



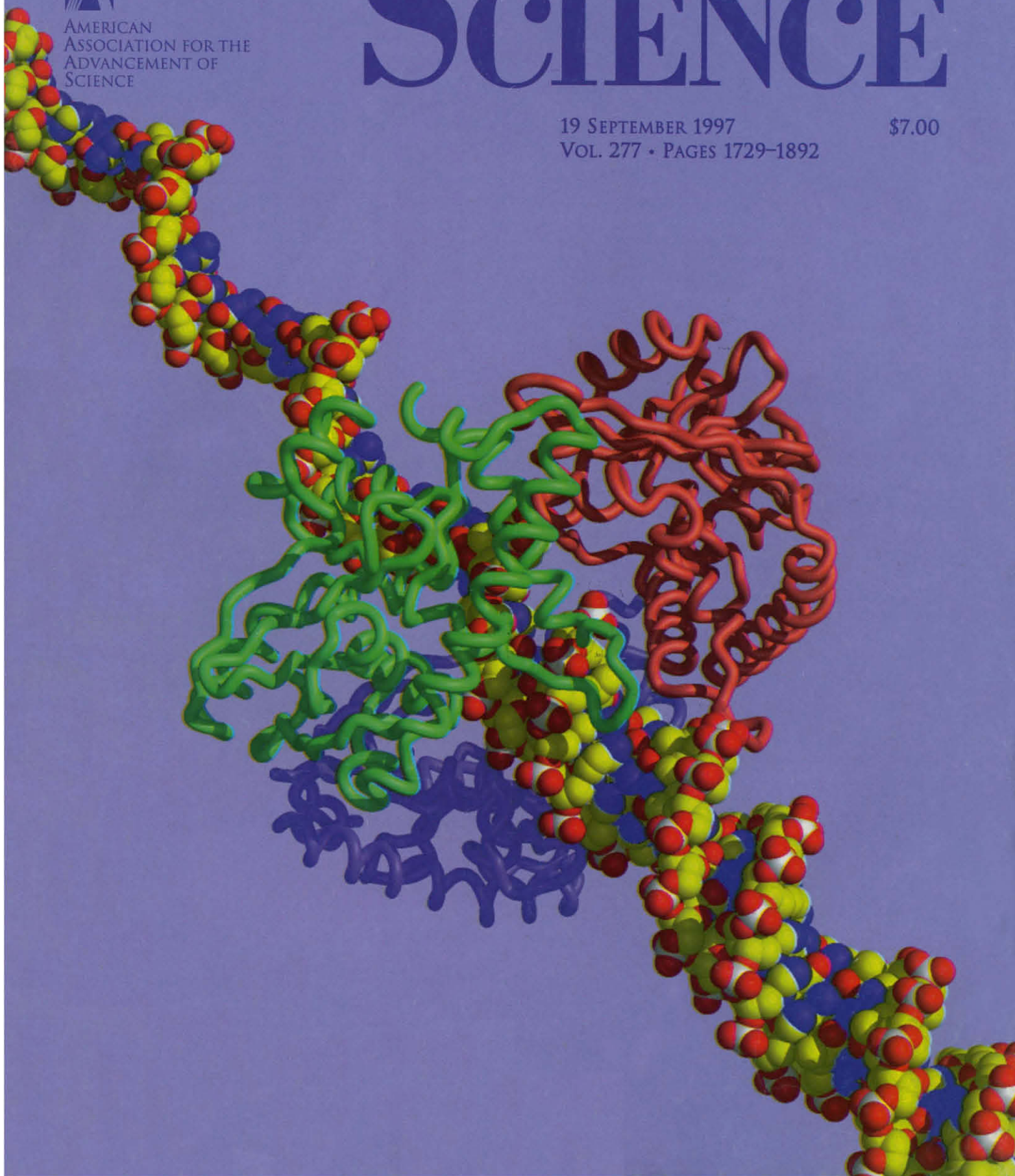
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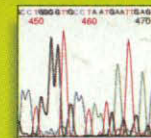


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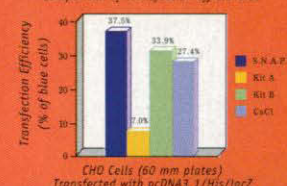


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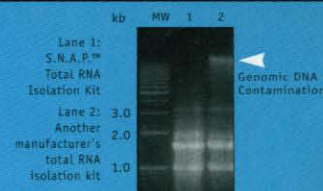
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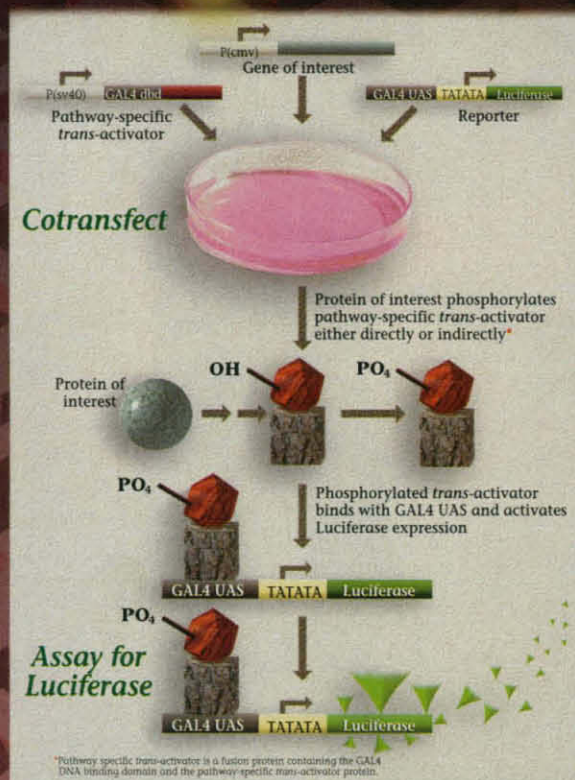
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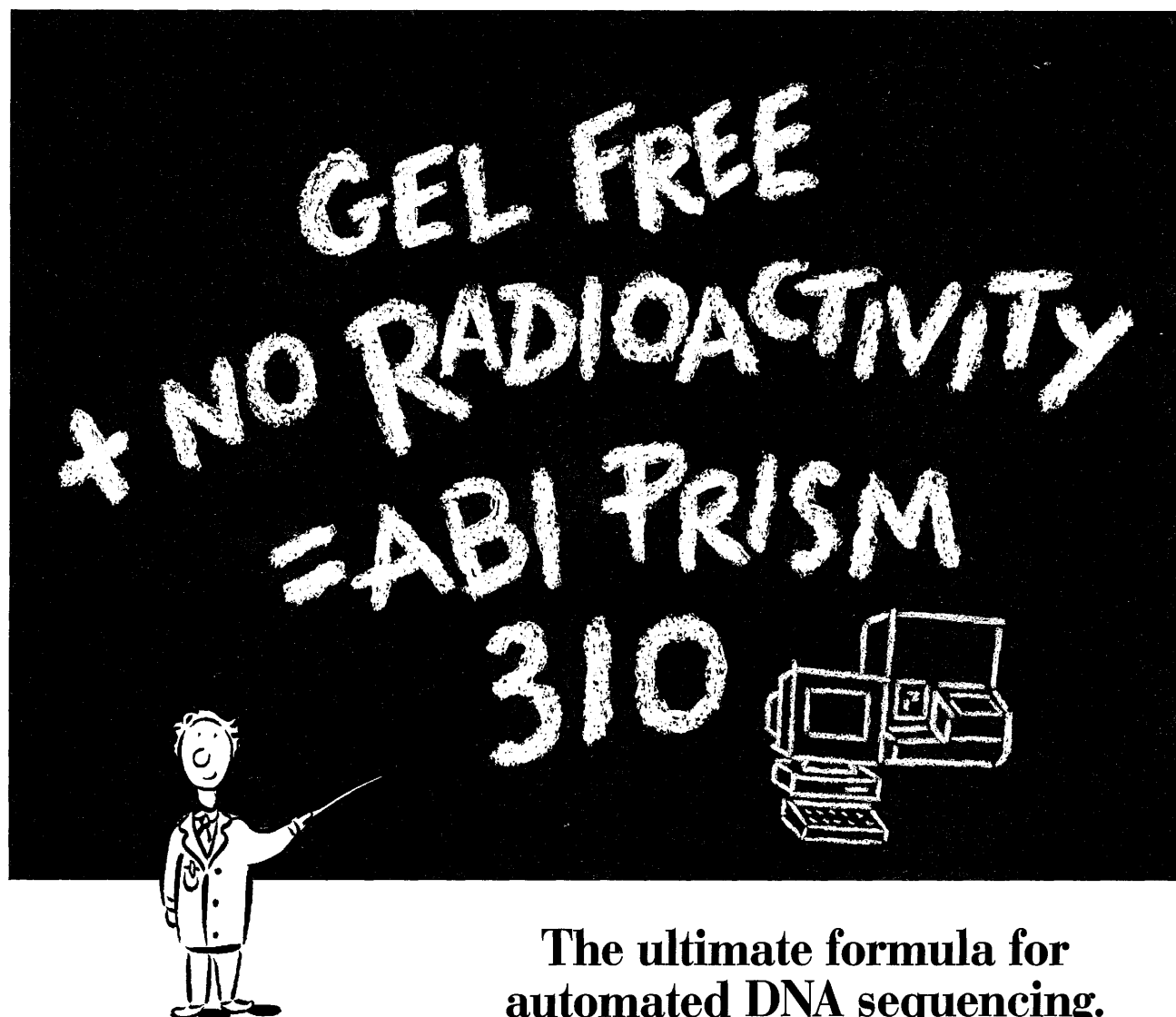
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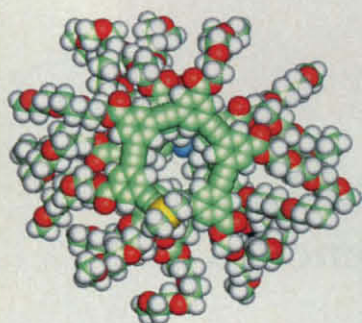


