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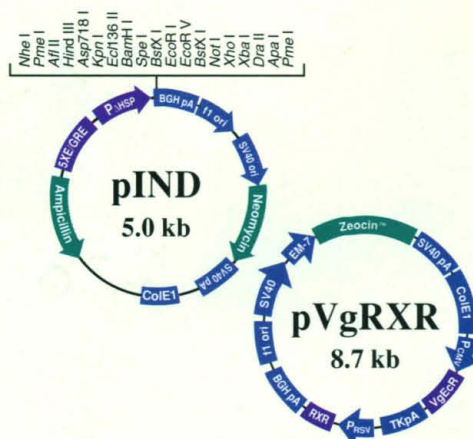
Developmental
Neuroscience

The First Truly Inducible Mammalian Expression System

Invitrogen's new Ecdysone-Inducible Mammalian Expression System is the first system to offer truly low basal expression and high inducibility. Based on a naturally evolved eukaryotic regulatory mechanism that triggers the molting process of insects, ecdysone-responsiveness transfers ideally into mammalian systems (1). This system is entirely unique. It does not rely on prokaryotic operator sites and the artificial nuclear localization of regulatory molecules to control induction as do other regulated systems. This makes the Ecdysone System extremely efficient and easy to use.

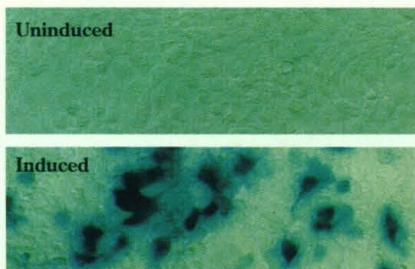
How it Works

For transient transfection and induction, simply clone your gene of interest into pIND, then cotransfect it into mammalian cells with the regulator vector, pVgRXR. Approximately six hours later, treat the transfected cells with the ecdysone analog, muristerone A and begin analysis. Depending on the sensitivity of the assay, you can begin detecting induced expression in as little as two hours. For stable transfection, use dual selection with Zeocin™ and G418.



Tightly Regulated

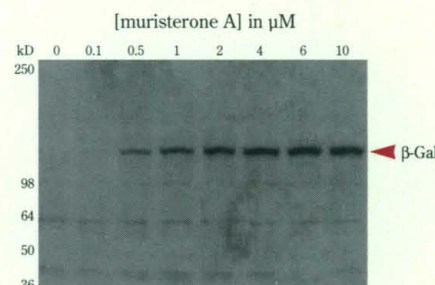
The slides below show a simple colorimetric assay of 293 cells cotransfected with pVgRXR and pIND/lacZ before and after muristerone treatment. This example vividly illustrates the Ecdysone System's tight control and capacity for high inducibility.



Uninduced and induced transiently transfected 293 cells stained with X-gal.

Specificity and Control

Induction is specific—exhibiting no pleiotropic effects on mammalian cells—and dose-dependent—allowing for thorough analysis of gene function through a full range of expression.



Dose-dependent β -galactosidase Induction. 293 cells stably transfected with pVgRXR and pIND/lacZ treated with increasing amounts of muristerone A. Equal amounts of isolated protein were separated by SDS-PAGE, blotted and detected by antibody and chemiluminescence.

COMPLETE KIT. The Ecdysone-Inducible Mammalian Expression System has everything you need to get started, including vectors, a positive control, sequencing primers, Zeocin™ antibiotic, and muristerone A. Stable cell lines expressing the regulator (pVgRXR) are also available. Call Invitrogen or visit our web site to learn more about the Ecdysone-Inducible Mammalian Expression System.

1. No, D. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93: 3346-3351.

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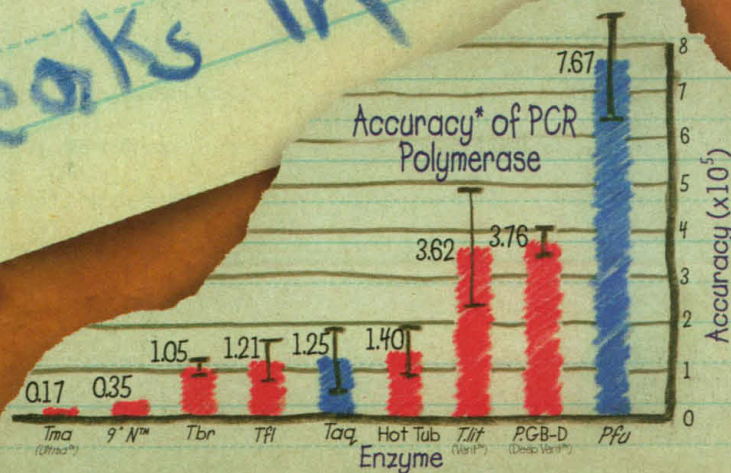
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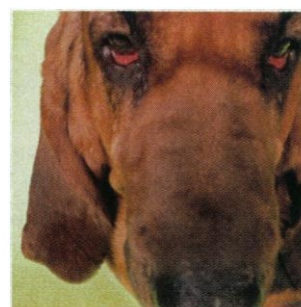
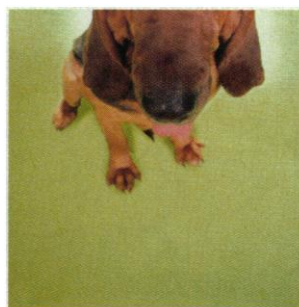
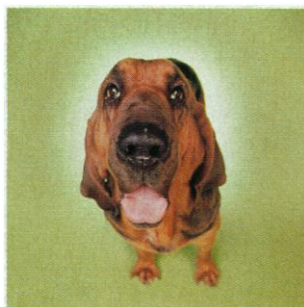
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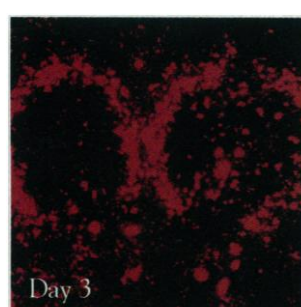
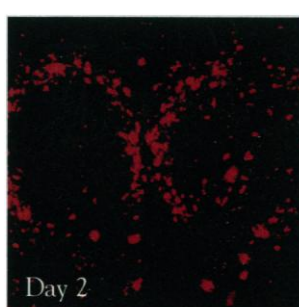
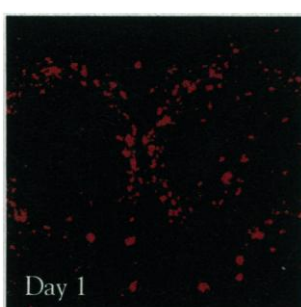
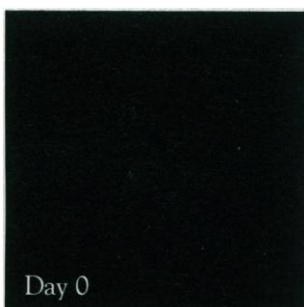
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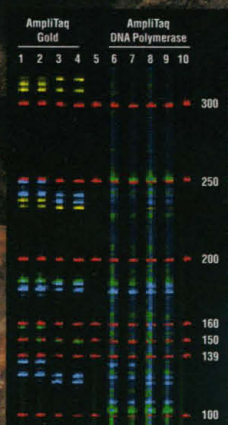
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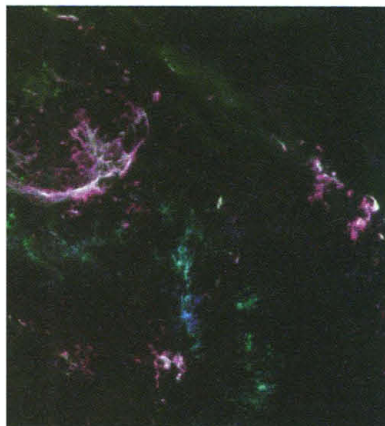
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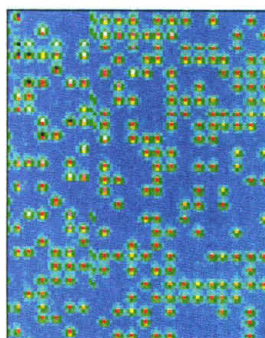
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COVER

