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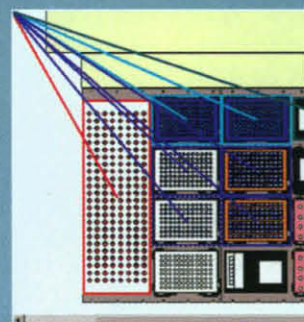
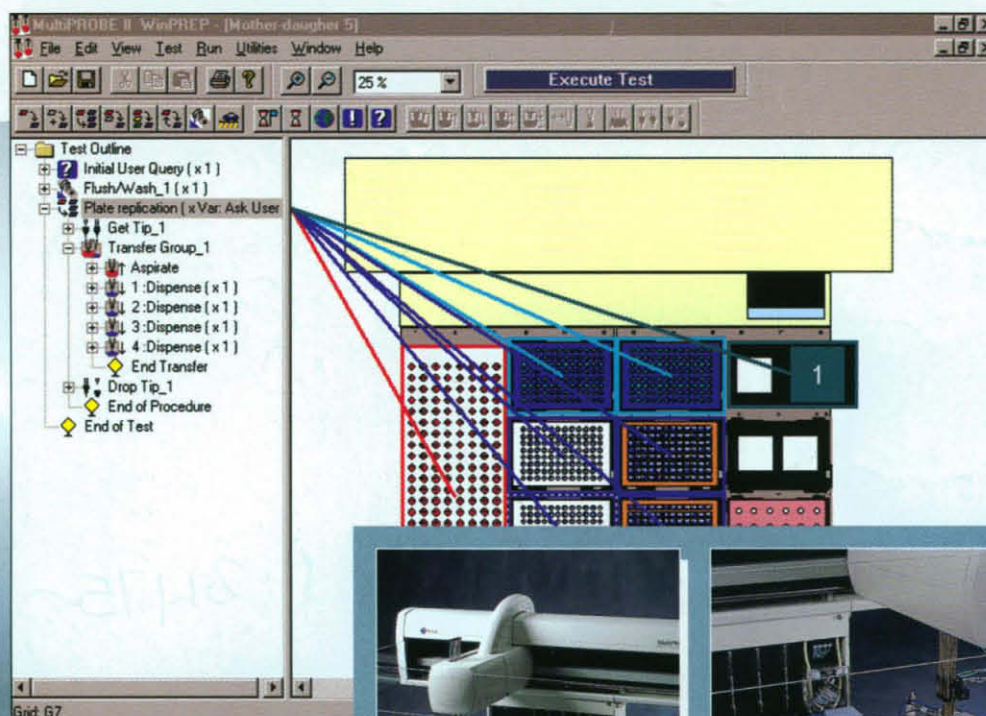
# SCIENCE

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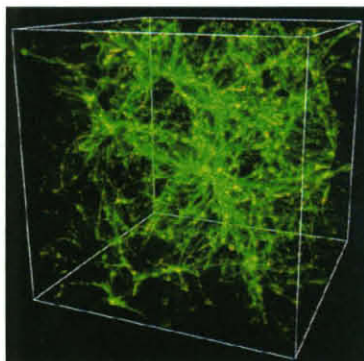
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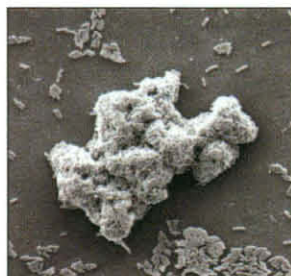
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## COVER

Three-dimensional microstructures were formed by contact printing a pattern on metal-coated glass capillaries (~2 millimeters in diameter), then etching the unprotected metal, electroplating onto the remaining metal structure, and subsequently dissolving the cap-

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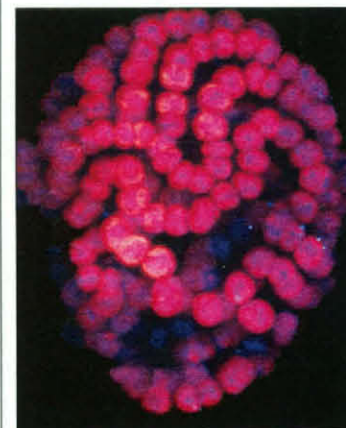
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**2073 & 2095**

Life inside ice

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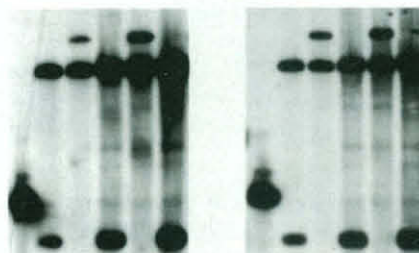
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Southern blot: Cosmid DNA digested with Not I and EcoR I, probed with a 1.1 kb probe labelled with AlkPhos Direct (left) and digoxigenin (right).  
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## Neptune weighs in on the Kuiper belt

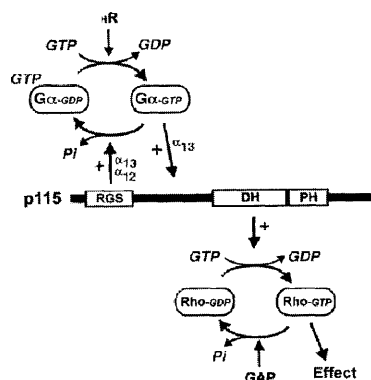
The Kuiper belt is a broad disk of kilometer-size or smaller objects beyond Neptune. The belt is assumed to contain debris ejected from the early solar system by the giant gaseous planets and is also considered to be a source of short-period comets. Although there have been many recent observations of some Kuiper belt objects, there are only estimates of the number of objects and the total mass of the belt. Ward and Hahn (p. 2104; see the commentary by Morbidelli, p. 2071) developed a spiral density wave model in which the interaction of the Kuiper belt with Neptune is used to account for the small eccentricity of Neptune. By using this model to fit Neptune's eccentricity, they estimate the mass of the Kuiper belt: about one Earth's mass worth of debris that extends to about 75 astronomical units.

