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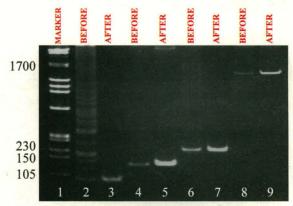
PCR primers and DNA templates vary in purity, GC content and amount of secondary structure. In addition, DNA may have chemical modifications, nucleic acid analogs or other characteristics that can inhibit amplification efficiency. To improve the yield and specificity of the desired PCR products, the buffer components of a specific amplification reaction can be modified. But the process is tedious and time-consuming.

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PCR OPTIMIZATION



Four primer/template sets were PCR-amplified using either standard Taq polymerase buffer (10mM Tris, 50mM KCl and 1.5mM MgCl₂) or individually optimized Opti-PrimeTM buffer systems. Lane 1: 1 ug of lambda Hind III/phi x 174 Hae III marker. Lanes 2&3: 105- bp PCR product of a human Gaucher's disease gene. Lanes 4&5: 150-bp PCR product of Bluescript® vector MCS. Lanes 6&7: 230-bp PCR product of an Epstein Barr viral nuclear antigen gene. Lanes 8&9: 1700-bp PCR product of a lac/ target gene from a transgenic mouse. Lanes 2,4,6 and 8 are of primer/template sets amplified using standard Taq polymerase buffer. Lanes 3,5,7 and 9 are of primer/template sets amplified using individually optimized Opti-Prime kit buffers.

*The PCR process is covered by patents owned by Hoffmann-La Roche Inc. Use of the PCR process requires a license.

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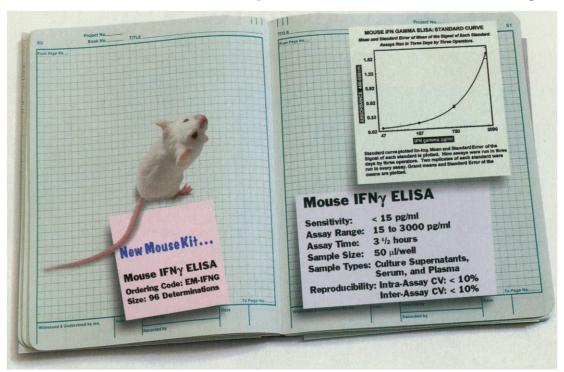
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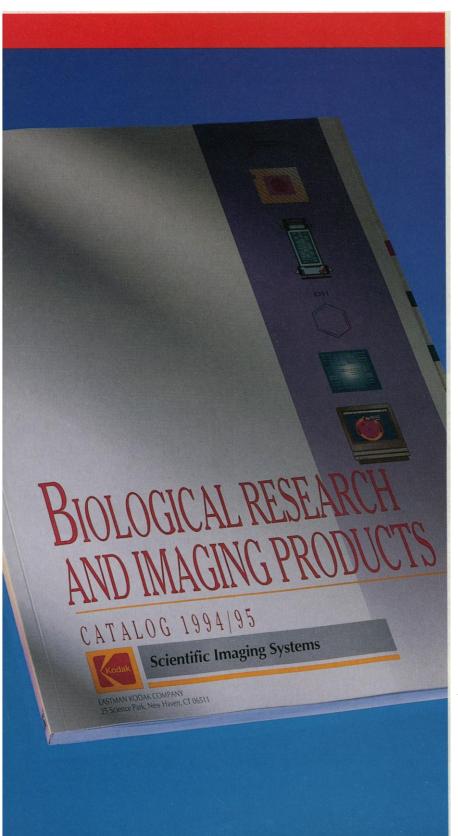
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ISSN 0036-8075 8 APRIL 1994 VOLUME 264 NUMBER 5156