Segmentation in the early embryonic hindbrain is revealed by *Hox* reporter gene expression in a transgenic mouse. The alternating blue- and brown-stained domains are rhombomeres, repeat units that underlie the hindbrain's segmented neural architecture. Development

of the nervous system, from embryonic patterning to early function, is the focus of this issue. See page 1109, the Editorial on page 1063, and the special section beginning on page 1099, which includes an article on Web sites of interest to neuroscientists. [Image: James Sharpe]



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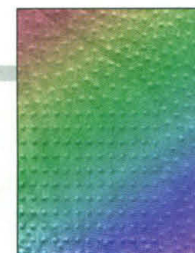
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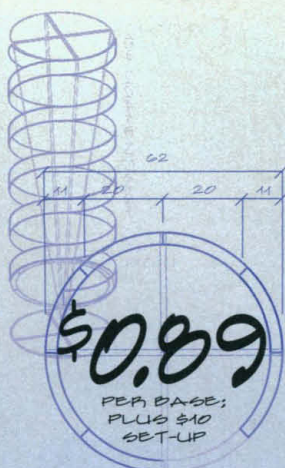
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Video clips showing vortex dynamics in superconducting films by Harada *et al.*
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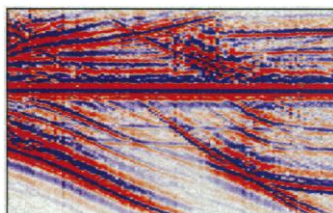
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THIS WEEK IN SCIENCE

edited by PHIL SZUROMI

Mantle minerals

Key information regarding the composition of the lower mantle, and thus the nature of convection in the Earth, is provided by matching seismic and mineralogical data of the transition between the upper and lower mantles that occurs at a depth of about 660 kilometers. Estabrook and Kind (p. 1179) show that seismic reflections



that were expected to be returned from just beneath the phase transition at 660 kilometers are not present. These data can be explained if the jump in *P*-wave velocity across the 660-kilometer transition is about 2%, or less than the previously expected value. This analysis implies that the lower and upper mantles have similar compositions.

Affecting climate

Whether the observed changes in climate during this century are the result of natural variability or human influences remains hotly debated. Tett *et al.* (p. 1170) have performed a set of climate simulations involving changes in three factors that are under anthropogenic influence and are believed to affect climate, namely sulfate aerosols, greenhouse gases, and stratospheric ozone. Detailed comparison with observations shows that best agreement is reached when the simulations are forced with a combination of these three factors, compared to simulations involving only

Tails of the ascidians

Chordates, which include vertebrates, normally have during some stage of development a notochord, an internal flexible rod of cells along their dorsal side. Tunicates (ascidians, or sea squirts) are among the simplest chordates and now provide genetic clues to notochord development. Most species, such as *Molgula oculata*, develop from a tailed tadpole larva, but some, such as its relative *M. occulta*, evolved to lack a tail and notochord structure as larvae. Tail expression can be restored, however, in hybrid embryos. Swalla and Jeffery (p. 1205; see the news story by Pennisi, p. 1082) have now found that the zinc finger gene *Manx* is expressed in cells that develop notochord features and is down-regulated in the tailless species.

subsets of these factors. Although many questions and uncertainties remain, recent climate changes are unlikely to be entirely due to unforced natural variability.

Photorefractive organic glass

Photorefractive (PR) materials have applications in optical devices and storage systems. Organic PR materials can be made with properties that rival or exceed those of the main inorganic PR material, lithium niobate. One problem, however, is that the chromophores used to generate the PR response must be dissolved in excess polymers for the material to remain in an optically useful glassy state. Lundquist *et al.* (p. 1182) now report a class of improved organic PR materials in which the chromophore itself forms a stable glass so that the polymer fraction can be decreased substantially.

