sciencemag.org/cgi/content/full/286/5445/1647a

Harte *et al.* (Reports, 9 Apr., p. 334) argued that the probability distribution of species is self-similar with respect to area and use this notion to reconcile and address several common species-area relations. Maddux and Athreya comment that the model of Harte *et al.* "implies that species are distributed in one of three trivial ways" and thus conclude that the law is "in general...invalid." Harte *et al.* respond that "patch...shape does matter" and that the prediction made by [their] theory about the dependence of species richness on patch shape is reasonable."

Polymorphisms Return in the IL-13 Promoter

The full text of these comments can be seen at www.sciencemag.org/cgi/content/full/286/5445/1647b

Interleukin-13 (IL-13) plays an important role in allergic responses. In a previous Technical Comment and Response (28 May 1999), Anderson *et al.* and Wills-Karp and Rosenwasser examined part of the IL-13 promoter region, found no polymorphisms there, and concluded that the promoter region was not a susceptibility locus for atopy. Van der Pouw Kraan *et al.* now report that they find evidence of polymorphism just outside the region examined earlier, a finding that Gillespie *et al.* confirm.

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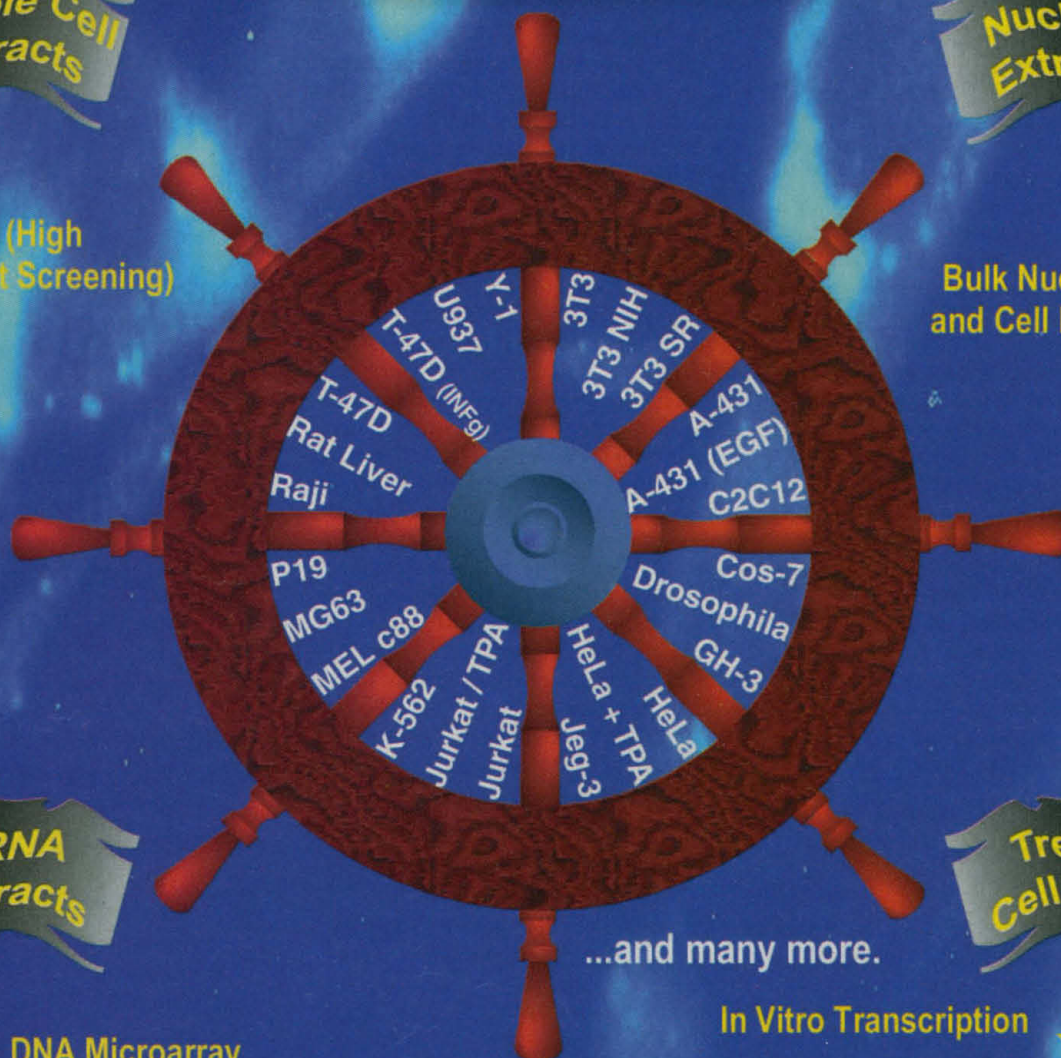
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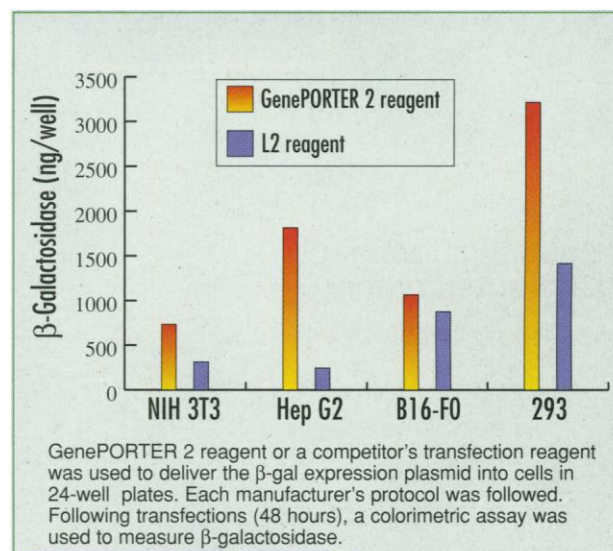
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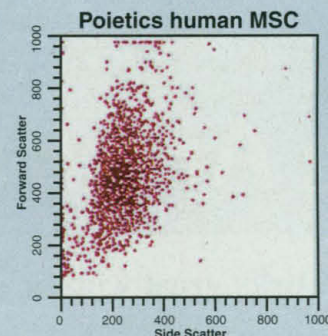
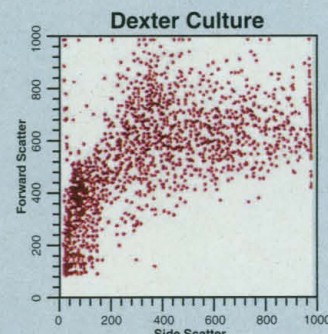