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1761 & 1796

Early date for mound complex



1764 & 1793

Polymers
moving into
the fold

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COVER

λ -Exonuclease binds to an end of double-stranded DNA and degrades one of the strands in a highly processive manner. The structure of the exonuclease consists of a toroidal trimer (~94 angstroms in diameter) and is presumed to enclose its substrate in the

manner illustrated. Its internal cavity is funnel-shaped, and the intact single-stranded DNA emerges from the narrow end of the funnel. See page 1824. [Image: Rhett Kovall, Allen Orville, and Brian W. Matthews (figure prepared with Midasplus software)]



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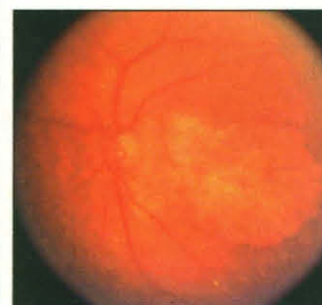
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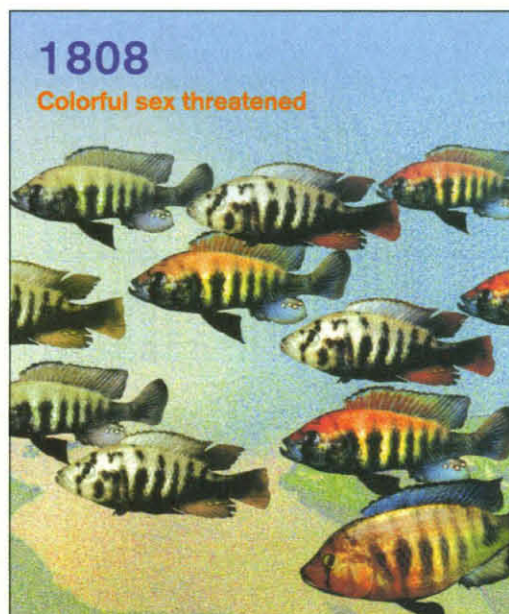
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1765 & 1805

Macular degeneration gene



Indicates accompanying feature

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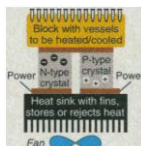
The Peltier-effect Thermal Cycler That Began A Technological Trend: The MJ RESEARCH PTC-100



Left to right: A PTC-100-96AgV with a speedy silver-gold block that can ramp at up to 2.5°C/sec, a PTC-100HB with improved Hot Bonnet heated lid that applies heat and pressure to the tops of tubes or plates for oil-free reactions, and a PTC-100-16MS that holds 16 slides & 24x0.2ml tubes for *in situ*.

How Peltier-effect Heat Pumps Actually Work

The Peltier effect is a solid-state physical phenomenon that was first described by Jean Peltier in the *Annales de chimie* in 1834. Peltier observed that when current passes through a bimetallic junction, one side gets hot and the other gets cold. In the 20th century, this "effect" has been combined with semiconductor technology to construct small, bi-directional heat pumps that are electronic in nature. These pumps are extremely precise and reliable, and they are the basis of MJ cyclers.



RIGHT WAY TO USE PELTIER

It is now widely accepted that Peltier heat pumps are the best technology for thermal cyclers—but exactly how these pumps are applied is critical. A design was pioneered with the PTC-100 where the pumps are mounted between a low-mass sample block and a large, efficient heat sink. Permanent mounting allows fast, efficient heat flow between the pumps and the block. The heat sink can store heat to be later pumped back into the sample block, or it can disperse the heat to outside air—this depends upon the action of a computer-controlled fan.

The Cycler That Went To War in the Persian Gulf and Battled Plague in India

When adversity flared in the Persian Gulf six years ago, the tools of molecular biology were brought to the front. Biological weapons were threatening; means to detect their use were needed. PTC-100s were there—and they did their duty.

In 1994, unsettling reports were circulating that plague had erupted in Surat, India. Indian authorities were addressing the problem as best they could, but the skills to detect *Y. pestis* had atrophied over time. Working with the WHO, MJ RESEARCH donated several PTC-100s, allowing Indian scientists to use molecular methods to battle the disease. In the final analysis, *only* these molecular tests* proved that the plague pathogen was ever present (see *Nature Med* 1:1237-9).

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



MJ Engineer Paul Titcomb in 1990, with a PTC-100 that was camouflaged by the U.S. Marine Corps for field use in *Operation Desert Storm*.