Timing deglaciation

Deglaciation following the Last Glacial Maximum was interrupted most notably by the Younger Dryas, which was a re-

turn to colder conditions about 12,000 years ago, but the origin of this event has been uncertain because it has been difficult to correlate in time the relevant climate records obtained from various regions around the North Atlantic. Björck *et al.* (p. 1155) examined several climate records, including Greenland ice cores and tree rings and lake sediments from northern Europe, to develop a chronology for the Younger Dryas and related climate shifts. They suggest that the Younger Dryas lasted until about 11,400 years ago and was caused by a change in the thermohaline circulation of the Atlantic Ocean.

Causing division

In dividing eukaryotic cells, exit from mitosis and initiation of anaphase requires the activation of the anaphase-promoting complex (APC), a group of proteins that mediates regulated proteolysis of proteins critical to the control of the cell cycle. Reports from Peters *et al.* and Zachariae *et al.* (pp. 1199 and 1201) describe a new subunit of the APC. The APC subunit appears to be evolutionarily conserved because the APC subunits from both yeast and

Xenopus laevis (a frog) are similar to a protein called BIME from *Aspergillus nidulans* (a fungus). The properties of BIME in *Aspergillus* indicate that the APC may also function to regulate entry into as well as exit from mitosis.

Caught napping

The differences between the chemistry of the brain while awake versus asleep has intrigued researchers for many years. Cirelli *et al.* (p. 1211) show that the locus coeruleus, a small region of the brain that communicates with large areas of the brain cortex and hippocampus, has a much lower pattern of activity in sleeping than in waking rats. The authors provide evidence which suggests that the locus coeruleus acts as a neuromodulator to control the activity of other brain areas during the sleep-wake cycle.

Mutating pathogens

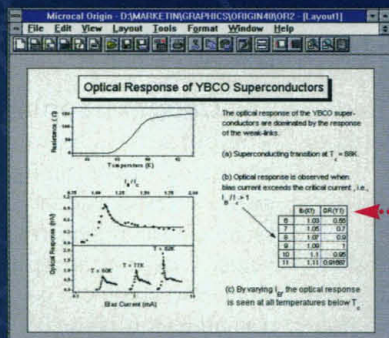
Methyl-directed mismatch repair (MMR) helps control the rate of mutations in newly synthesized DNA and gene transfer between species. LeClerc *et al.* (p. 1208) examined isolates of bacterial pathogen species associated with food-transmitted human diseases, including *Escherichia coli* and *Salmonella enteritidis*, and found that they had an unexpectedly high mutation rate. In all of the cases examined, the hypermutability was due to defects in MMR. The authors suggest that hypermutability may help account for the rapid increase in antibiotic resistance seen recently in bacterial pathogens (see the news story by Grady, p. 1081).