## Perovskite to the core

Magnesium (Mg)- and iron (Fe)-rich perovskite is considered to be a major component of the lower mantle, yet previous high-pressure and high-temperature experiments suggested that the mineral decomposed into its component oxides, Mg- and Fe-rich oxide and silica, SiO<sub>2</sub>. Such decomposition would alter the rheology (flow characteristics) of the lower mantle and require changes to any model of the composition of the lower mantle. Serghiou *et al.* (p. 2093) conducted three different experiments at 75 to 100 gigapascals and 3000 Kelvin to try to observe this decomposition. They concluded that perovskite remains as a single stable phase to the pressure and temperature limits of their experiments and does not decompose. Thus, mod-

## Coupling G proteins to Rho

A mechanism for signaling between receptor-coupled heterotrimeric guanine nucleotide binding protein (G protein) and the small or monomeric guanine nucleotide-binding protein Rho has been established (see the commentary by Hall, p. 2074). Hart *et al.* (p. 2112) and Kozasa *et al.* (p. 2109) show that Rho participates in signaling pathways activated by the  $\alpha$  subunits of the G<sub>12</sub> subfamily of G proteins. Such signals influence various cell functions from transformation and development to cell death. Activated G $\alpha_{13}$  was found to bind directly and stimulate a guanine nucleotide exchange factor for Rho known as p115RhoGEF (an activator of Rho). The p115RhoGEF protein interacted with G $\alpha$  proteins through a domain with similarity to an analogous region in RGS proteins (regulators of G protein signaling). Furthermore, p115RhoGEF showed specific guanosine triphosphatase-activating stimulation for G $\alpha_{12}$  and G $\alpha_{13}$ . The results indicate that p115RhoGEF directly couples G proteins to Rho and that other proteins with RGS domains might also serve as effectors in G protein signaling pathways.



els of the composition and rheology of the lower mantle can assume that perovskite is a major component.

## From silt to stone

Thousands of years ago, the Mesopotamians lived in the desert between the Tigris and Euphrates rivers with few natural resources to draw upon. Stone *et al.* (p. 2091) conducted a surface survey of the early second millennium B.C. site of Mashkan-shapir (about 80 kilometers south of Baghdad) and found fragments and slabs of basalt. Through petrographic analysis and petrologic modeling, they determined that the basalt was synthetically produced by the Mesopotamians rather than being transported from any volcanic source region. The authors hypothesize that the "synthetic

basalt" was deliberately produced for use as tools and that the production of synthetic basalt required the innovative synergy of the metallurgical and ceramic industries of that time.

## From 2D to 3D

Three-dimensional microscopic objects have been formed from two-dimensional patterns through a combination of soft lithography and electrochemistry. Jackman *et al.* (p. 2089; see the cover) transfer microcontact-printed patterns to glass cylinders and then electroplate metals onto the pattern. After etching away the glass substrate, the remaining metal structure can be further manipulated. In one case, a structure is pulled apart to form a wire cube, and in another, two substrates are used to produce linked rings.

## Molecule or metal?

Small clusters of metal atoms, as they increase in size, should exhibit a transition from behaving like large molecules to exhibiting metallic behavior. Chen *et al.* (p. 2098) observed this in electrochemical studies of well-defined gold clusters (1.1 to 1.9 nanometers in diameter) protected with organic monolayers. Small clusters showed redox behavior and a large central energy gap, whereas larger clusters showed metallic double-layer capacitance and Coulomb staircase responses.

## Flexing polymers

Electrostrictive materials exhibit strain (change their size) in an applied electric field; examples include piezoelectric crystals. For actuator applications, large strains (several percent) and high-energy density to produce large forces are desirable. Zhang *et al.* (p. 2101) show that electron irradiation dramatically improves the electrostrictive response of the ferroelectric copolymer, poly(vinylidene fluoride-trifluoroethylene). They suggest that irradiation breaks up the ferroelectric domains and converts the material into a relaxor ferroelectric.

## Shock patterns

Large-scale atomistic simulations of complex deformations of materials have only recently become possible with the availability of massively parallel supercomputers. Such deformation processes are also difficult to track experimentally in real time, and therefore simulations are particularly important. Holian and Lomdahl (p. 2085) simulate the progression of shock

(Continued on page 2023)



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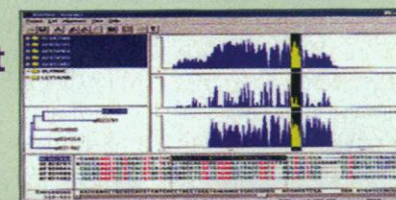
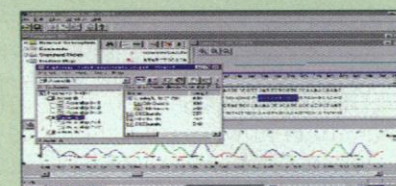
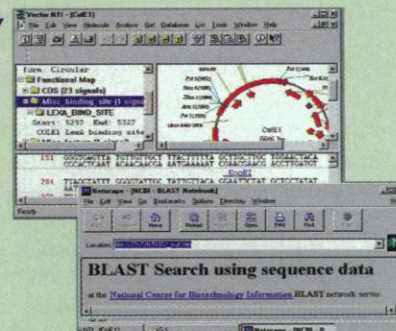
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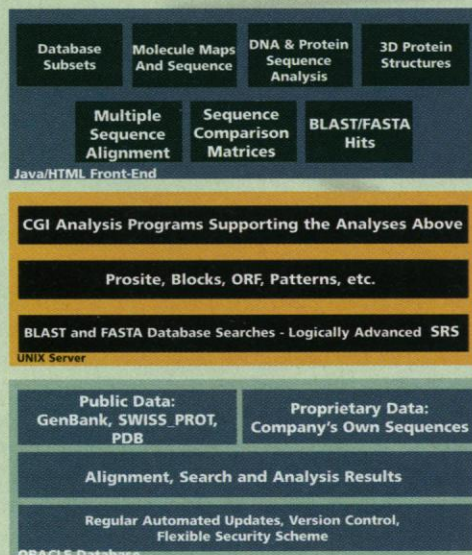
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(Continued from page 2021)

waves through crystal cells comprising several million atoms. They show that stacking fault patterns that evolve are not a function of the size of the modeling cell and that introducing an artificial extended defect lowers the energy barrier for the formation of faults.