A MUCH-COPIED DESIGN

First Introduced in 1988 and Improved Over Time—It Still Outperforms Most Cyclers

WATERTOWN, Mass. — In 1986 and 1987, much "buzz" was circulating in the biology community about a new method for identifying and amplifying sequences of DNA that was being developed by the Cetus Corporation. A tantalizing paper describing an early form of PCR* had appeared in *Science* in late '85, but a critical refinement—the use of thermostable polymerase enzyme—was not described officially until '88. At that time, the need for significant numbers of instruments to thermally cycle tiny aliquots of biological reagent first became apparent.

The partner of the Cetus Corporation, The Perkin-Elmer Corporation, concentrated on the development of a thermal cycler based upon mechanical vapor compression technology. MJ RESEARCH, INC.—then a year-old start-up company—recognized the opportunity and began development of instrument based upon the more promising Peltier-effect heat pump. Several other companies in the U.S. and England also began development of thermal cyclers—mostly based upon resistive heating and passive air or water cooling (though one other US firm also developed a variant of the Peltier cycler).

In late '88, MJ RESEARCH introduced the world's first Peltier thermal cycler—the PTC-100. Since that time, the instrument's design has been modified, crafted, tweaked—and perfected. To this day, the PTC-100 outperforms most competing cyclers in uniformity, speed, accuracy, and capacity—even though at least twenty other manufacturers have since entered the market. MJ has introduced newer designs—the Mini-Cycler, DNA Engine, and Tetrad—yet the PTC-100 remains a respected workhorse of science.

But perhaps the greatest compliment to this pioneering design is the ubiquitous imitation. Though it was scorned at first by some, the PTC-100's configuration is now mimicked by virtually every manufacturer in every new introduction.

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THIS WEEK IN SCIENCE

edited by BROOKS HANSON

Small steps in solution

Charging of small structures, such as quantum dots or single molecules, can occur in a series of discrete one-electron (or coulomb) steps. Fan and Bard (p. 1791) show that with sensitive detection methods, such charging steps can also be observed for nanometer-scale electrodes in solutions containing reactants that form an oxidation-reduction couple.

Folding conjugated polymers

A relative simple polymer made up of identical aromatic hydrocarbon units displays solvent-driven folding behavior. Nelson *et al.* (p. 1793; see the news story by Pennisi, p. 1764) show that a polymer with a phenylacetylene backbone folds into a compact helical structure, and the transition depends on chain length, solvent, and temperature. Non-specific forces drive the transition, as there are no hydrogen-bonding interactions.

A light touch

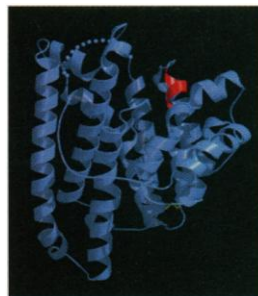
Certain chalcogenide glasses are known to exhibit photoinduced anisotropy; these materials absorb or refract polarized light differently in various directions. Krecmer *et al.* (p. 1799; see the Perspective by Tanaka, p. 1786) show that upon absorption of polarized light, the films contract parallel to the electric vector of the polarized light and dilate orthogonal to it. The effect may be utilized in nanotechnology, where positioning with a precision to within a nanometer is required.

Early mounds

Early Native Americans built several complex mounds at Watson Brake, Louisiana, marking one of the beginnings of complex society in the Western

Origins of sterol diversity

Isoprenoids are found as, for example, visual pigments, reproductive and mating hormones and steroids, and membrane constituents. Abnormalities in their biosynthesis can lead to heart disease and cancer, whereas isoprenoids such as taxol offer promise as beneficial drugs. The diverse isoprenoids are generated from compounds with 5, 10, 15 and 20 carbon atoms that form substrates for many enzymes, primarily cyclases, as discussed in a Perspective by Sacchettini and Poulter (p. 1788). Lesburg *et al.* (p. 1820), Starks *et al.* (p. 1815), and Wendt (p. 1811) describe the structures of three of these cyclases and discuss the mechanism of protonation.



Hemisphere. Saunders *et al.* (p. 1796; see the news story by Pringle, p. 1761) obtained many radiocarbon dates from the mounds and show that these were built about 5000 to 5400 years ago, much earlier than was thought. Evidently the mounds were occupied seasonally.

Carbohydrate clues to disease

A molecular basis for a fatal, neurodegenerative disease, late infantile neuronal ceroid lipofuscinosis, has been found with an approach that should be useful in studying other lysosomal storage diseases. Sleat *et al.* (p. 1802) show that newly synthesized soluble lysosomal enzymes contain a modified carbohydrate, mannose-6-phosphate (man-6-P) that acts as a targeting signal. The receptor for man-6-P was used to pull out the modified proteins and look for differences between patients and controls. The sequence of the gene resembles proteases, and it is deficient in the brains of patients with the disease.

Genetics of vision loss

Age-related macular degeneration (AMD) is the primary cause of severe vision loss among the

elderly. Allikmets *et al.* (p. 1805; see the news story by Pennisi, p. 1765) examined 167 unrelated AMD patients and found that 26 of them had mutations in ABCR, a gene that codes for an adenosine triphosphate-binding transporter protein in retinal photoreceptors. Mutations in the same gene had been identified in patients with Stargardt's disease, an inherited form of macular dystrophy. This discovery may lead to presymptomatic testing for AMD and new strategies for prevention and therapy.

All wrapped up

The structure of the DNA exonuclease from bacteriophage lambda has been determined, and it reveals some of the reasons for its functions. Kovall and Matthews (p. 1824) found that the functional exonuclease would wrap around the DNA. The tunnel formed by the protein accommodates double-stranded DNA at one end but only single-stranded DNA at the other. In between lie the active degradative sites. The assembled structure does not leave a DNA strand until it falls off the end, or its own subunits dissociate; thus, the observed biochemical function is processivity.

Cichlid loss: A murky tale

The colorful cichlid fish in Lake Victoria, notable for their rapid evolution, are now also notable for their rapid decline: Half of all species have disappeared in recent years. Seehausen *et al.* provide a likely explanation (p. 1808). Cichlid coloration and species diversity are maintained by color-associative mating, which is correlated with light conditions. Eutrophication of the lake is increasing the turbidity of the water, which is leading to the breakdown of reproductive barriers and loss of diversity. Thus, diversity is being rapidly lost in the absence of classical extinction.

Getting a good grip

The primary structure of one of the collagens secreted by the foot of the mussel is presented by Coyne *et al.* (p. 1830; see the Perspective by Engel, p. 1795). Flanking the central structural portion, which forms the hallmark collagen triple helix, are domains with sequences similar to those found in elastin (a chief component of tendons and ligaments) and spider silk. Outside of these domains are zinc-binding sequences that may help bundle individual molecules into the tough but pliable byssal threads that anchor the mussel to rock.

Using vitamin D

Vitamin D is critical to healthy bones and a responsive immune system. Its absence, or inability to metabolize it, results in rickets. Vitamin D, available from dietary sources, must be converted within the body into the physiologically active form. Takeyama *et al.* (p. 1827) have identified in mouse the enzyme that manages this step, 1 α -hydroxylase that resides in the kidney.