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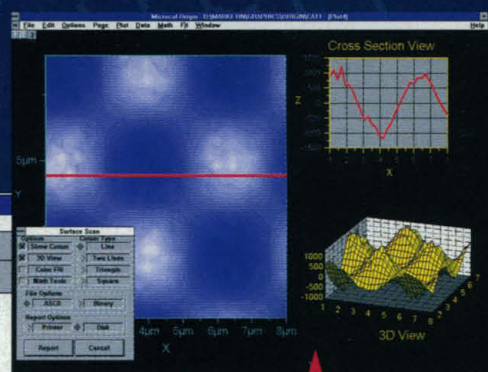
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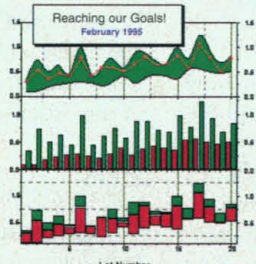
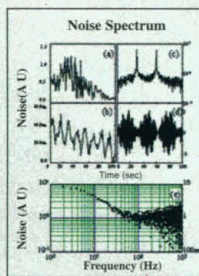
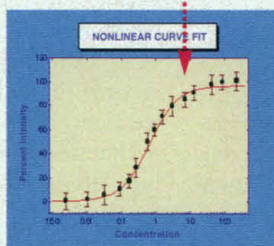
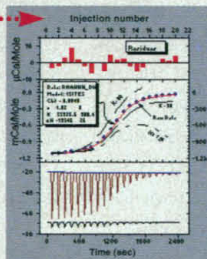
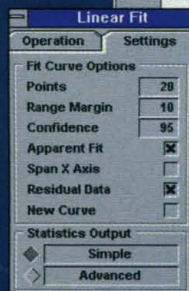
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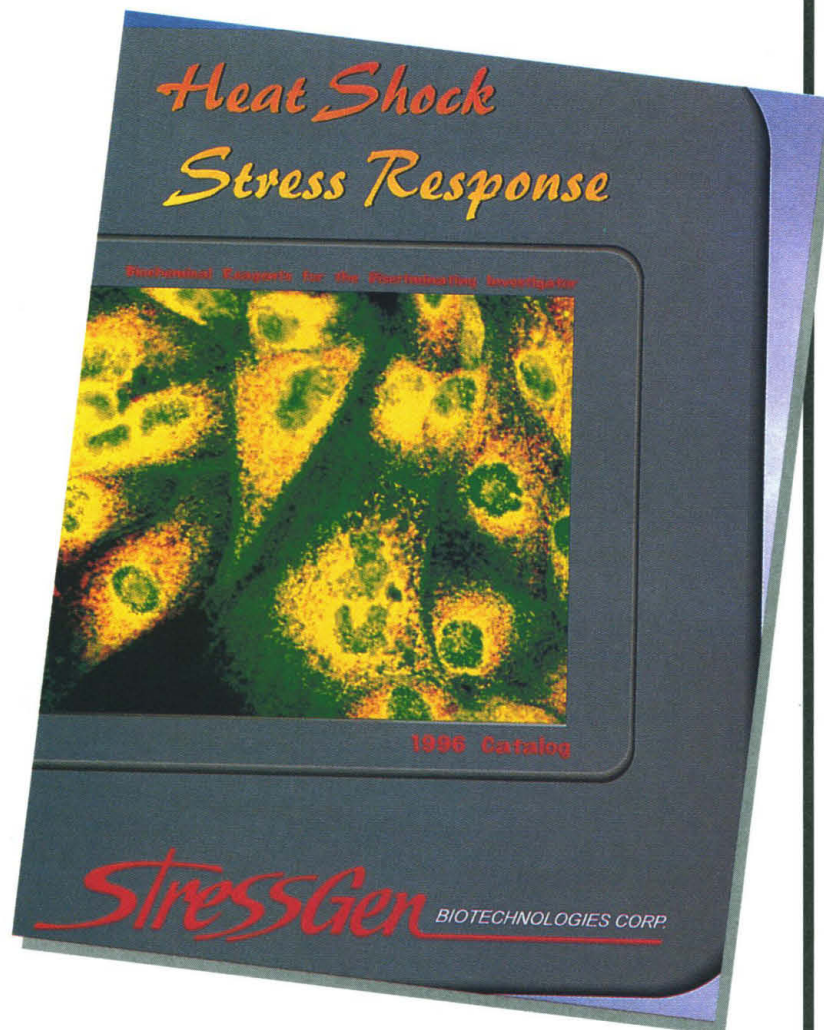
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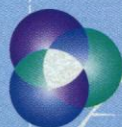
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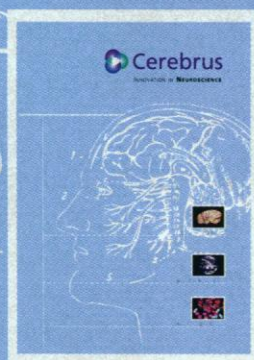
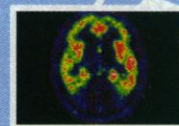
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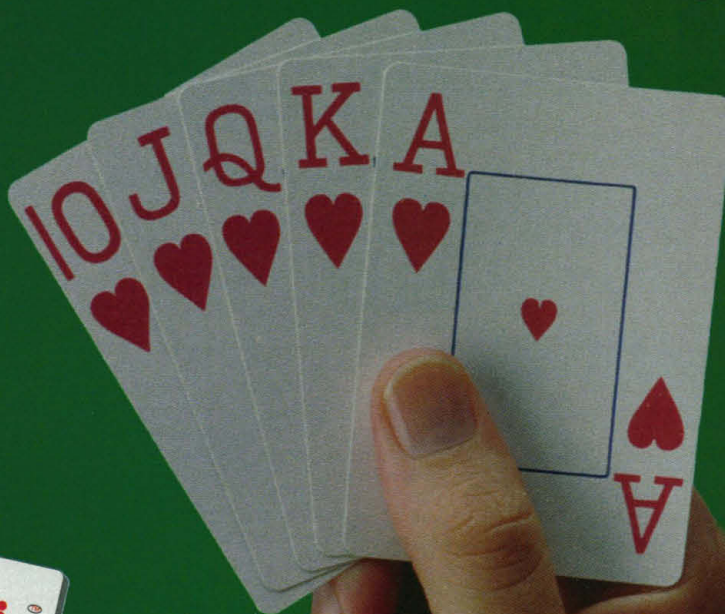
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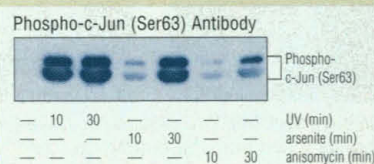
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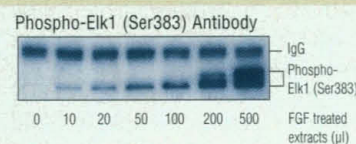
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Analysis Using: SAPK/JNK assay kit



SAPK/JNK activity in extracts of treated SK-N-MC cells was analyzed by c-Jun "pull down"/kinase assay. Phosphorylation of c-Jun at Ser63 was visualized by immunoblotting with phospho-c-Jun (Ser63) antibody.

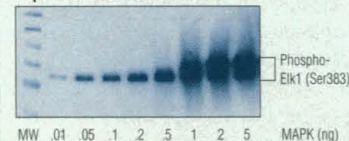
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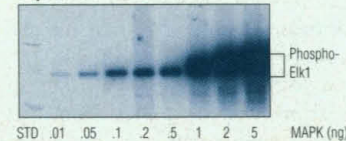
MAP Kinase activity in extracts of FGF-treated SK-N-MC cells was analyzed by phospho-MAPK antibody IP/Kinase assay using Elk1 as a substrate. Phosphorylation of Elk1 at Ser383 was visualized by immunoblotting with phospho-Elk1 (Ser383) antibody.

Sensitivity Comparison: phospho-specific antibody vs. radioactivity

(A) phospho-specific antibody exposure time: 30 seconds



(B) radioactivity [³²P] exposure time: 24 hours



MAPK-induced phosphorylation of Elk1 was measured by quantitative immunoblotting with phospho-specific Elk1 (Ser383) antibody (A) and compared to direct measurement of phosphate incorporation using [³²P]-ATP (B). MW = NEB's Biotinylated Protein Marker, Cat. No. 7710.