Science

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE



192 & 221 Barbara Mikulski speaks on science

NEWS The Hand on Your Purse Strings 192 "If we get into a fight, I'm not a pacifist." Livermore Lab Chief Nuckolls Resigns 195 Under Pressure Report: All's Fair in NSF Major Awards 196 Nicotine Research: Key Study 196 Unveiled-11 Years Late SIDS Paper Triggers a Murder Charge 197 Livermore Physicists Ask for the Sun 198 Deep-Trench Research Waits on Balky 199 Japanese Submersible In Pittsburgh, Physicists Get Down 200 to the Nitty-Gritty Ecologists Dare to Ask: How Much Does 202 Diversity Matter? Trail of Toxins Leads Through Conference Rooms in Dallas

Leroy Hood: Thinking Big in Seattle UW Team Reaches Out to Grade- and High-School Students		206
POLICY FORUM		
Science in the National Interest B. A. Mikulski		221
PERSPECTIVES		
Self-Organization in Living Cells B. Hess and A. Mikhailov		223
The Evolution of Genetic Intelligence D. S. Thaler		224
ARTICLE		
Modulated Magnetic Phases in Rare Earth Metallic Systems T. Chattopadhyay		226
RESEARCH ARTICLE		
Neural Mechanisms for Forming a Perceptual Decision C. D. Salzman and W. T. Newsome		231

SPECIAL NEWS REPORT

DEPARTMENTS

THIS WEEK IN SCIENCE	181	Elephant Man's Disease: M. R. Wallace • Pho	
EDITORIAL Chemicals: Perceptions Versus Facts	183	ics Lesson: I. Hinchliffe SCIENCESCOPE 1	91
LETTERS	185	RANDOM SAMPLES 2	05
Misrepresentation and Fantasy: R. N. Cahn, Jackson, C. Quigg • A National Institute for Environment: P. D. Saundry • Adaptive Opti Astronomy: F. Seitz • Space Science Crunc Katz; H. Tananbaum • National Protein Nucleic Acid Databases: E. Adman, M. Geller	r the less in the J. and t, M.	Choosing Big Technologies, reviewed by R. W. Sm • Games of Life, P. Yodzis • A Natural History Shells, W. B. Saunders • STM and SFM in Biolo C. Bustamante • Vignettes • Books Received	y of
 Cohen, N. M. Allewell, B. S. Baker, J. Villafr Smallpox Virus: Better to Store?: P. He 		PRODUCTS & MATERIALS 2	99

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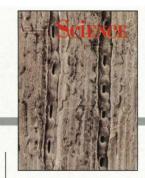
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COVER

Scanning electron micrograph of stomatal rows from a modern limber pine needle (image width is ~1 mm). The density of stomata (pores that permit a plant to exchange gases with the atmosphere) of needles from fossil pack rat middens decreased 17% during the last

deglaciation, concomitant with a 30% increase in atmospheric CO₂. See page 239. [Micrograph: Peter K. Van de Water, Department of Geosciences, and David Bentley, Division of Biotechnology, Arizona Technology Laboratory, University of Arizona, Tucson]



Stellar Luminosity Variations and Global Warming P. Foukal Trends in Stomatal Density and 3C/12C Ratios of Pinus flexilis Needles During Last Glacial-Interglacial Cycle P. K. Van de Water, S. W. Leavitt, J. L. Betancourt Quantifying Global Warming from the Retreat of Glaciers J. Oerlemans

Morphological Bifurcations Involving
Reaction-Diffusion Processes During
Microtubule Formation
J. Tabony

Molecular Nanotube Aggregates of β- and γ-Cyclodextrins Linked by Diphenylhexatrienes G. Li and L. B. McGown

Binding and Suppression of the Myc
Transcriptional Activation Domain by p107
W. Gu, K. Bhatia, I. T. Magrath, C. V. Dang, R. Dalla-Favera

Related Target Enhancers for Dorsal and NF-kB Signaling Pathways S. González-Crespo and M. Levine

Recombination in Adaptive Mutation Z 258 R. S. Harris, S. Longerich, S. M. Rosenberg