### Bug ice

Antarctica contains several permanently ice-covered lakes in its dry valleys. Priscu *et al.* (p. 2095) describe a microbial ecosystem living within the ice cover of these lakes. The microbes survive in water inclusions within the ice. Food for the ecosystem is provided when sediments, including organic matter, blow onto the ice and slowly sink through it as the ice ablates at the surface. In a commentary, Psenner and Sattler (p. 2073) provide comparisons with environments in other often frozen lakes.

### Bacteria versus insects

Toxins are part of the battle between host and pathogen but can also be put to use to control agricultural pests. Bowen *et al.* (p. 2129; see the news story by Strauss, p. 2050) identified a bacterial toxin that may be useful in engineering pest-resistant plants. The toxin is synthesized by a bacterium, which is carried into the insect by its mutualistic nematode. The released bacteria then synthesize the deadly toxin, whereupon the host insect glows with bioluminescence emitted by the bacteria. The nematode then feasts on both bacteria and insect carcasses. The potent toxin is encoded by a group of related gene complexes.

### Bacterial virulence in the gut

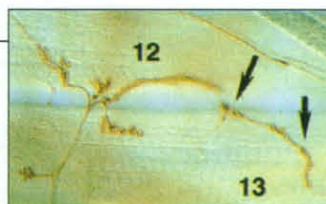
Certain gut bacteria can cause serious diarrhea. Bieber *et al.* (p. 2114; see the news story by Weidenbach, p. 2048) examine the role of protrusions from the surface of disease-causing *Escherichia coli*—the pili—in virulence. The presence of pili helps the bug to bind to the gut wall, which leads to changes in the gut cell that interfere with absorption and so causes diarrhea. Furthermore, bugs that have pili that were not capable of promoting bacterial motility were also less virulent. These results suggest that adherence of the bacteria and their subsequent dispersal in the gut are both factors in increasing the severity of symptoms.

### Protection by folic acid

The risk that babies might develop with neural tube defects is greatly diminished when the mother has sufficient folic acid intake. Fleming and Copp (p. 2107), using mice carrying certain mutations that promote neural tube defects, have analyzed the protective effects of added folic acid. The results indicate that the supplementary folic acid corrects a metabolic defect in pyrimidine biosynthesis but that methionine has an exacerbating effect.

### Connecting brain to brawn

Wiring up the nervous system in early embryogenesis involves axons extending into unknown territory, and at least in some cases following guidance cues that lead them only to the general area of their final connection. Shishido *et al.* (p. 2118) analyze some of the mechanisms that take over as the final details



of correct connection are resolved. Analyzing neuromuscular junctions in *Drosophila*, they find that a transmembrane protein encoded by the gene *capricious* helps define exactly which muscle is innervated by a particular neuron.

### Forgetting after a day

Long-term potentiation (LTP) is an increase in the strength of synaptic transmission between neurons. In the rat, one of the critical functions of the brain region known as the hippocampus is to encode its spatial environment. Kentros *et al.* (p. 2121) provide another piece to support the chain of reasoning that LTP, in this case the type that depends upon transmission via *N*-methyl-D-aspartate (NMDA) receptors, is the cellular correlate of learning, in this case spatial learning. By using pharmacologic blockade

of NMDA receptors, these authors demonstrate that this mode of synaptic transmission is necessary for long-term (24-hour) retention of a newly learned environment but not for short-term retention (1.5 hours) or for navigating in an old and familiar environment.

### Edge of the abyss

What factors cause the extinction of populations of large carnivores held in small, sometimes fragmented, reserves? Population size, perhaps, or the density of the species? A large survey by Woodroffe and Ginsberg (p. 2126) shows that achieving high numbers is certainly a factor, but not a strong one. The critical issue is range size. Those species that range most widely—such as bears, wolves, and wild dogs—are at greatest risk of local extinction. The simple explanation for this is that they are more often exposed to edges of reserves and, thereby, to the biggest threat to their existence—conflict with human activity.

## Technical Comment Summaries

### Dating Rock Surfaces

Publications in *Science* [R. I. Dorn *et al.*, *Science* 231, 830 (1986)] and elsewhere originally suggested that radiocarbon ages could be obtained from organic matter trapped in a rock surface layer and that these ages could be used to infer the age that a rock surface was exposed. This approach has been most often used to infer the ages of archaeological samples and geomorphic features, such as petroglyphs and lake shorelines.

W. Beck *et al.* (p. 2132) comment that many of the radiocarbon samples that have been dated in their laboratories contain a mixture of carbon sources, rendering the radiocarbon ages ambiguous. They also comment that they were unable to extract similar carbon sources from their field samples. In response, Dorn (p. 2135) points out that the presence of mixtures of carbon sources with different ages in rock surface layers has been previously noted and occurs naturally (see the news story by Malakoff, p. 2041).

The full text of these comments and figures can also be seen at [www.sciencemag.org/cgi/content/full/280/5372/2132](http://www.sciencemag.org/cgi/content/full/280/5372/2132)



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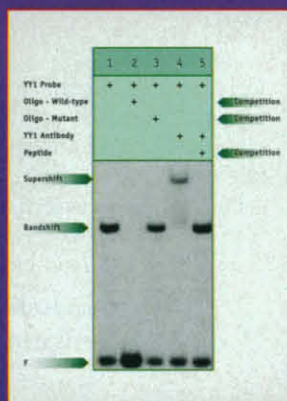