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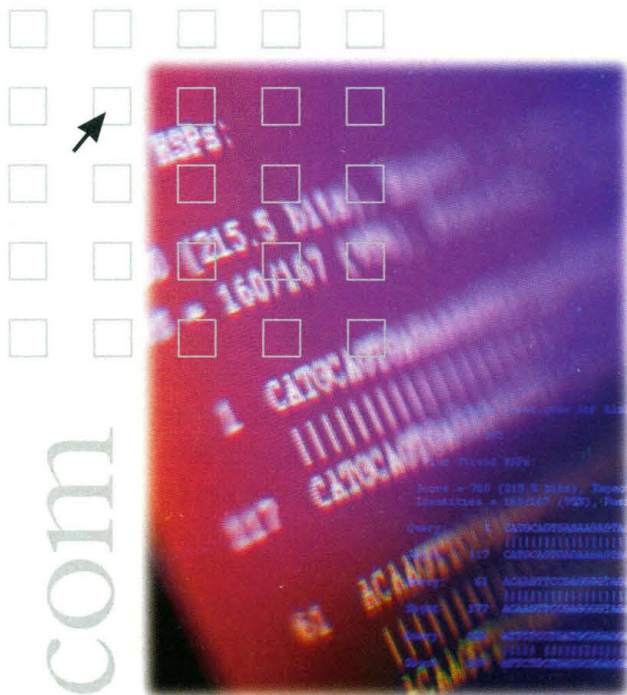


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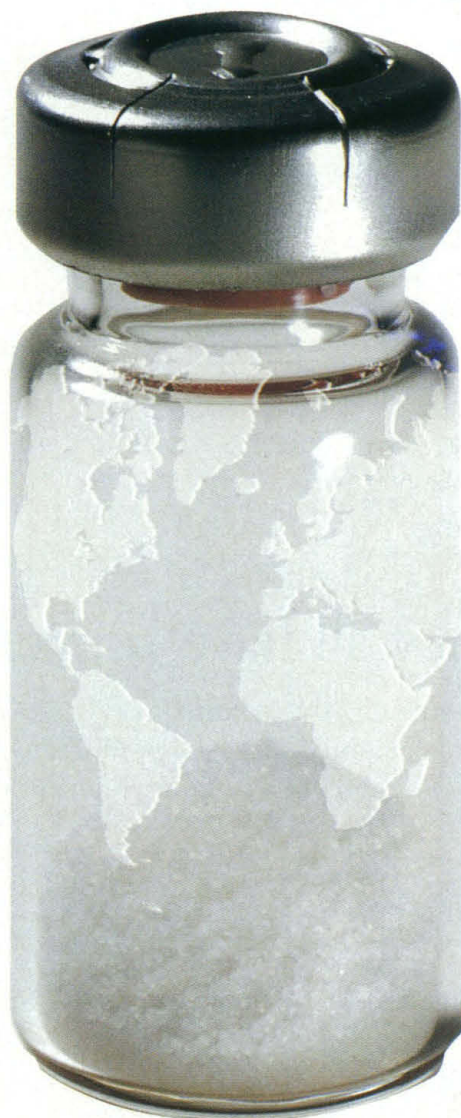
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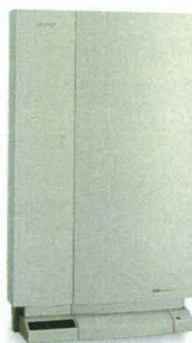
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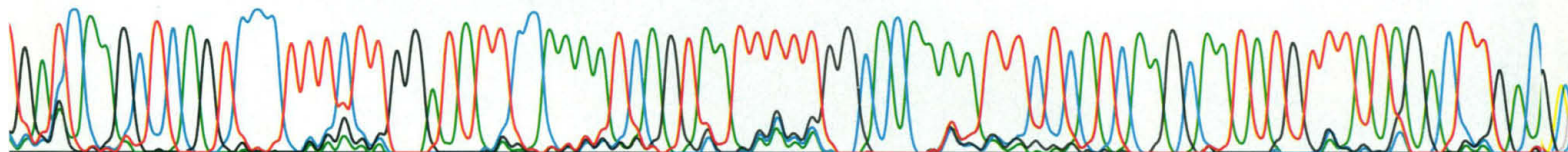
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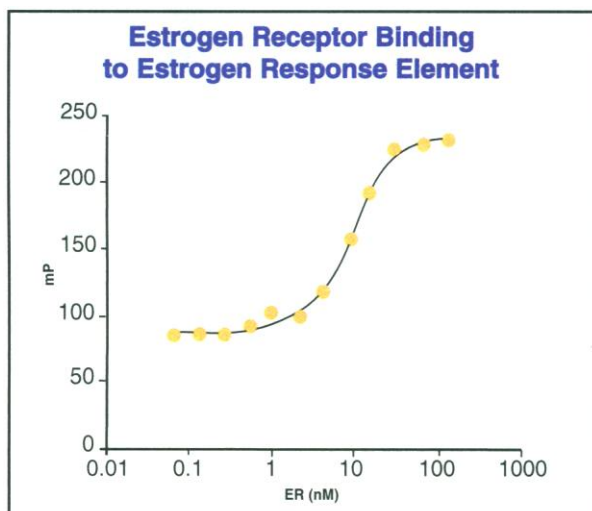


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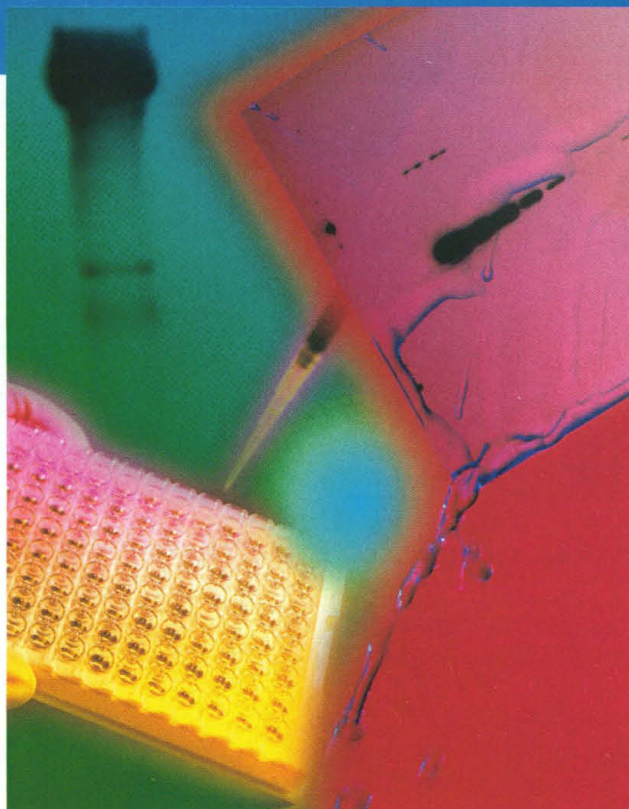
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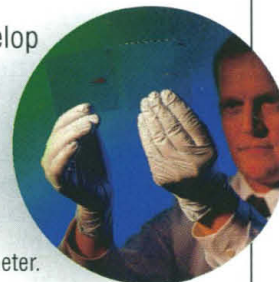