Isolation of S. cerevisiae snRNPs: 261
Comparison of U1 and U4/U6.U5 to
Their Human Counterparts
P. Fabrizio, S. Esser, B. Kastner, R. Lührmann

Mutational Isolation of a Sieve for Editing in a Transfer RNA Synthetase
E. Schmidt and P. Schimmel

Stimulation of Human γδ T Cells by
Nonpeptidic Mycobacterial Ligands
P. Constant, F. Davodeau, M.-A. Peyrat, Y. Poquet, G. Puzo, M. Bonneville, J.-J. Fournié

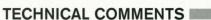
Telomere-Led Premeiotic Chromosome 270 Movement in Fission Yeast Y. Chikashige, D.-Q. Ding, H. Funabiki, T. Haraguchi, S. Mashiko, M. Yanagida, Y. Hiraoka

Requirement for the Yeast Gene LON in Intramitochondrial Proteolysis and Maintenance of Respiration

C. K. Suzuki, K. Suda, N. Wang, G. Schatz

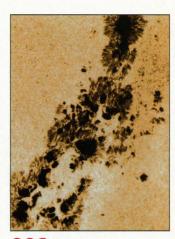
Soluble β-Amyloid Induction of
Alzheimer's Phenotype for Human
Fibroblast K⁺ Channels

R. Etcheberrigaray, E. Ito, C. S. Kim, D. L. Alkon

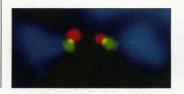


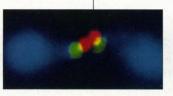
High-Pressure Melting of (Mg,Fe)SiO₃-Perovskite
D. L. Heinz, E. Knittle, J. S. Sweeney, Q. Williams, R. Jeanloz; R. Boehler and A. Zerr

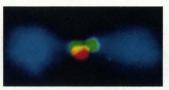
Statistical Analyses of Soil Quality
D. A. Wardle; J. P. Reganold



238 Inconstant sun







270
Telomeres (green) cluster near spindle pole body (red) in yeast karyogamy

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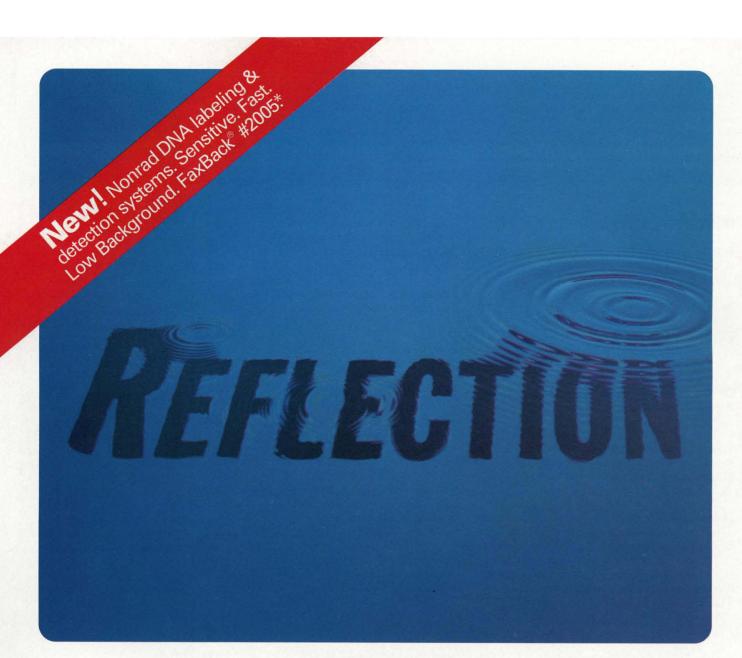
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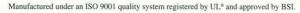
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Decisions, decisions

Perceptual judgments, such as deciding the direction of motion of an object, require processing of neuronal signals within the cerebral cortex. Salzman and Newsome (p. 231) explored how this processing takes place by recording from and stimulating neurons in the visual cortex of rhesus monkeys while the monkeys performed a motion discrimination task. The monkeys' performance was best described as being governed by a "winner-take-all" decisionmaking mechanism that favored one stimulus or the other, rather than by a vector averaging of the two signals, a mechanism used in other parts of the brain.