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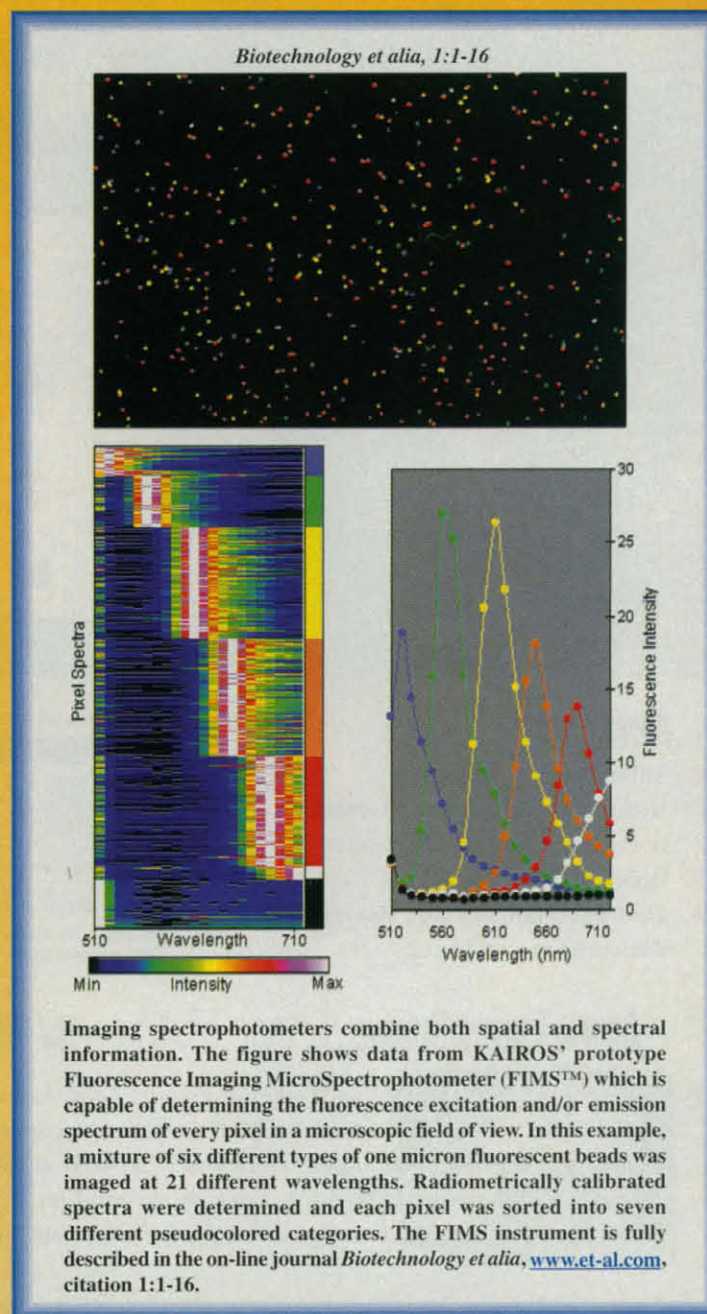
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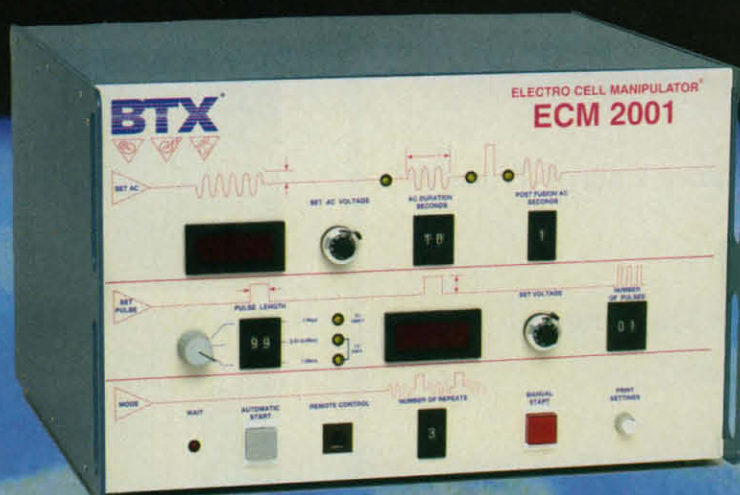
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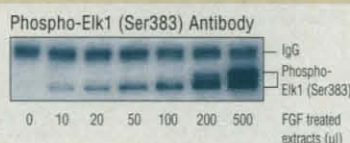
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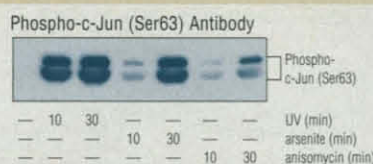
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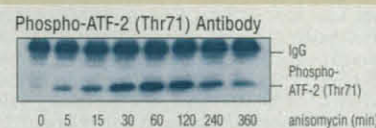
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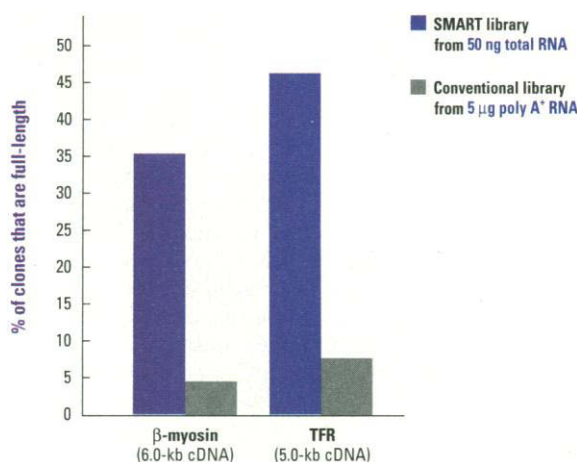
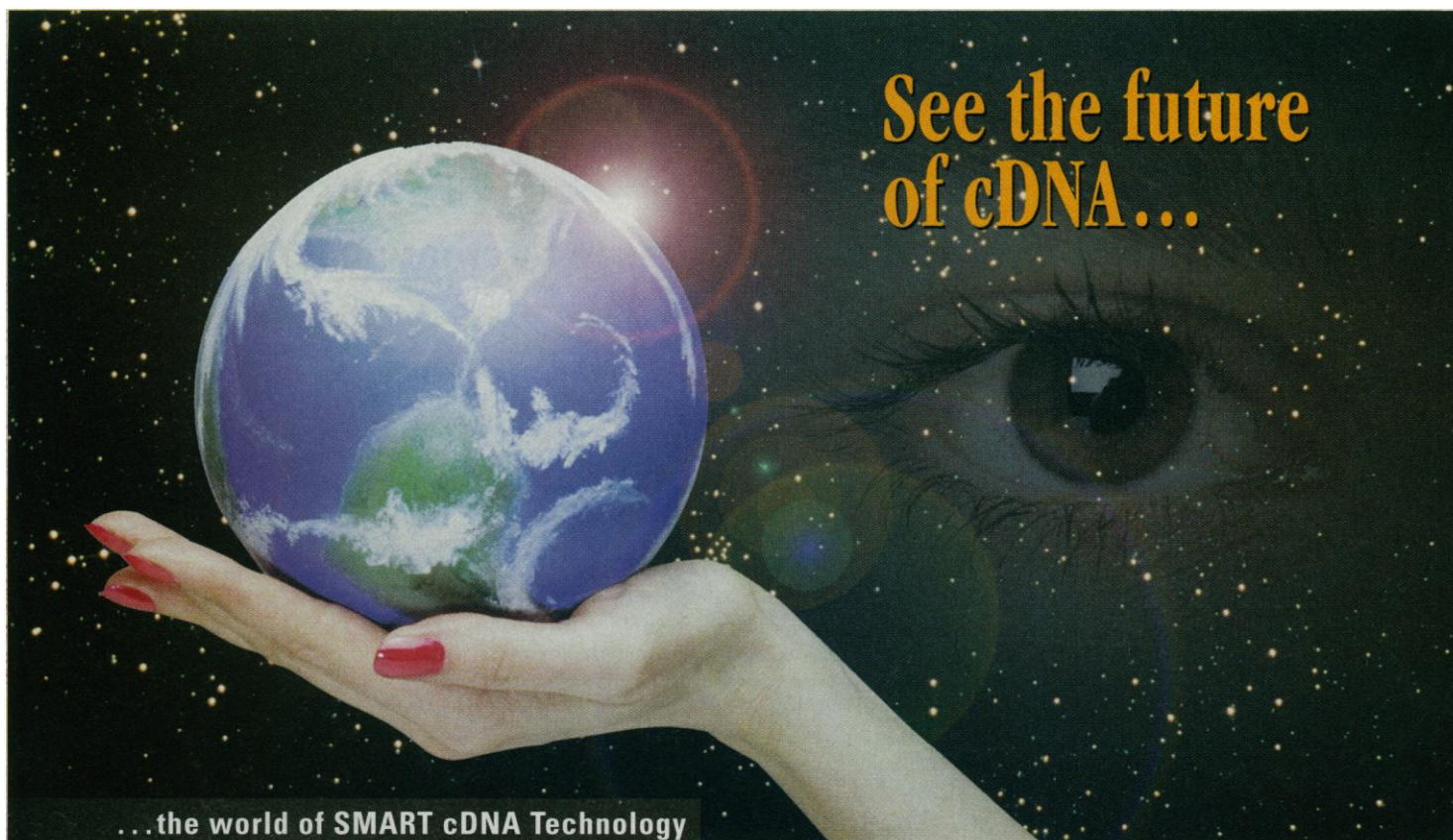