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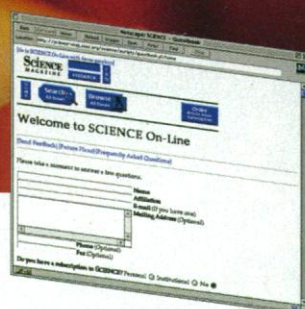
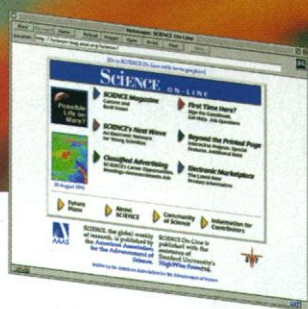
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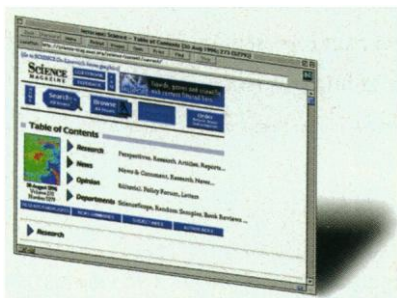
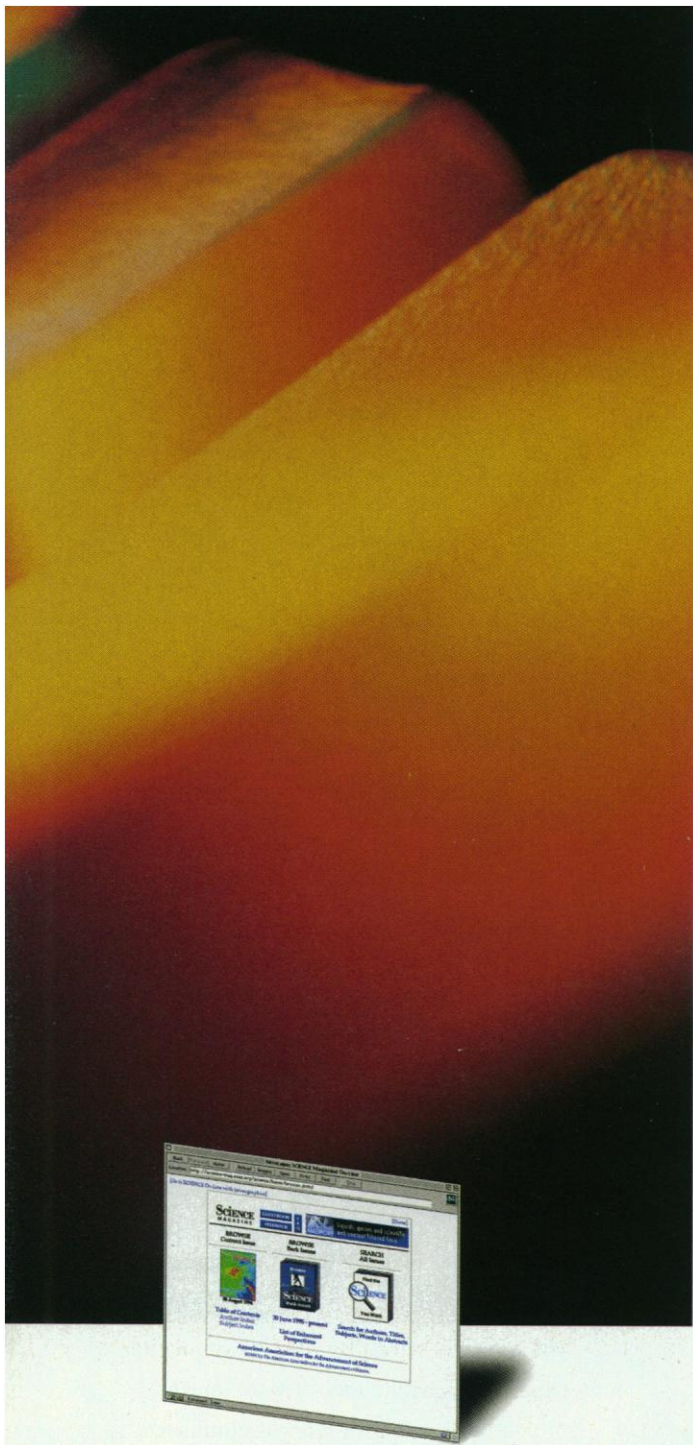
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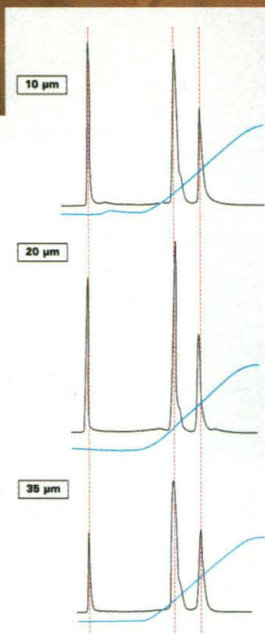
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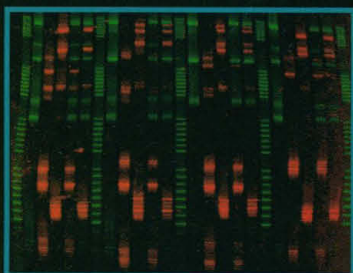
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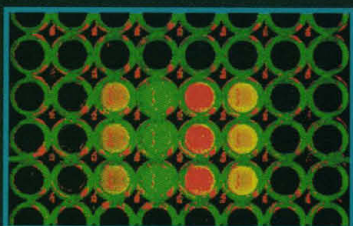
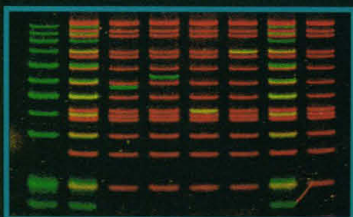
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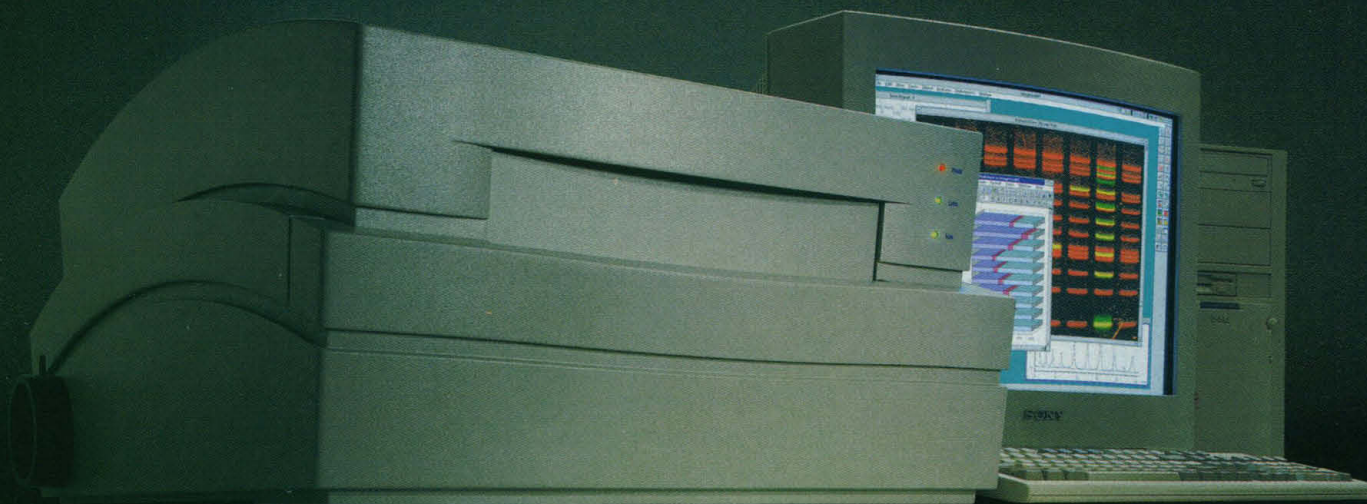
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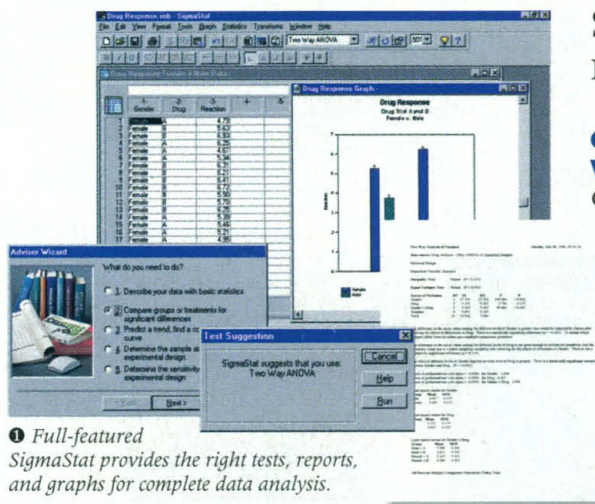