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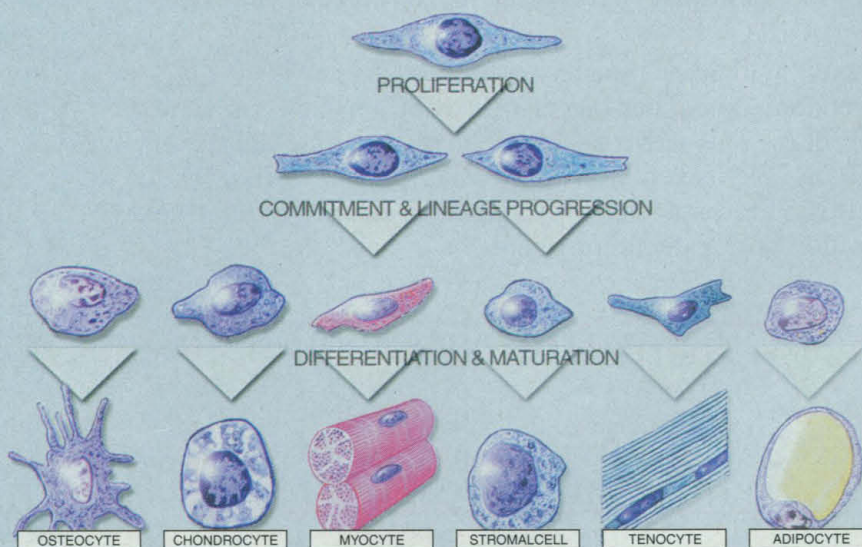


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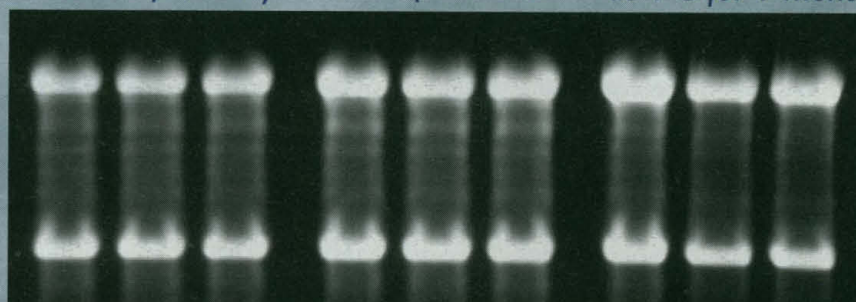
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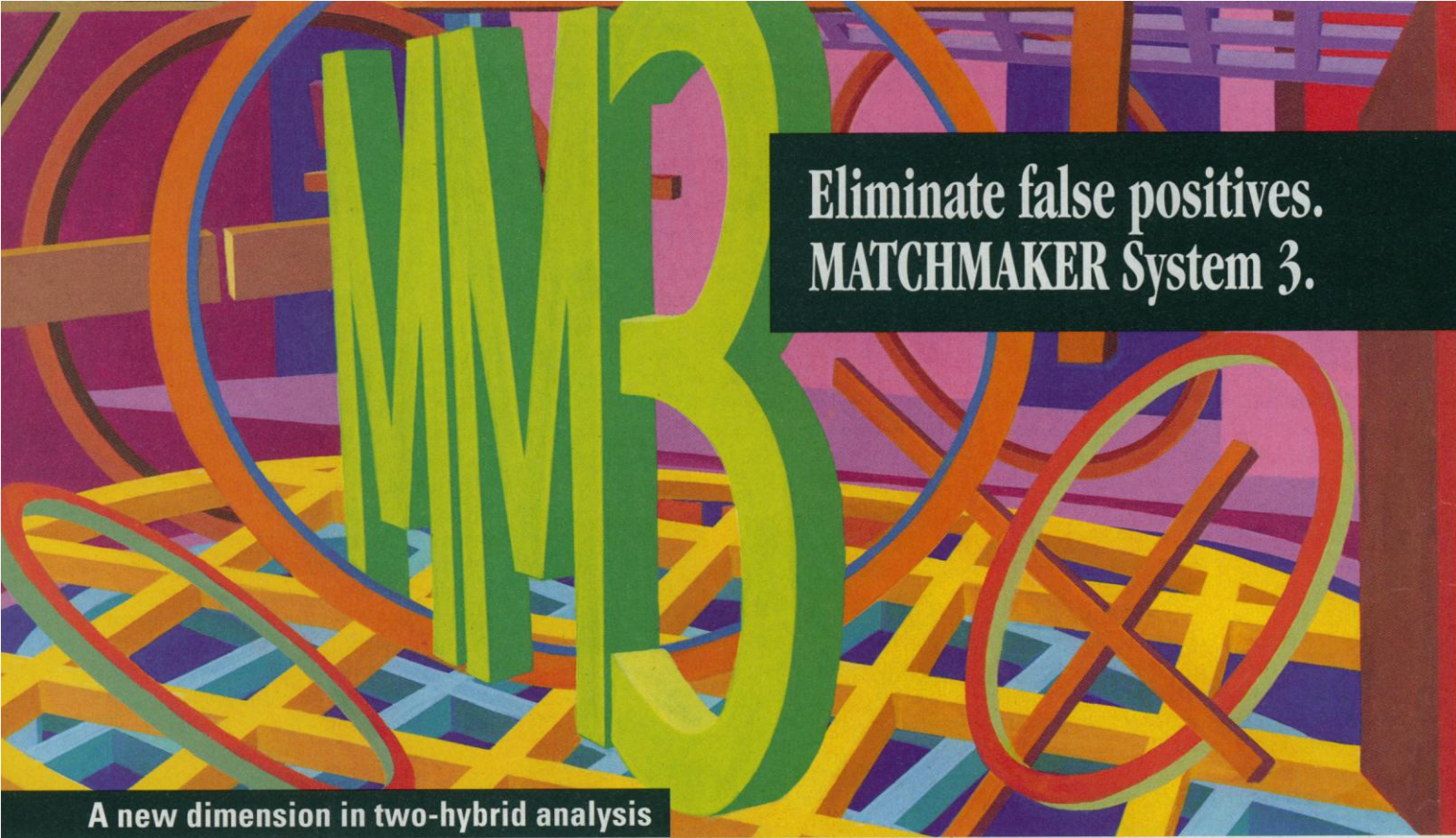
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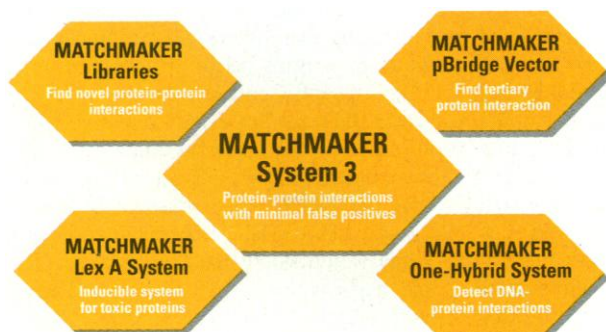
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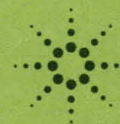
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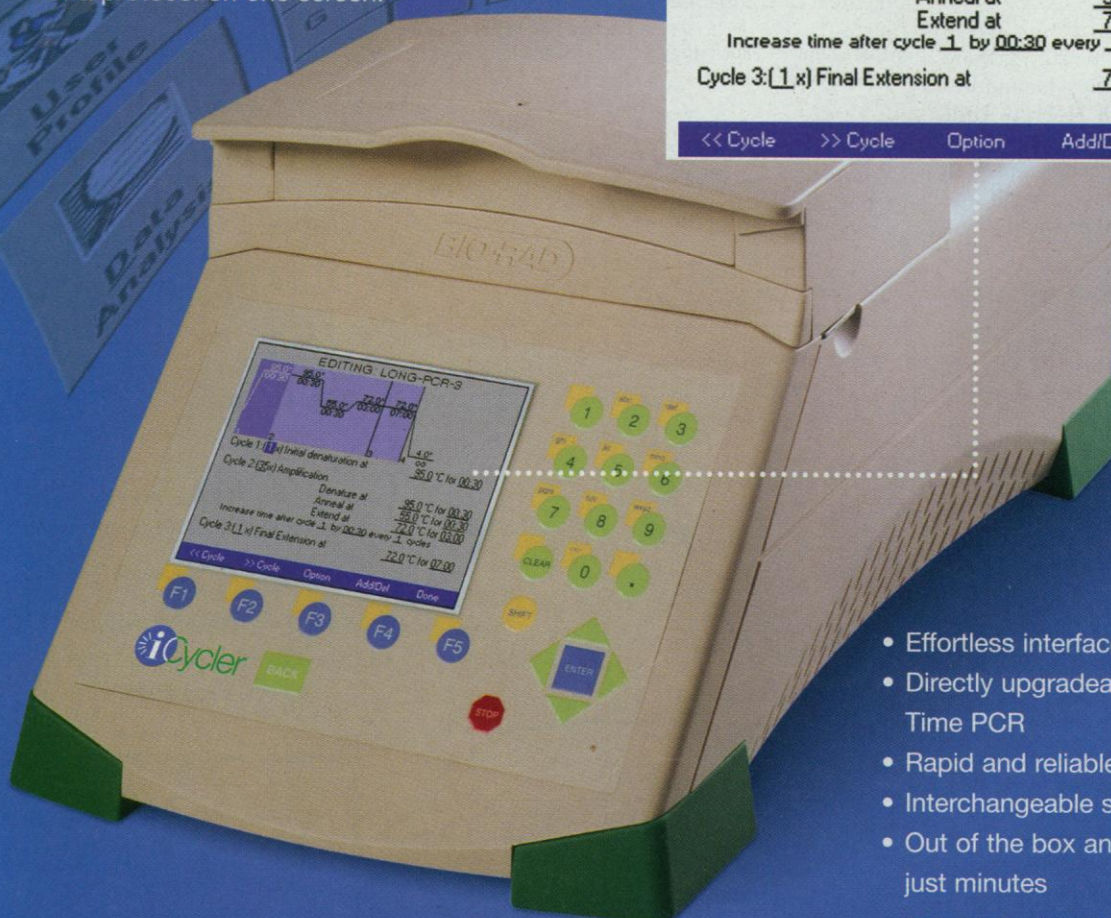