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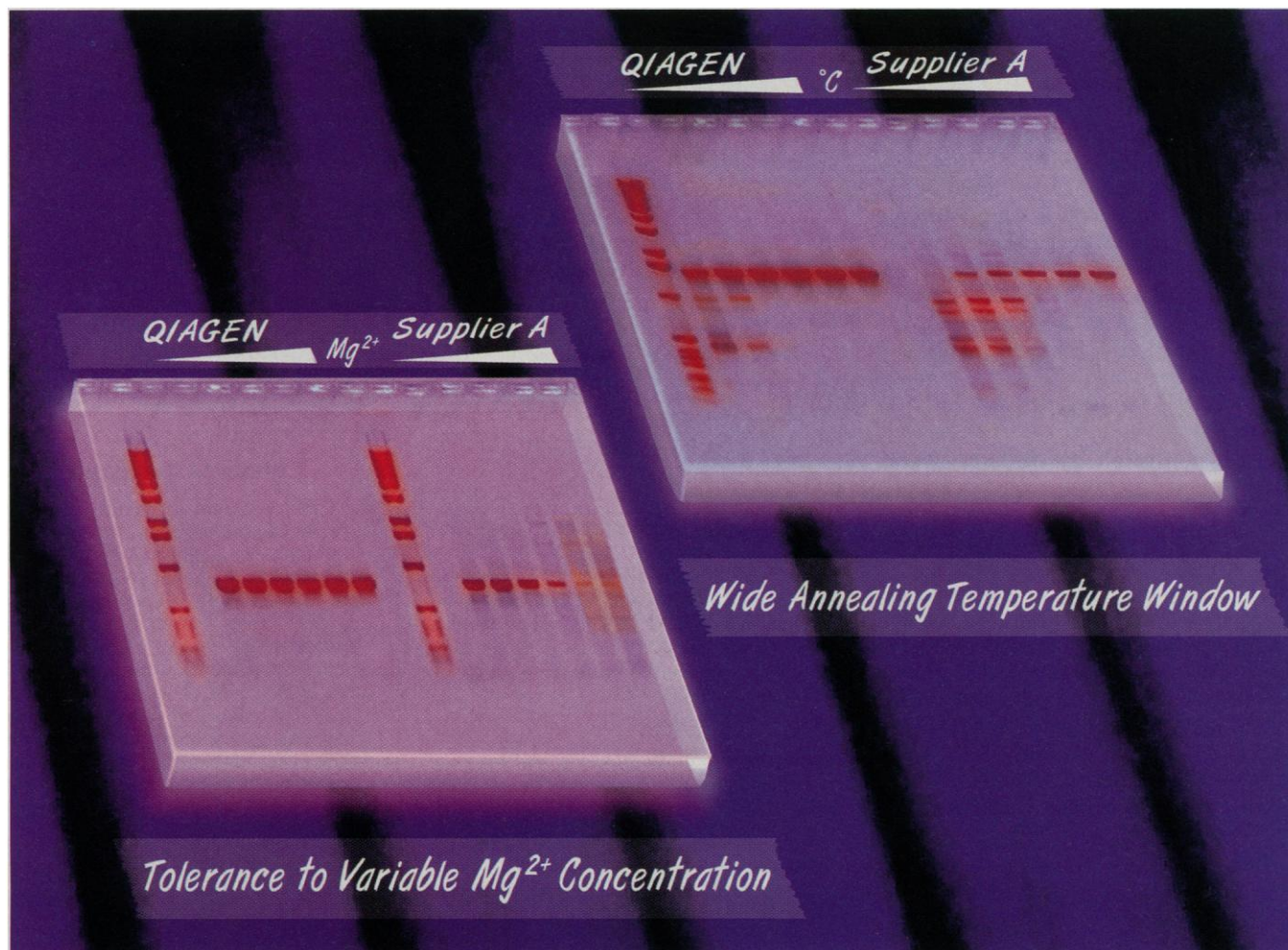
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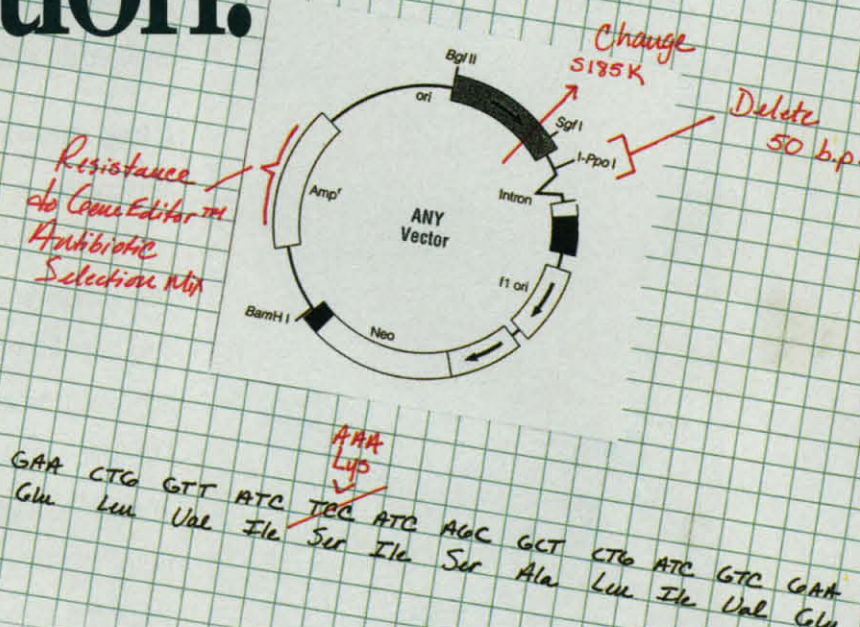
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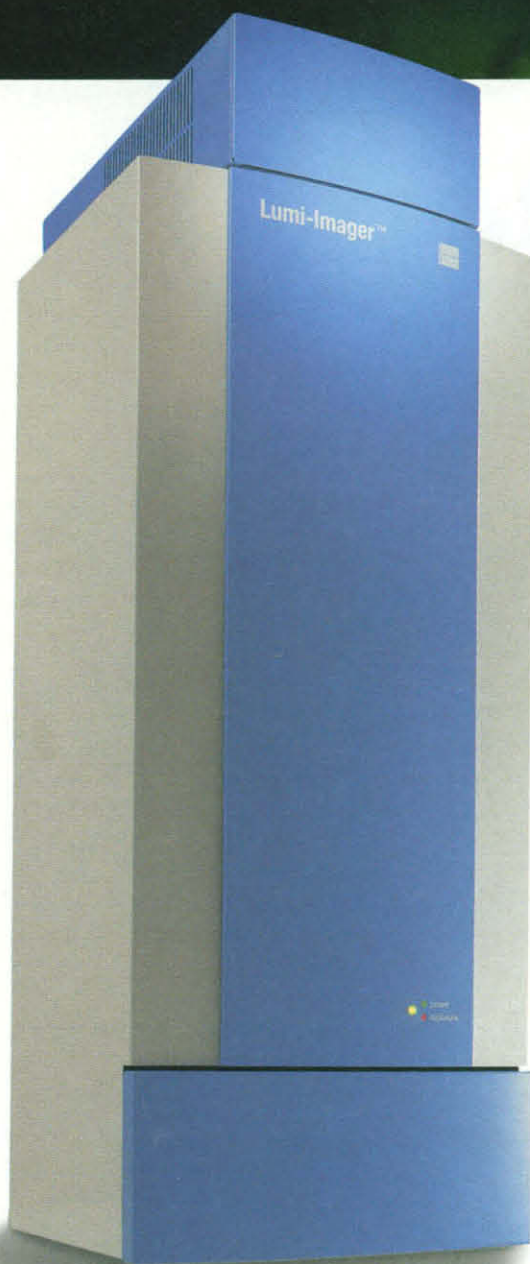
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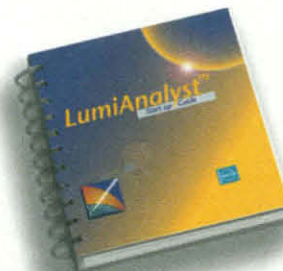