Enhancer coupling

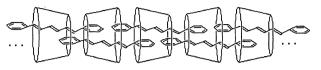
Although two rel-related molecules, dorsal and nuclear factor κB (NF-κB), regulate two diverse processes, dorsal-ventral patterning in Drosophila and hematopoiesis in mammals, the signaling pathway that these transcription factors participate in appears conserved. González-Crespo and Levine (p. 255) show that this conservation extends into the nucleus to the enhancers to which these factors bind. When coupled to a reporter gene, a target enhancer of NF- κ B, the κ immunoglobulin light chain enhancer, can generate stripes in the Drosophila embryo in a pattern similar to that generated by the rhomboid enhancer. Thus, conserved enhancers can couple similar signaling pathways to diverse genes.

Myc pockets

The Myc protein is a key transcription factor that is aberrantly expressed in many cancers. Gu

Hooking cyclodextrins into nanotubes

One approach for making nanotubes is to string cyclodextrins (CDs), which are cyclic polysaccharides, along a polymer chain. Li and McGown (p. 249) now show that rigid nanotube aggregates can be formed from larger CDs by sonicating aqueous solutions of



these molecules with rigid diphenylhexatriene (DPH) molecules. A variety of characterization methods suggests that the average nanotube contains 20 $\beta\text{-CDs}$ or 30 $\gamma\text{-CDs}$ linked by DPH molecules, which potentially could act as "molecular wires."

et al. (p. 251) show that Myc associates in vivo with p107, a protein related to the tumor suppressor Rb. This interaction. involving the transactivation domain of Myc and the pocket domain of p107, results in suppression of Myc's transactivation function. Mutant forms of Myc from Burkitt lymphoma cells are not functionally suppressed by p107, although they still bind to it. Disruption of a regulatory interaction between Myc and p107 may be important in tumorigenesis.

Climate proxies

Valley glaciers, which occur in many continents and climate belts, are sensitive records of climate change and can be used to establish an independent estimate of global climate change provided that their dynamics are understood. Oerlemans (p. 243) shows that the record of retreat (that is, changes in glacial length) of many valley glaciers is coherent globally after accounting for variations in glacial geometry and climate sensitivity. The mean rate of retreat is consistent with a global warming of 0.66 kelvin during the past 100 years.

The sun's luminosity is not

exactly constant but changes during the 11-year magnetic cycle as well as on longer time scales, which raises the question of whether some part of global climate change might be attributable to variations in solar output. By looking at more than a century's worth of solar data from the Royal Greenwich Observatory, Foukal (p. 238) concludes that luminosity variations large enough to have a significant effect on the Earth's climate during the past few millennia can be ruled out because the concomitant changes in magnetic activity would have shown up in the carbon-14 and beryllium-10 records.

The morphology of stomata of leaves of many plants change systematically with atmospheric carbon dioxide (CO₂) concentrations. The large numbers of accurately dated fossils needed to use such a record can be obtained from pine needles preserved in pack rat middens. Van de Water et al. (p. 239; see cover) measured stomatal densities and carbon-13/carbon-12 ratios from 41 middens from the western United States with dates from about 30,000 years ago. The data imply that CO₂ concentrations increased abruptly by about 30 percent 15,000 years ago, in accord with the ice core record, and that water use efficiency in pine increased by about 15 percent.

