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* U.S. Patent Number 5,128,256

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Pierce linked in disappearance of slow, radioactive Oligonucleotide labeling

Further investigation uncovers development of new OligoLinkTM Derivatization and Alkaline Phosphatase Conjugation Kits.

By Pierce Research Staff

vidence is mounting against Pierce in the case of the mysterious disappearance of traditional oligonucleotide labeling. The accused has reportedly replaced the conventional time-consuming radioactive labeling procedure with fast, easy-to-use kits for labeling any oligo or other nucleic acid probe. According to eyewitness accounts, users of the new kits avoid the need for custom conjugation, derivatized nucleotides or enzyme reactions.

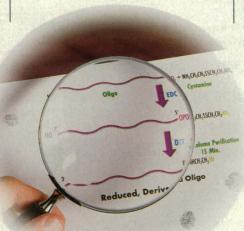
The kits are described as being a sensitive, non-radioactive labeling method, and have drawn considerable attention, as alkaline phosphatase-labeled probes detected with chemiluminescent substrates are the most sensitive and viable safe alternative to radioisotopes.

Sources close to the case have learned that the new kits are *versatile*, allowing users to *label* an oligonucleotide with any conjugate, including cross-linkers, fluorescent labels or biotinylation reagents. There are also reports claiming that probes labeled with this "OligoLinkTM" remain stable for months—or even years.

Detectives on the case are hindered by the unusually large number of crime scenes to investigate—reported as being hundreds of labs worldwide. Officials believe a recovery of the missing labeling procedure is highly unlikely.

Watch for these suspects - and their M.O.

The Pierce $OligoLink^{TM}$ Derivatization Kit utilizes a simple cross-linking step to



end modify any oligonucleotide with a 5'phosphate group. The resulting derivatized oligo can then be coupled to the label or protein of your choice, or attached to a solid support. If you want to label with alkaline phosphatase, the Pierce OligoLink™ Alkaline Phosphatase Conjugation Kit allows users to form stable oligonucleotide-alkaline phosphatase conjugates which are useful in detecting the presence of specific sequences in colony lifts, Southern Blots and DNA Dot Blots. This kit has been

optimized for use with the OligoLink™ Derivatization Kit, but it will conjugate any oligo containing a free sulfhydryl group. Because any synthetic oligo can be labeled with this alkaline phosphatase conjugation kit, the preparation of custom conjugates is not needed.

Also Wanted: OligoLinkTM Hybridization Kit-believed to be headed for the area soon.

Product # Description U.S. Price

17700 OligoLink™ Derivatization Kit \$175

Includes sufficient materials for two phosphorylated oligonucleotide derivatizations.

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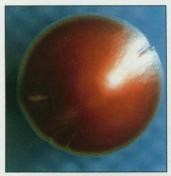
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Science

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Shaded topography of the 12-kilometer-long Asal rift (Djibouti, East Africa). Colors indicate elevations, from -150 meters below (dark blue) to 350 meters above (purple) sea level. The topography results from the tectonic dismemberment over the past 100,000 years of a large central volcano (Fieale) that formed astride the rift zone 300,000 to 100,000 years ago. Reconstruction of this volcano indicates a spreading rate across the rift of 17 to 29 millimeters per year. See page 1677. [Image: J.-B. De Chabalier and J.-P. Avouac]



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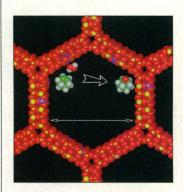
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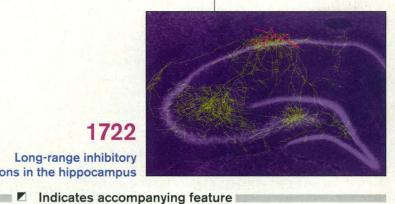
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Long-range inhibitory neurons in the hippocampus