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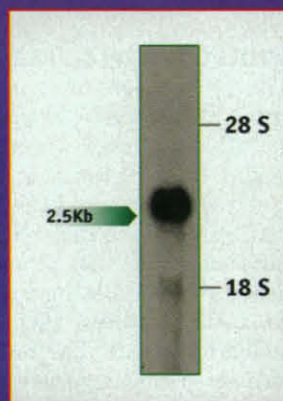
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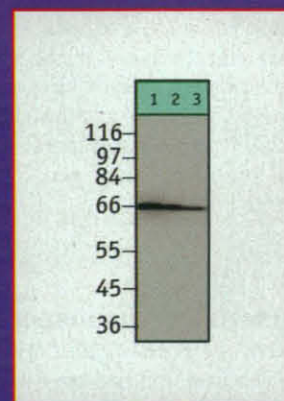
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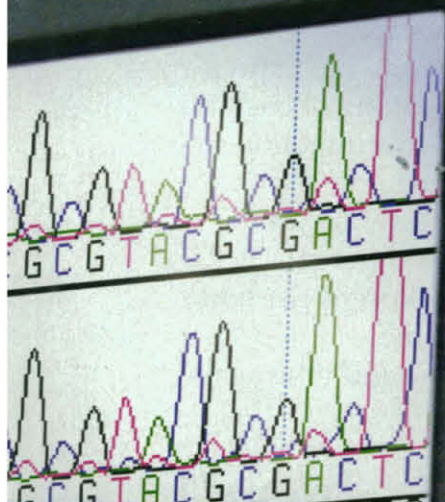
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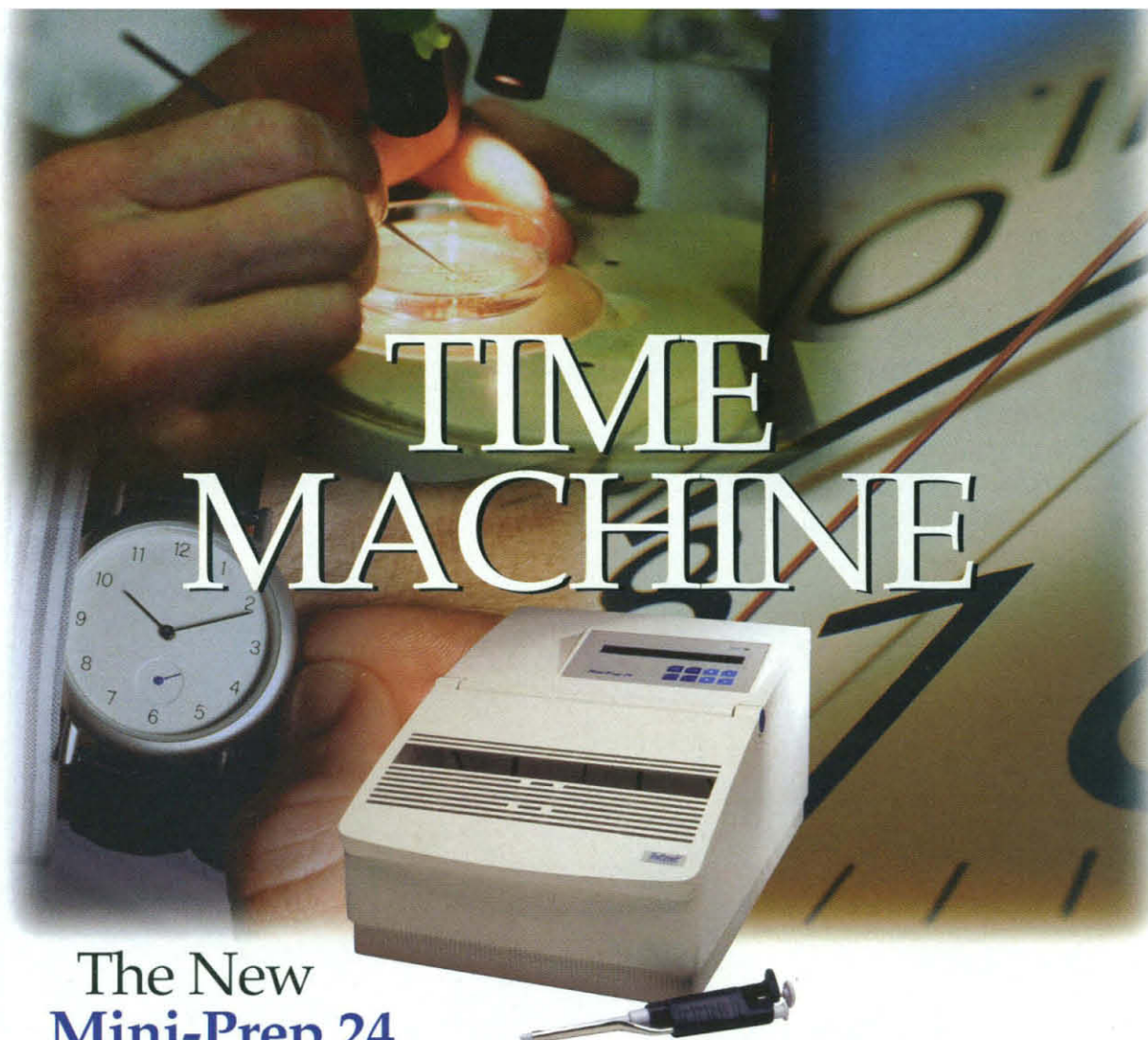
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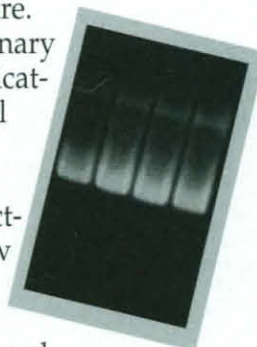
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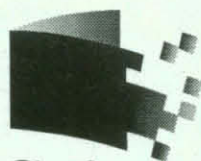
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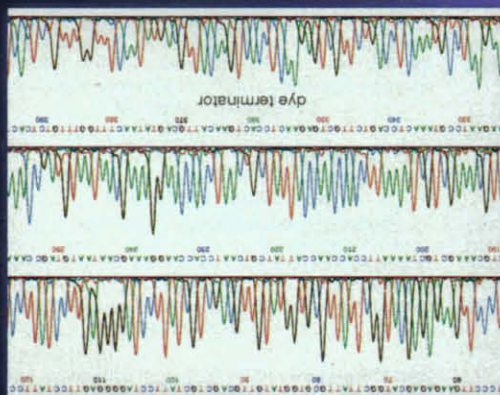
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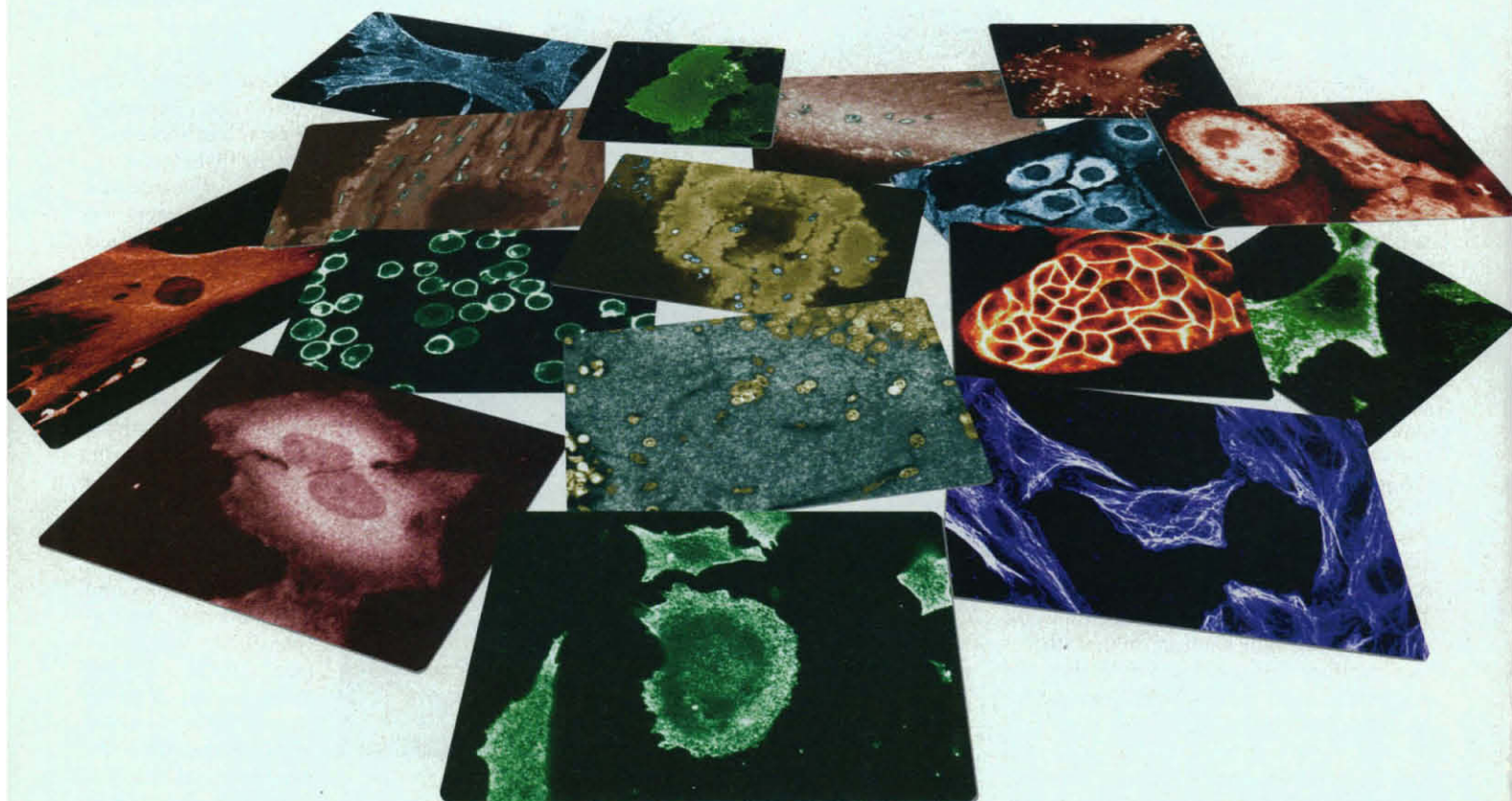
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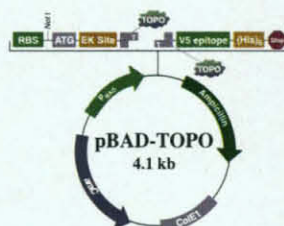
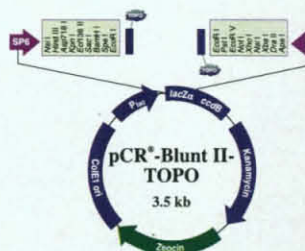
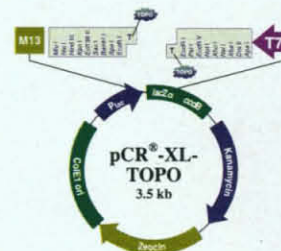
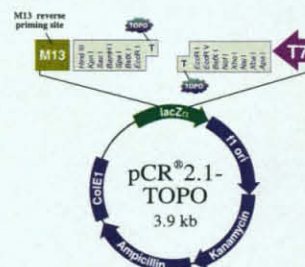
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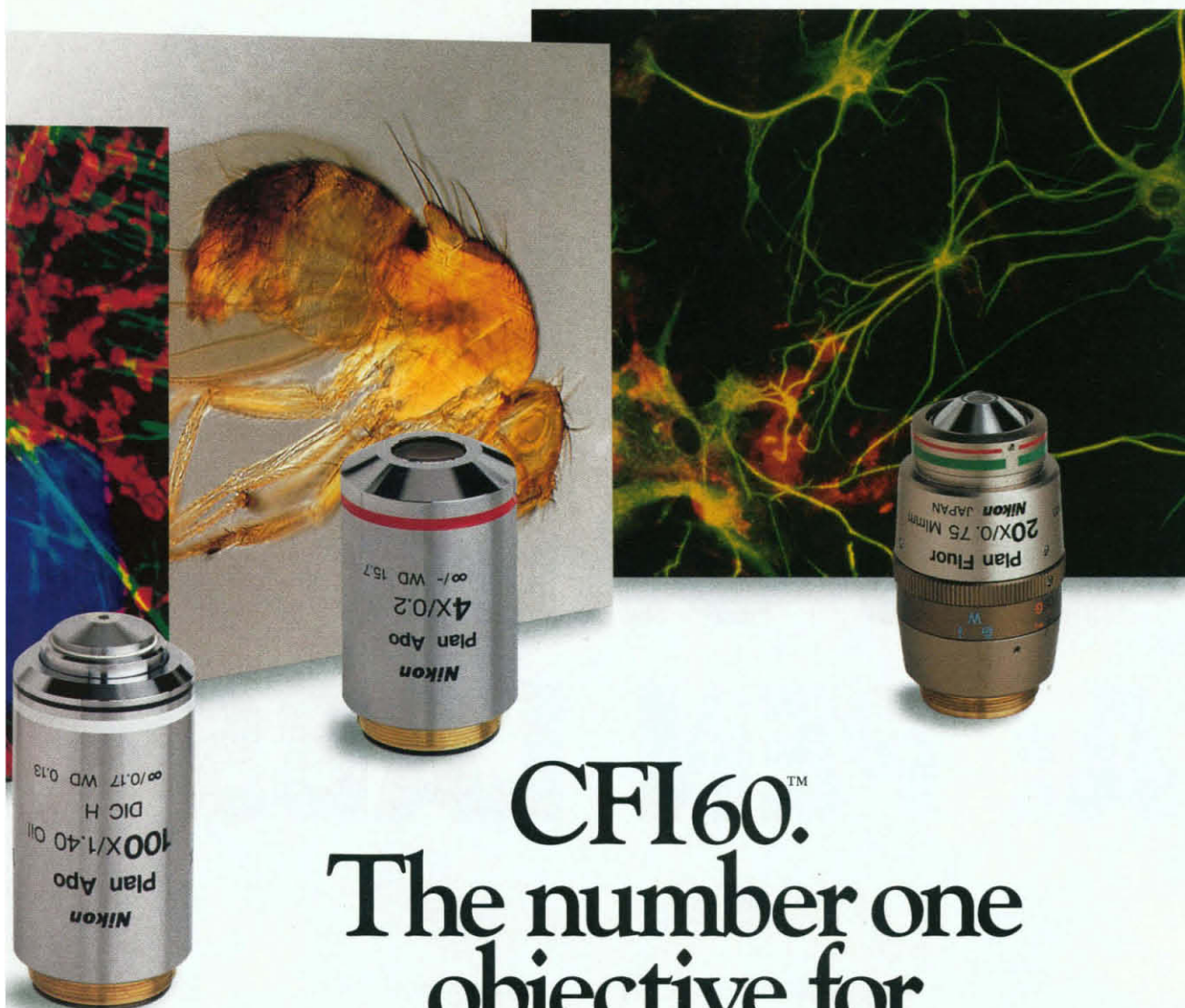