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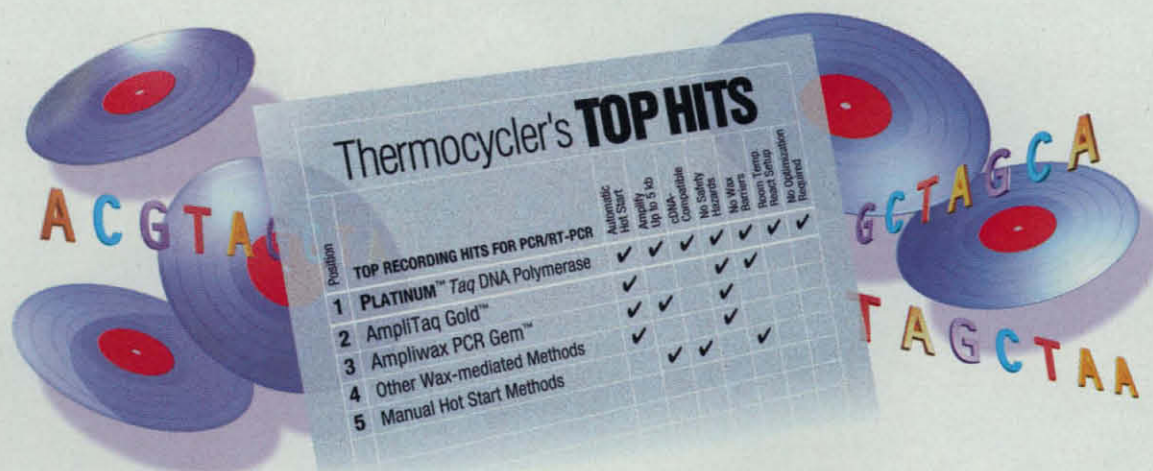
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Fig. 1. Sensitive detection of integrated HPV in SiHa cells using TSA-Direct (Cyanine 3 FISH). Biotinylated HPV-16 E6 DNA probe (1000 bp) hybridized to cultured SiHa cells. TSA fluorescence detection used Streptavidin-HRP followed by Cyanine 3 Tyramide. Slide counterstained with Hoechst 33342 (Molecular Probes, Inc.) and evaluated using separate tetramethylrhodamine and DAPI filters. Photo taken on KODAK 1000 speed film with 5 second (Cyanine 3 Tyramide) and 0.5 second (Hoechst 33342) double exposure using a 100X objective.

Fig. 1

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2b. TSA-Enhanced Fluorescence Detection

Tetramethylrhodamine filter

2c. Standard Fluorescence Detection.

2d. TSA-Enhanced Fluorescence Detection.

Multiband pass filter

Figs. 2a-d. Comparison of HPV fluorescence detection using Cy™3-conjugated Streptavidin versus TSA-Direct (Cyanine 3 FISH). Biotinylated HPV-16 E6 DNA probe hybridized to cultured CaSki cells.

2a-b. Standard fluorescence detection carried out with Cy™3-conjugated Streptavidin (Jackson ImmunoResearch Laboratories, Inc.). TSA-enhanced fluorescence used Streptavidin-HRP followed by Cyanine 3 Tyramide. Slides counterstained with Hoechst 33342 (Molecular Probes, Inc.) and evaluated using a tetramethylrhodamine filter. Photos taken using KODAK 1000 speed film with a 1 second exposure using a 40X objective.

2c-d. Protocol same as above but counterstained slides evaluated using a multiband pass filter. Photos taken using KODAK 1000 speed film with a 1 second exposure using a 40X objective.

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3a. Standard Fluorescence Detection

3b. TSA-Enhanced Fluorescence Detection

Fig. 3a-b. Comparison of standard fluorescence detection using Cy™3-conjugated Streptavidin versus TSA-Direct (Cyanine 3). Courtesy of Kevin Roth, M.D., Ph.D., Washington University School of Medicine, St. Louis, MO. Bouin's fixed, paraffin embedded mouse intestinal tissue, deparaffinized and incubated with biotinylated wheat germ agglutinin. Sections incubated with Cy3-conjugated Streptavidin (3a) or with Streptavidin-HRP followed by Cyanine 3 Tyramide (3b). Wheat Germ Agglutinin labels intestinal epithelial cells at the base of the crypts.