Not by peptides alone

Human $\gamma\delta$ T cells respond to antigens from mycobacteria, but the nature of these antigens has been difficult to determine. Constant et al. (p. 267) isolated four structurally related ligands from a Mycobacteria tuberculosis strain that stimulated the proliferation of a subset of human $\gamma \delta T$ cells. Nuclear magnetic resonance and mass spectroscopy revealed that one of these antigens, TUBag4, contains a 5' triphosphorylated thymidine group. The stimulatory pathway for these nonpeptide antigens is still unclear but may eventually provide insight into autoimmune diseases involving $\gamma\delta$ T cells.

Taking the lead

It has been generally thought that centromeres proceed first when chromosomes move. Chikashige et al. (p. 270) studied premeiotic chromosome movement in fission yeast. Telomeres proceed first in premeiotic chromosome movement until chromosome movement until chromosome segregation, at which point the centromeres lead like they do in mitosis. The function of this initial telomere movement is unknown but may facilitate homologous pairing.

Hot or not so hot?

Recent high-pressure experiments have yielded drastically different melting temperatures for MgSiO₃ perovskite, the major phase in the lower mantle. Heinz *et al.* (p. 279) and Boehler and Zerr (p. 280) discuss some of the differences in the results.

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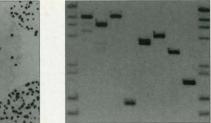
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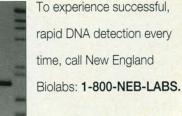
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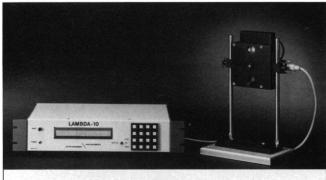
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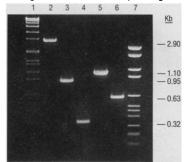
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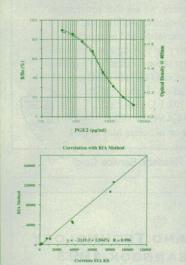


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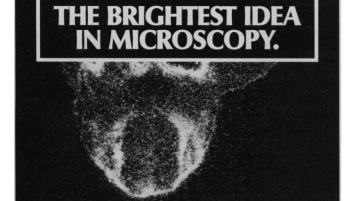
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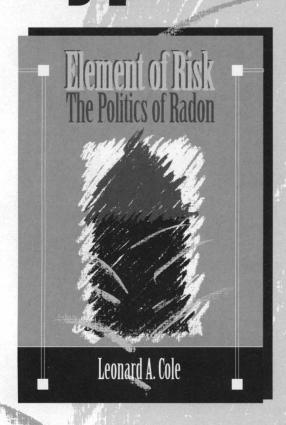
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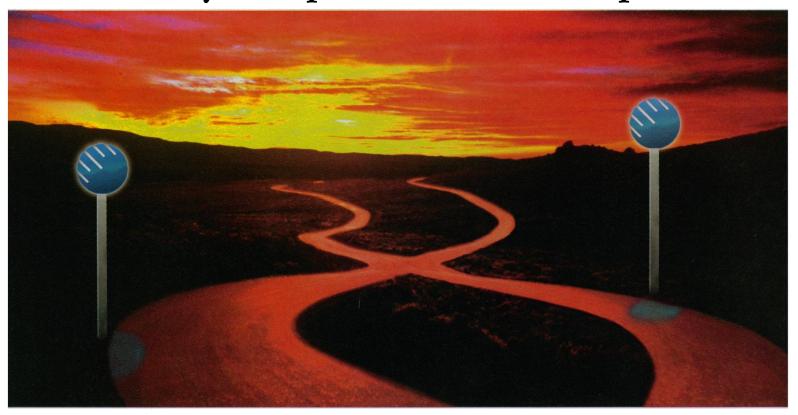
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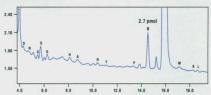
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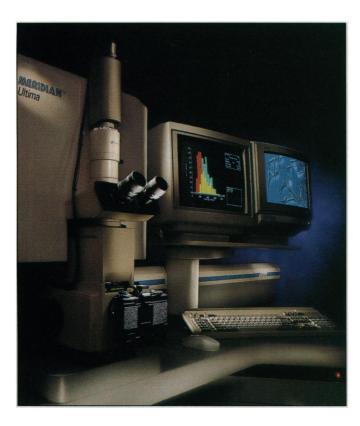
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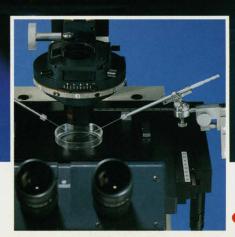
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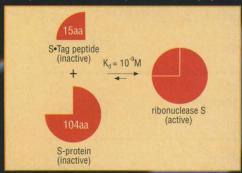