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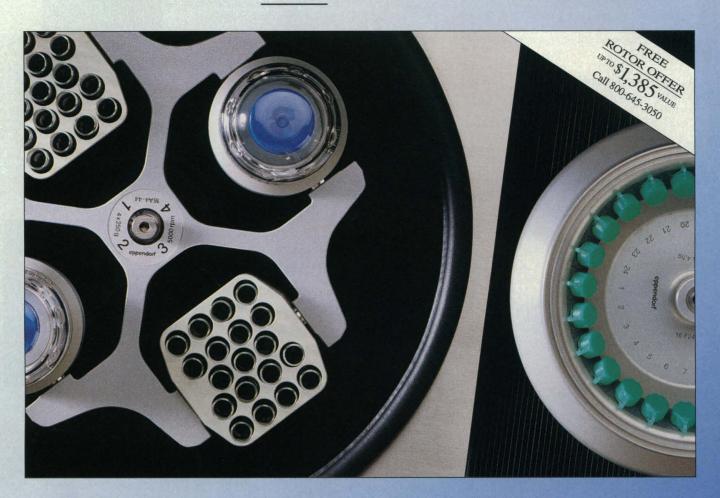
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THIS WEEK IN SCIENCE

edited by PHIL SZUROMI

Bulking up

How many metal atoms are needed in a cluster for it to start exhibiting the properties of the bulk metal? Billas et al. (p. 1682) measured the magnetic moment of different sizes of clusters of iron, cobalt, and nickel. Ferromagnetism is observed for even the smallest clusters, but for 30 atoms or fewer the cluster's magnetic moment is atomlike. Bulk magnetism becomes the norm for clusters of several hundreds of atoms.

Not the culprit

Astronomers have documented a number of absorption and emission features that appear in infrared spectra of a wide variety of astronomical sources, but the molecules responsible for these features have not yet been identified. Polycyclic aromatic hydrocarbons (PAHs) have been frequently mentioned as candidates for the task, but Schlemmer et al. (p. 1686) provide laboratory evidence against this hypothesis. A sensitive photon-counting technique was used to obtain emission spectra of gas-phase naphthalene and pyrene, and strong features were found at wavelengths where no astronomical emission or absorption is known. Because the spectra of PAHs of all kinds, in both neutral and ionized forms, are closely related, these specific results cast doubt on the PAH hypothesis in general.

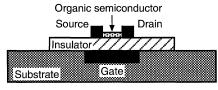
Two for one

Binding between biomolecules is often studied through a Stern-Volmer experiment: The excited-state decay of probe molecule is monitored for different concentrations of a quencher. For weakly bound complexes, it

Putting it on plastic

Although one thrust of semiconductor research is miniaturization, another is to make the various device elements with organic-based

molecules and materials. Garnier et al. (p. 1684) have constructed such a polymer-based transistor whose active element consists of thiophene



oligomers 40 nanometers in thickness. Different polymer layers are built up with a printing technique, thus avoiding costly processing steps under high vacuum conditions.

can be difficult to observe a limiting value. Zhou and Hoffman (p. 1693) show that weakly bound complexes can be analyzed by performing the titration in reverse, holding the quencher constant, and varying the probe concentration. They then show that, over a wide range of ionic strengths (including physiological conditions), the 2:1 complex of cytochrome c and cytochrome c peroxidase, rather than a 1:1 complex, is the one that has a high rate for electron transfer.

Well connected

The hippocampus is a part of the brain that appears to participate in some forms of learning and memory. It receives input from other parts of the cortex through the perforant pathway that targets the dentate granule cells of the hippocampus. These cells send their output to the CA3 region via mossy fibers, and the cells of CA3 send their output to the CA1 region via the Schaeffer collaterals. Sik et al. (p. 1722) have reconstructed two inhibitory CA1 neurons that, unlike known locally ramifying inhibitory neurons, project back to what are thought to be earlier stages of processing in the hilar region and granule cell layer. These connections could help to organize individual neurons into populations that are responsible for temporally coherent activity of the hippocampus.