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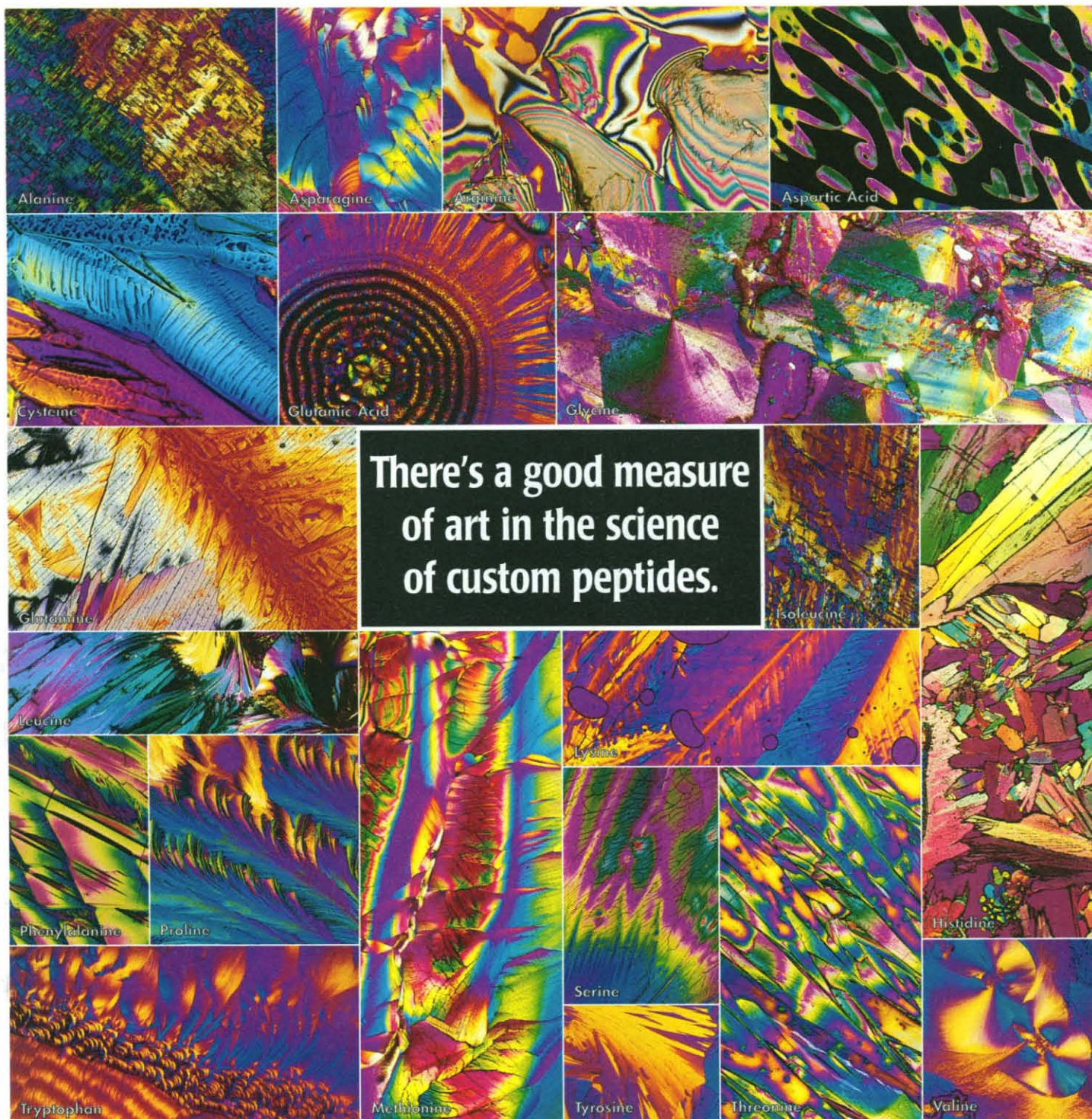