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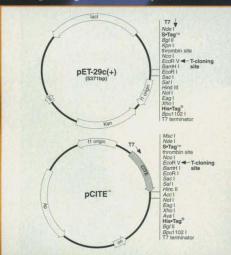
S•Tag™ System

Novagen's new S•Tag** System is a protein tagging system based on the interaction of the 15aa S•Tag peptide with the 104aa S-protein derived from pancreatic ribonuclease A. This interaction was first characterized by Richards and colleagues (1), who observed that the S-protein:S-peptide complex (known as ribonuclease S) maintained enzymatic activity, whereas neither component had activity by itself.



These characteristics make the S•Tag peptide an ideal tag for recombinant proteins, since any fusion protein can be conveniently detected and purified using the S-protein (2). The unique property of reconstituting enzymatic activity by the S•Tag peptide:S-protein interaction allows sensitive, quantitative measurement of any fusion protein by a simple assay.

Vectors available for making S•Tag fusions are pET-29a-c(+) for T7 RNA polymerase-driven expression in *E. coli*, and pCITE¹¹ for optimal production of proteins *in vitro* with Novagen's Single Tube Protein' System.



Kits available for detection of S•Tag fusion proteins include the S•Tag Rapid Assay Kit for quantitative measurement and the S•Tag Western Blot Kit for detection of fusion proteins on blots. The S•Tag Purification Kit takes advantage of the S•Tag peptide:S-protein interaction for rapid affinity purification of fusion proteins on a small to medium scale, under native conditions.

References

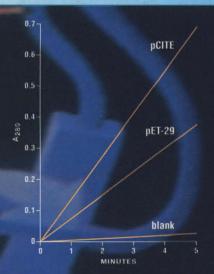
- Richards, F.M. and Wyckoff, H.W. (1971) in "The Enzymes," Vol. IV (Boyer, P.D., Ed.), pp. 647-806. Academic Press, New York.
- Kim, J.-S. and Raines, R.T. (1993) Protein Science 2, 348-356.

*Patent pending

Cool assays.

S•Tag Rapid Assay Kit

- Quantitative determination of recombinant proteins in minutes
- Uses 15aa S•Tag sequence; no radioactivity or other special labels
- No purification necessary; works with crude extracts and in vitro translation reactions
- Homogeneous; no solid phase, no washes, no antibodies
- No special equipment; uses standard UV spectrophotometer
- · Low cost, optimized reagents
- · Detects 20fmol target protein in a 5 minute incubation
- Accurate determination based on molar concentration
- · Independent of target protein size, composition
- · Not affected by endogenous amino acid pools



Hot results.

S-Tag Western Blot Kit

- · Sensitive blot detection of S.Tag fusion proteins
- . Go from blot to bands in 30 minutes

Plus purification.

S-Tag Purification Kit

- · Rapid small to medium scale affinity purification
- Non-denaturing conditions for maximum recovery of active target proteins

S•Tag purification of β-galactosidase fusion protein made from a pET-29 recombinant, Crude extract (left) and purified protein (right).