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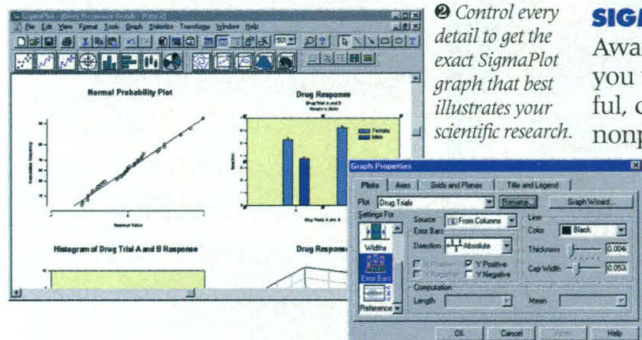
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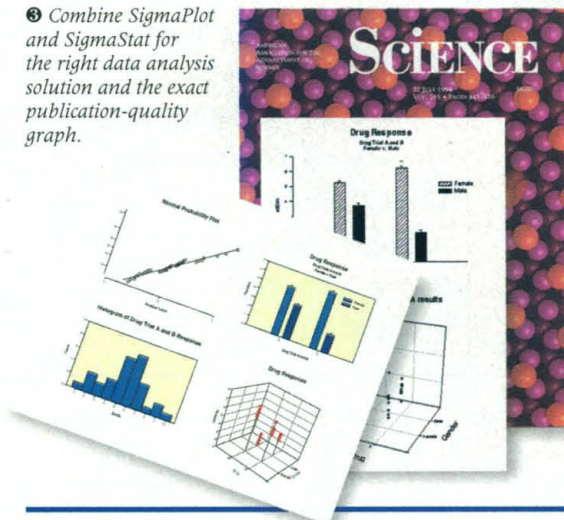
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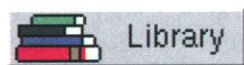
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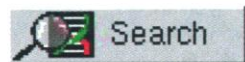
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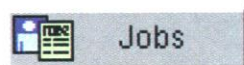
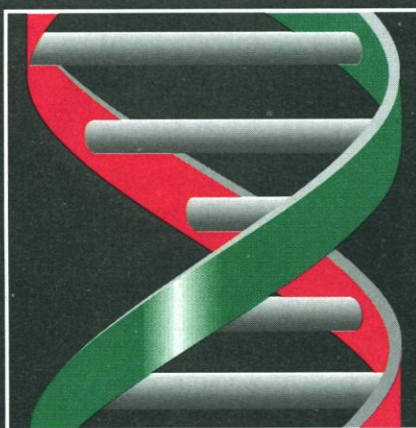
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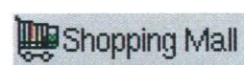
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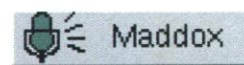