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Fig. 1. Sensitive detection of integrated HPV in SiHa cells using TSA-Direct (Cyanine 3 FISH). Biotinylated HPV-16 E6 DNA probe (1000 bp) hybridized to cultured SiHa cells. TSA fluorescence detection used Streptavidin-HRP followed by Cyanine 3 Tyramide. 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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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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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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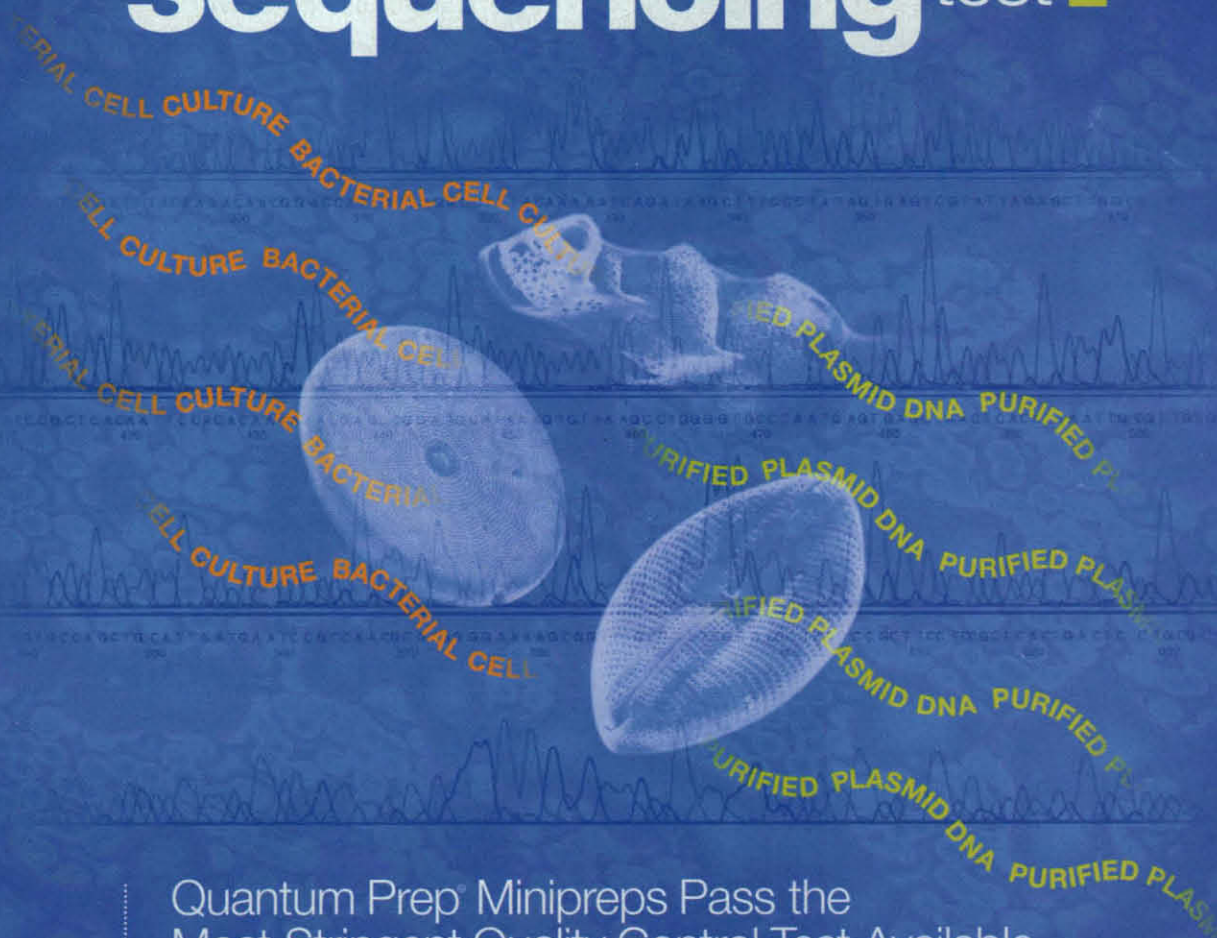
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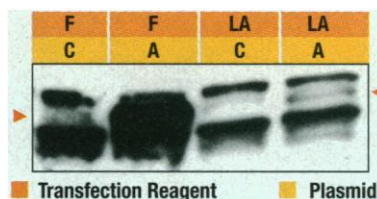
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S.T. Crooke (Eds.)