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BIO-OPTICA MILANO SPA Milano, Italy PHONE: 39-2-26-40-274 FAX: 39-2-21-53-000 Northern blot of rat growth hormone (rGH) mRNA (1 kb)

Southern blot of EcoR1 rGH gene fragment (11.4 kb)



Western blot of rGH (22 kD)



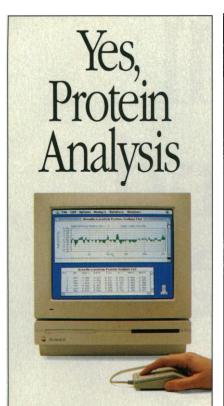
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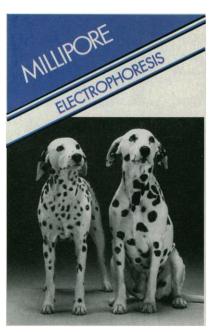




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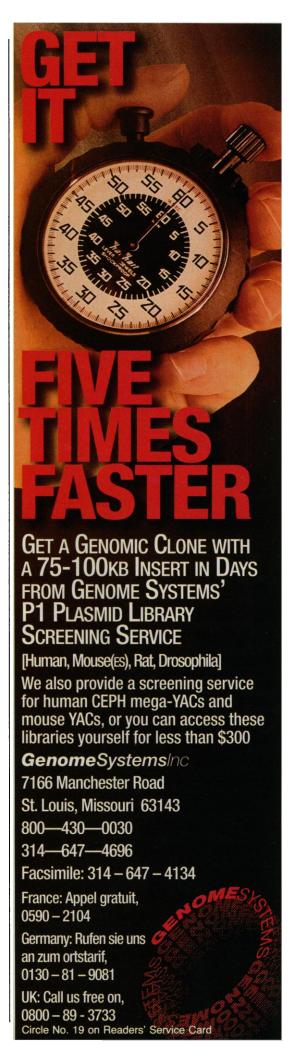
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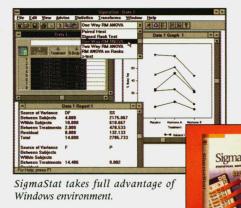
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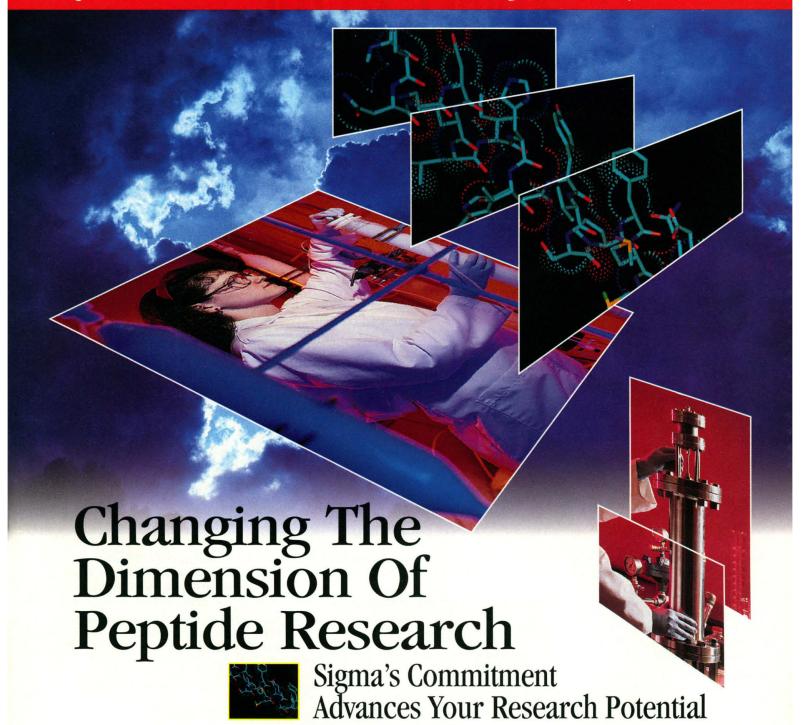








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