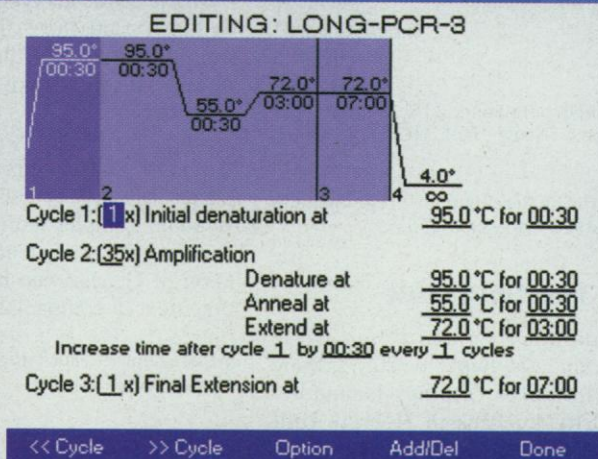
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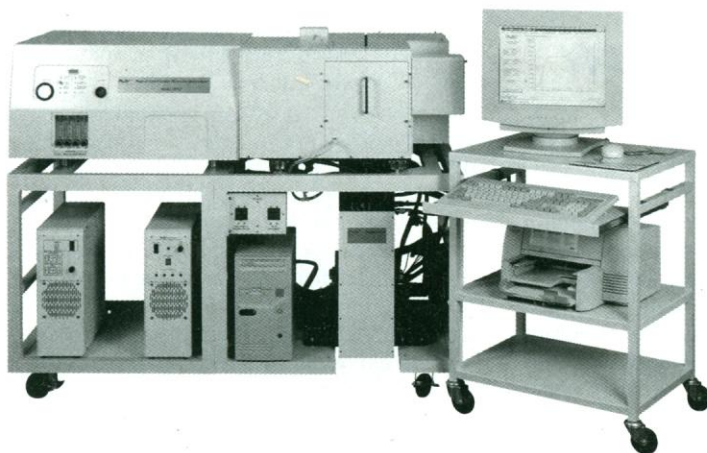
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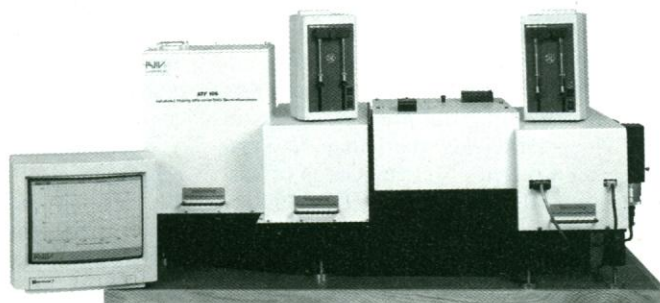
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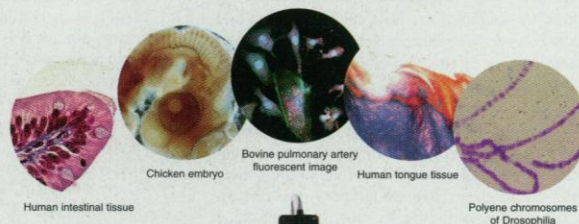
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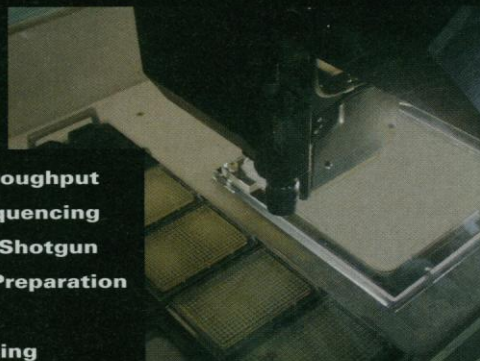
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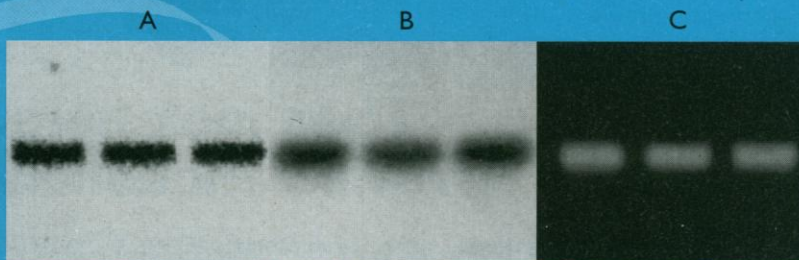
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