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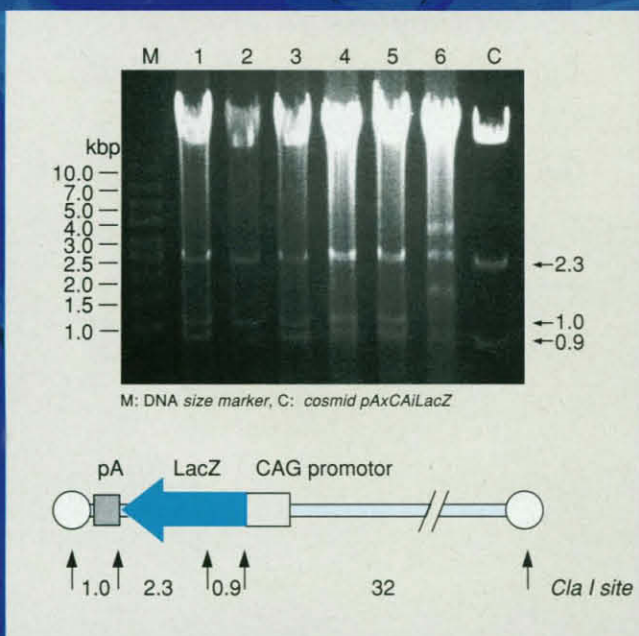
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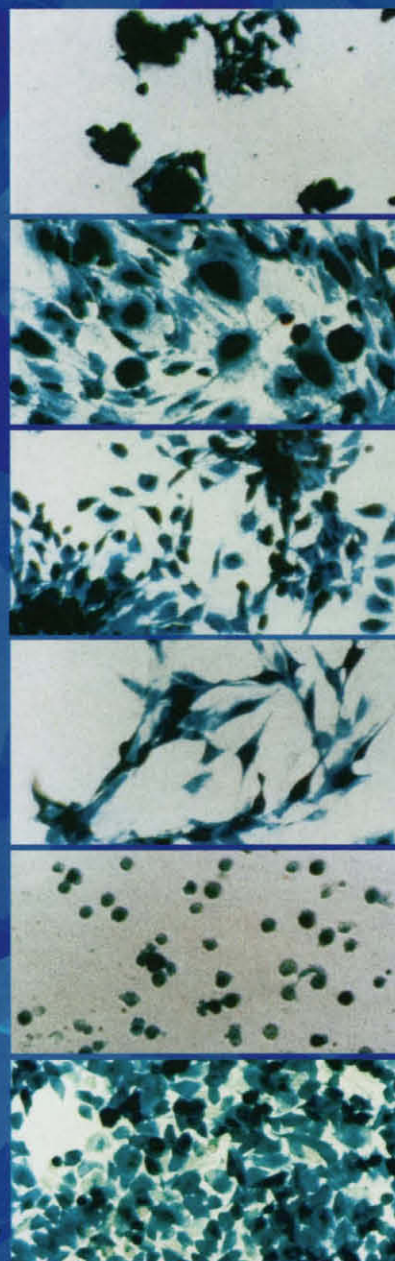
\* Method Principle: Miyake, S. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**, 1320  
Application (Transgenesis): Tsukui, T. *et al.* (1996) *Nature Biotech.* **14**, 982