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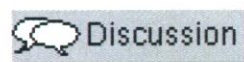


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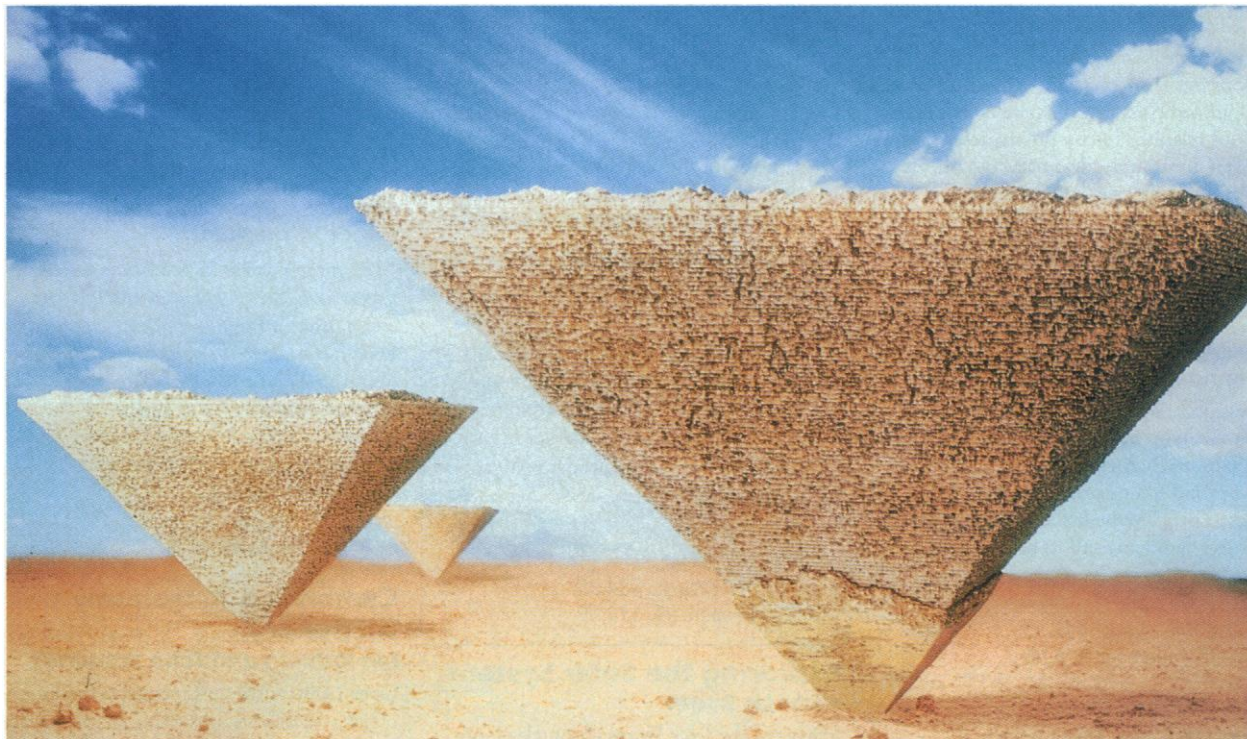
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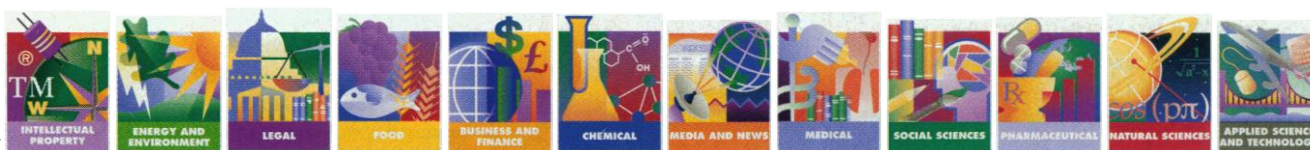
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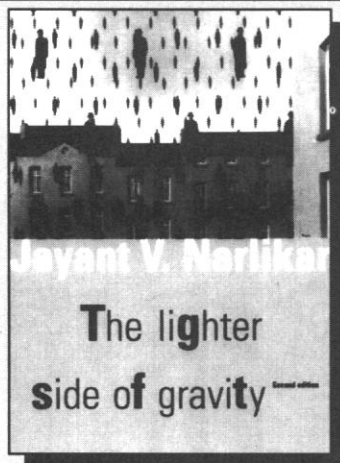
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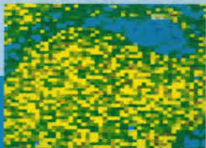
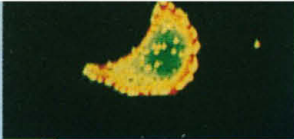
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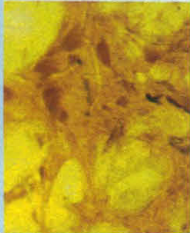
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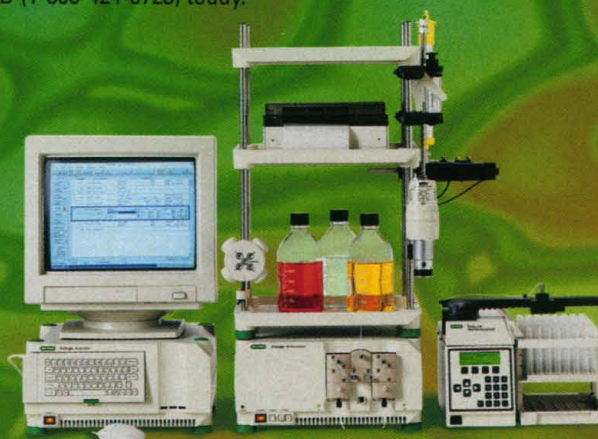
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