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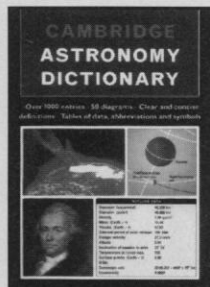
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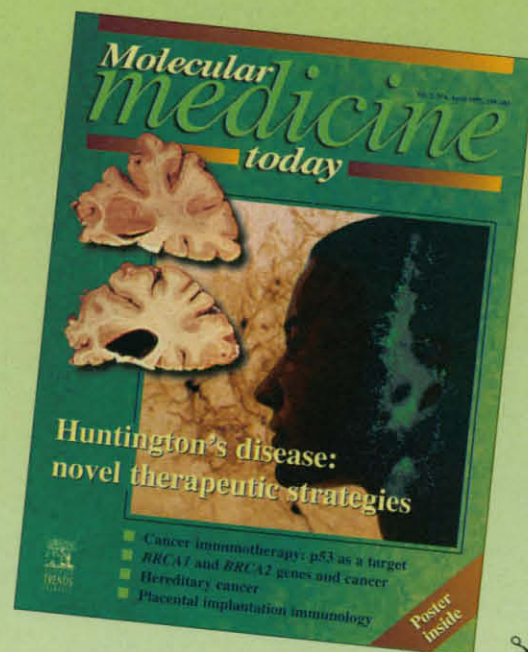
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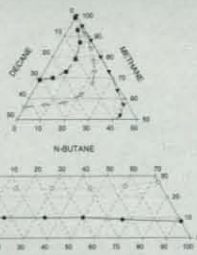
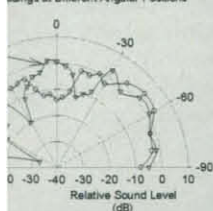
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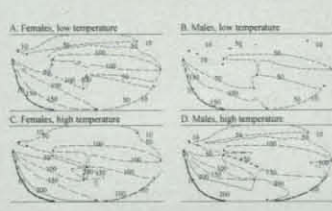
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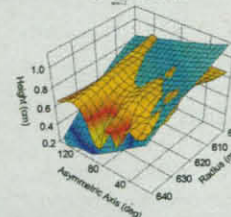


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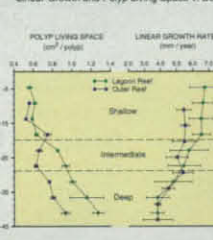


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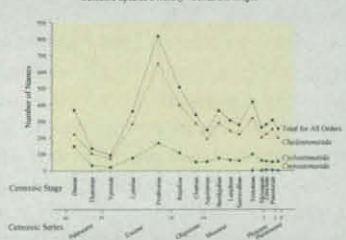
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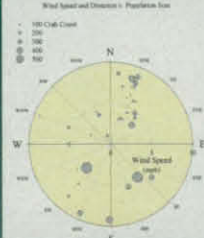
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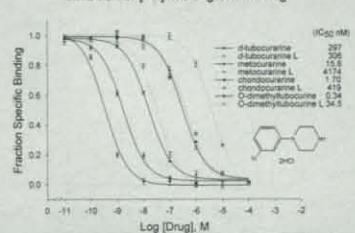
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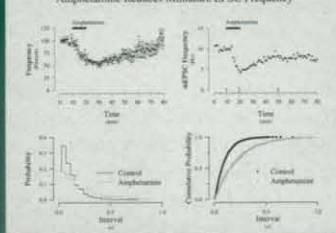
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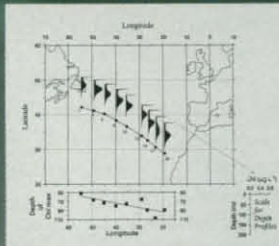
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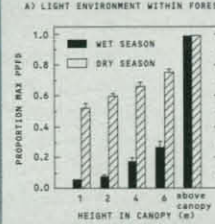
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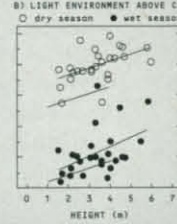
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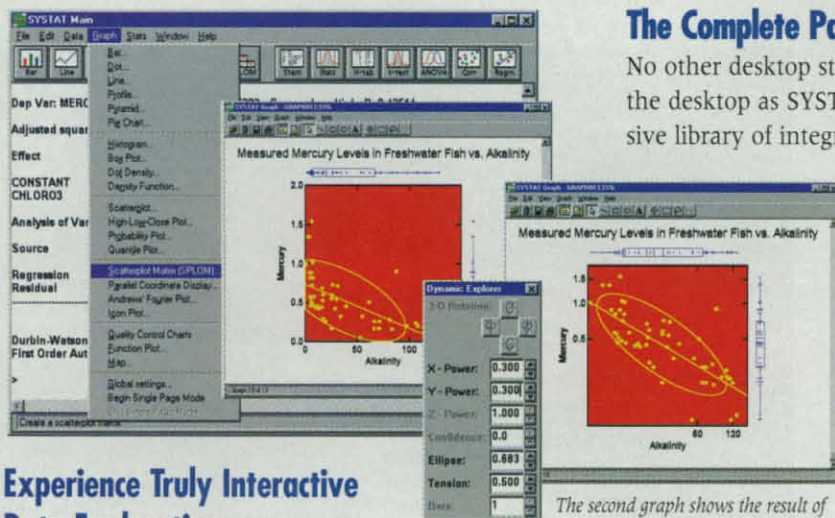
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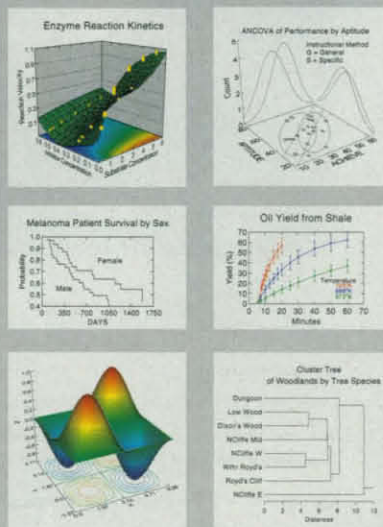
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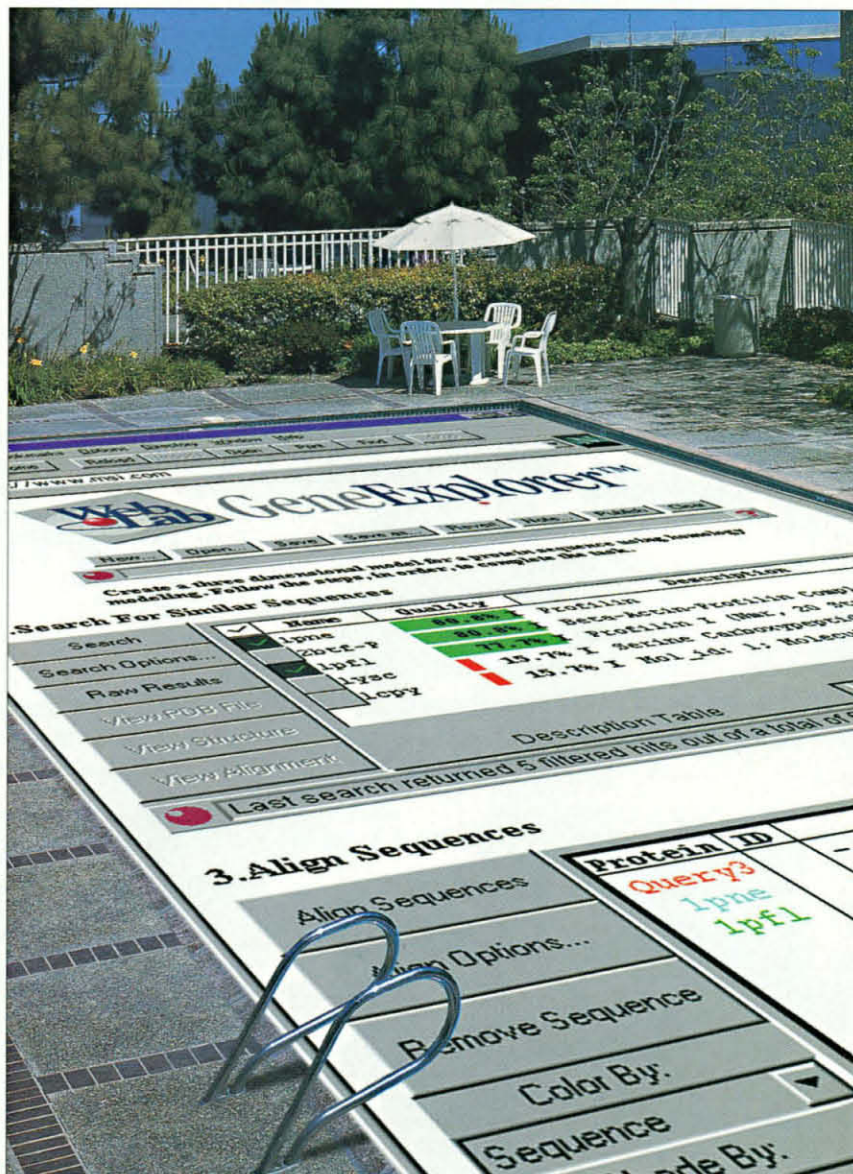
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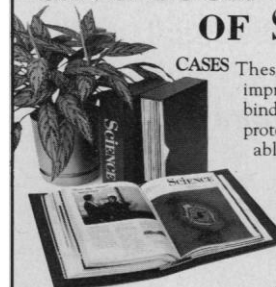