Unleashing hormones

A variety of plant hormones are found in conjugated, seemingly inactive, forms that nevertheless may contribute to regulation of the amount and location of the active, unconjugated form of the hormone. Szerszen et al. (p. 1699) have cloned the maize gene iaglu, which controls the first step in the conjugation pathway for the growth-promoting hormone, indole-3-acetic acid. Hybridization analysis suggests that homologous genes occur in other plants as well. Manipulation of such hormoneconjugation genes may provide a means to control plant growth without the application of exogenous hormones.

Variable control

Alternative splicing of mRNA is an important means of gene regulation. In vitro, SF family proteins and hnRNP A1 have opposing effects on the choice of the 5' splice site. Cáceres *et al.* (p. 1706) overexpressed these

proteins in HeLa cells and found that SF2/ASF and hnRNP A1 mediate 5' splice site choice in vivo. Whereas SF2/ASF activates the proximal 5' splice site and prevents exon skipping, hnRNP A1 promotes distal 5' splice site choice. Thus, variable ratios of such splicing factors may regulate differential splicing in vivo.

Metazoan Myxozoa

The phylum Myxozoa includes a collection of obligate endoparasites that have characteristics of both protozoans and metazoans. Smothers et al. (p. 1719) have concluded from analysis of 18S ribosomal RNA sequence data that although the Myxozoa have been considered part of the kingdom Protista, they are more appropriately grouped with the multicellular metazoa. Myxozoa may share an evolutionary history with bilateral animals, and thus offer an extreme example of the loss of complex characteristics that can occur during evolution.

Pores and pain

Local anesthetics reduce or prevent pain by blocking Na+ channels that are required for the generation of action potentials in nerves. The channels can exist in either a closed resting state, an open state, or an inactivated state that occurs when an open channel has just closed. The local anesthetics interact differently with channels in each of these states. Ragsdale et al. (p. 1724) have used site-directed mutagenesis to reveal the amino acid residues that contribute to drug binding in the various states and their relation to the pore of the channel.

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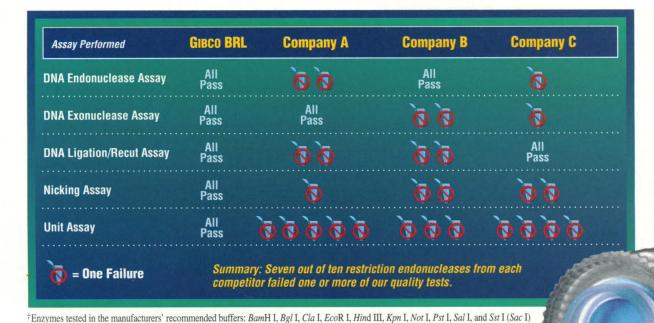
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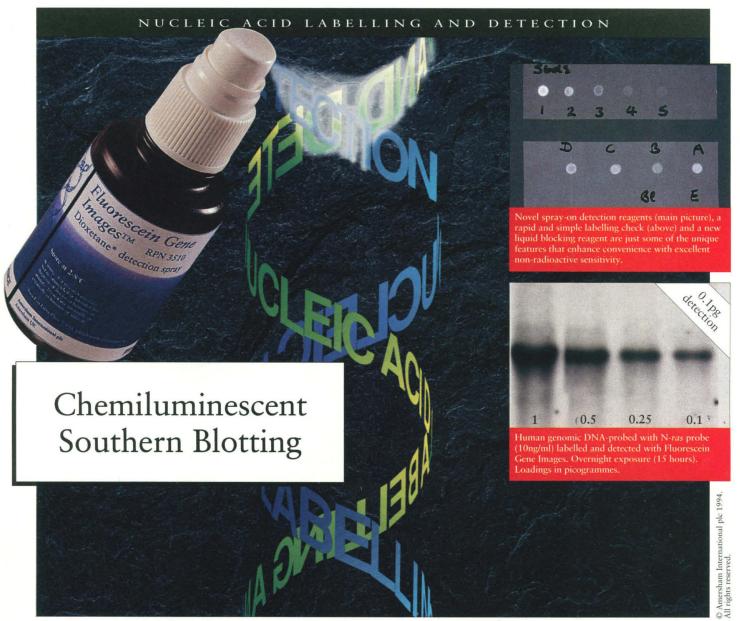
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GRAINCOLLECTION