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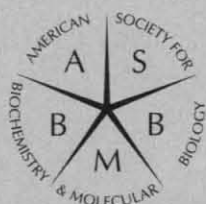
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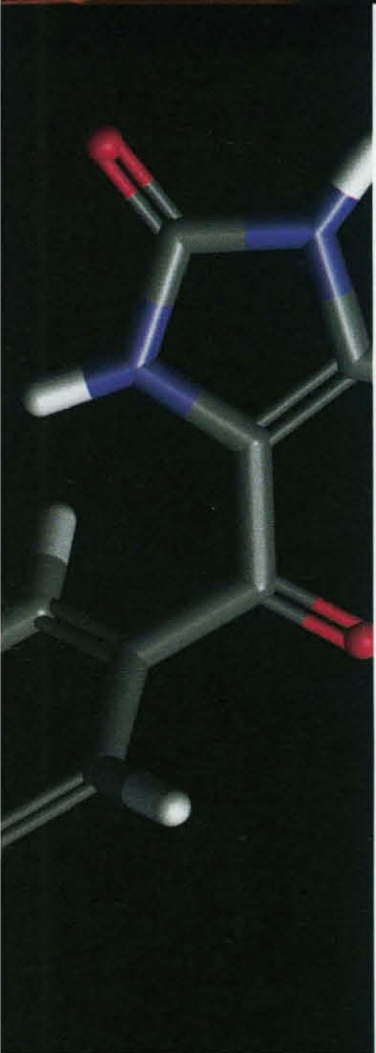
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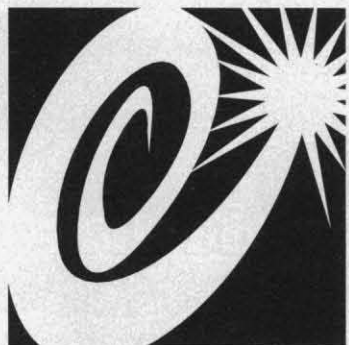
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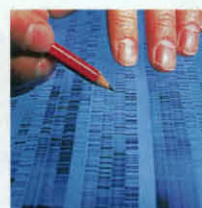
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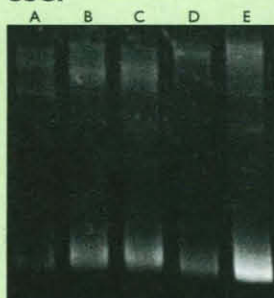
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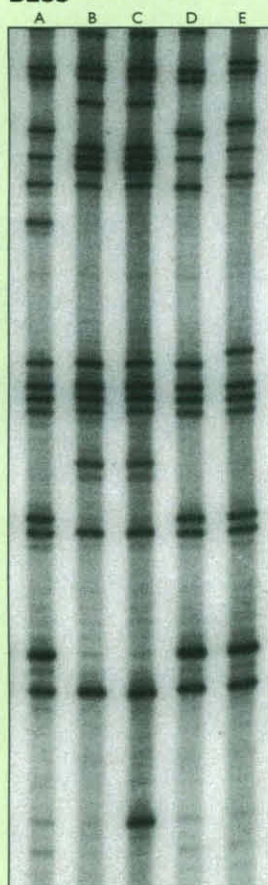
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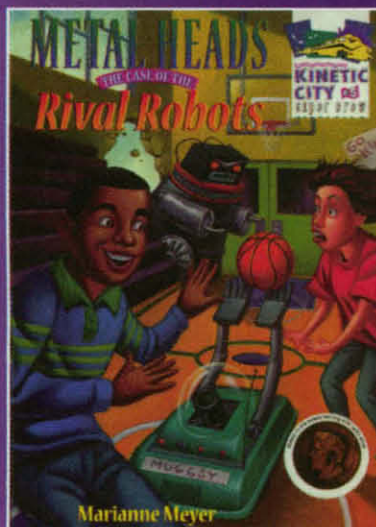
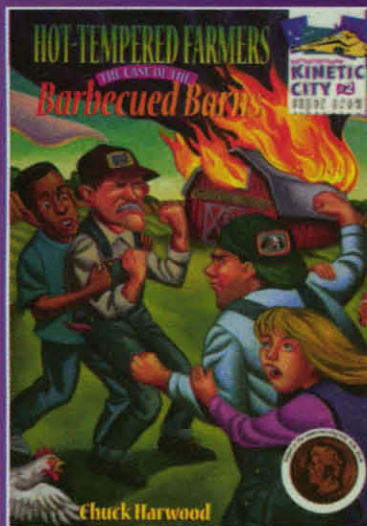
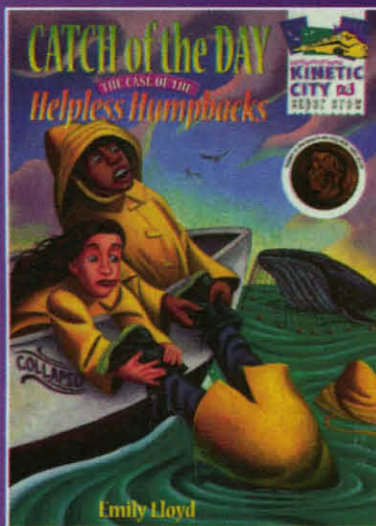
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