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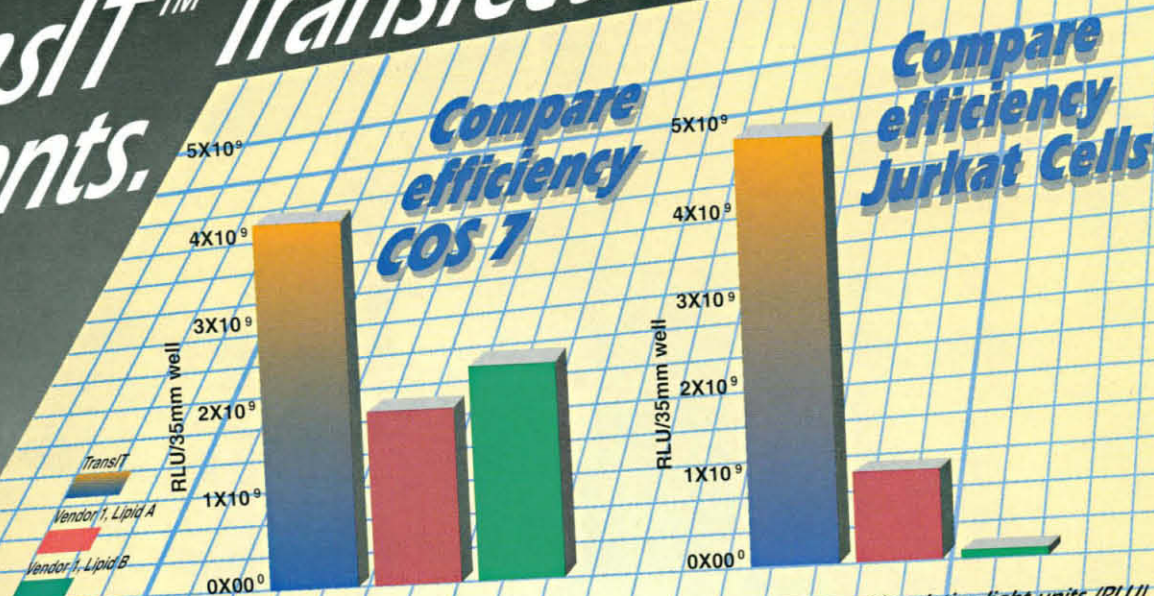
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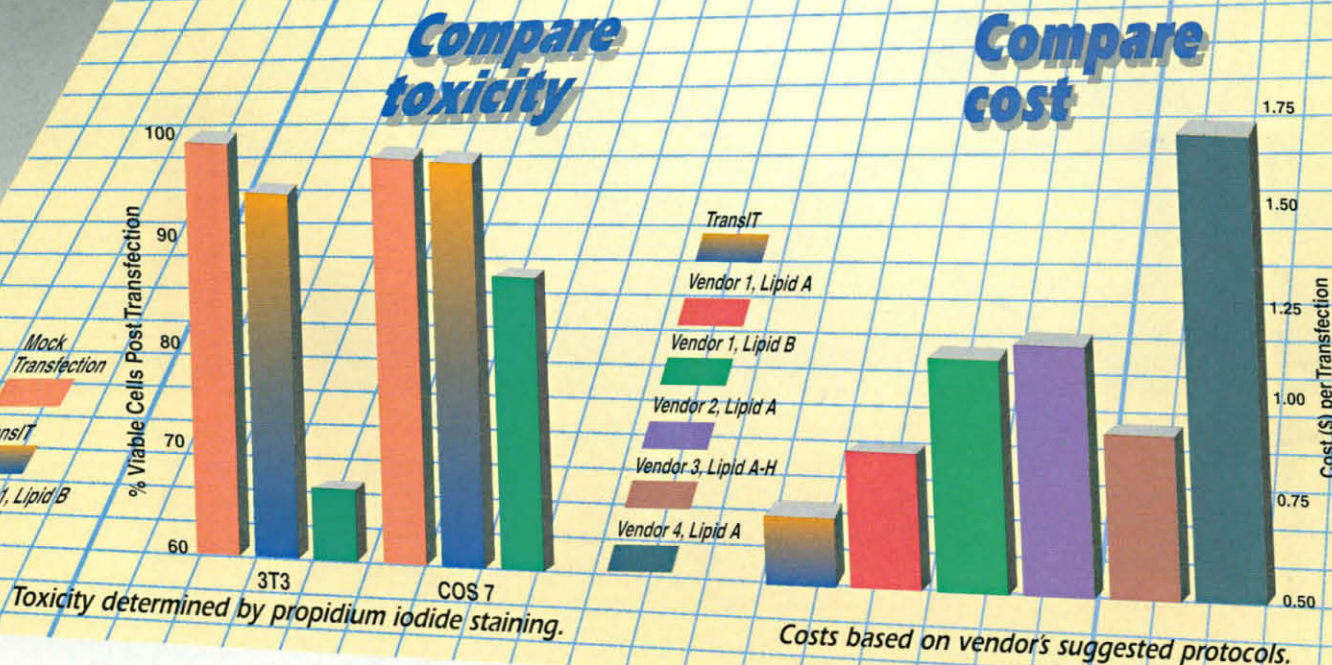
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