HUMANS' NATURAL ECOLOGICAL NICHE

SERGIO TREVIÑO
Translated by:

Rebeca San Martín-Feeney

The logically harmonious thesis contained in this work will revolutionize our current way of thinking about human origins and behavior.

Ernst Mayr, Professor of Zoology, Emeritus, at Harvard University, considered by many to be the greatest evolutionist of the century, has written to the author:

"...l am rather inclined to accept your thesis of the role of graincollecting in the history of mankind, persuaded by your arguments and those of others.

Thank you very much for your interesting and closely arqued book!"

How did bipedalism, the loss of body hair, and tool use originate? After more than 130 years of scientific research, the origin of these basic human characteristics is still unknown. Bipedalism, the loss of body hair, and tool use originated 14 million years ago, when our very distant ancestors, *Ramapithecus*, turned graincollectors. Erroneously, we still equate biological evolution with "progress", and therefore, believe humans descend from knuckle-walking, hairy, unskilled tool users who resembled the living great apes, when, in fact these apes descend from bipedal, naked, skilled tool users, who resemble modern man.









The large intestine, humans' largest internal organ, is presently used only to absorb water and electrolytes, although its sacculated nature indicates an evolutionary adaptation to digest cellulose. This intestine's movements are so slow that the first radiologist to observe it said it presented a picture of still life. Much of this inactivity can be attributed to mankind's omnivorous diet. Nonetheless, it follows that, when following an exclusively granivorous, cellulose-digesting diet, our large intestine proves to be much more useful and efficient, since our ancestors up to 50 thousand years ago always used it to digest cellulose fiber. We are presently neglecting a very useful capability that our ancestors adaptively acquired. Humans, as all other primates, were meant to be vegetarian cellulosedigesters and have slim bodies. The size of the human mouth is small for almost any type of omnivorous feeding, making this feature, as well as our powerful teeth, characteristic more of seed-eaters than of carnivorous or omnivorous mammals.

It is inconceivable to think that hominids and their protohominid ancestors lived in the savannas for millions of years and never developed the practice of feeding from gramineous seeds until the discovery of agriculture, or until fire was used to cook food. If we take into consideration that early hominids were already bipeds and tool users, and the seeds from grasses would lightly touch their hands as they walked in the long-grass grasslands, it would be illogical to assume that, in spite of the many vicissitudes they suffered during so many million years of living in the savannas, they never tried to feed from these seeds or that they never thought of removing the seeds with their hands. The author argues that when injuring themselves by removing the seeds, they used a natural small stone tool to protect their hands, which achieved an unanticipated advantage: they improved their efficiency in removing and threshing seeds.

We invite you to read this book and explore in it the scientific bases of these arguments, upholding the importance of the role of graincollecting in human evolution and behavior.

293 pp., 6x9 hardcover ISBN: 0-533-09415-1

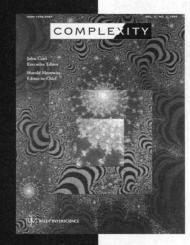
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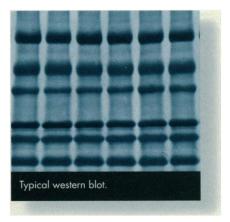
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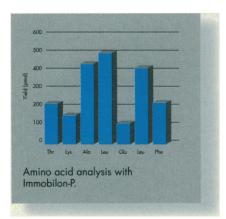
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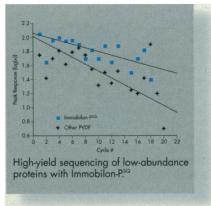
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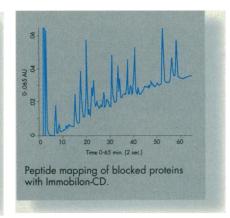




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HUMPBACK WHALE STILL HUNTED, mtDNA SHOWS

EARTHTRUST TEAM **GOES UNDERCOVER**

PCR* in Hotel Rooms

HONOLULU - A team working with the environmental group Earthtrust has adapted the tools of molecular biology to document the illegal sale of whale meat. The poaching of endangered species of whales had long been suspected, but due to a technicality of international law, actual data

were always difficult to collect. Although whale meat is com-

monly sold in several nations, scientists are restricted from taking tissue samples across national borders—even if only to conduct DNA analysis.

Enter Earthtrust, the Hawaii-based group that first exposed the slaughter of dolphins by deep-sea driftnetters. In 1993, their scientific team of Scott Baker of the University of Auckland & Steve Palumbi of the University of Hawaii developed a protocol for extracting and copying DNA that works well in the field. Baker then flew to Japan to test samples of whale meat obtained in advance by a collection team consisting of an off-duty wildlife officer and a linguist. This team had scouted fish markets and department stores to obtain samples and videotape of whale meat for sale. They relayed the samples to Baker, who set up his lab gear in a hotel room.

Under the rules of the International Whaling Commission, Japanese whalers are permitted to harvest a small number of Southern Hemisphere minke whales-a less threatened species-for population analysis, then sell the meat to finance their efforts. It had long been suspected that through this legal loophole, much illicit trade in endangered species was occurring. But data were lacking, and the CITES treaty-an international agreement designed to protect threatened animals worldwide-prohibits the transportation of products from endangered species across national borders. So Baker extracted DNA from the processed whale meat samples, then amplified mtDNA using

a portable MiniCycler thermal cycler donated by MJ RESEARCH. In order to adhere to the CITES regulations, Baker separated the synthetic, copied DNA from true whale DNA by using biotinylated primers and streptavidin-coated magnetic beads. He then returned to Hawaii with only copied DNA, which he and Palumbi sequenced and analyzed. Baker & Palumbi's data show that among 16

samples identified, 7 were from Southern minke whale, one from North Atlantic minke, one from humpback, 4 from fin whale, 2 from dolphin, and one was likely a beaked whale. These data were presented to the IWC in May and published in a recent issue of Science. Many groups hope that this new method of portable DNA analysis will help authorities enforce existing law, as well as obviate any future need for lethal "research whaling".

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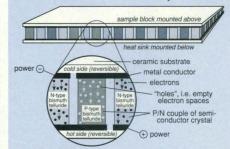
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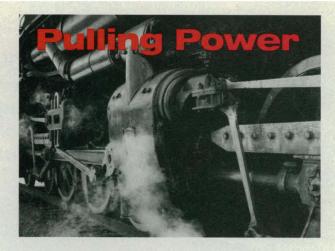
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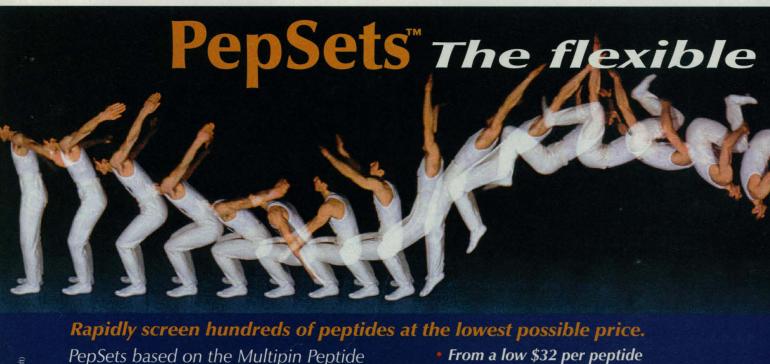
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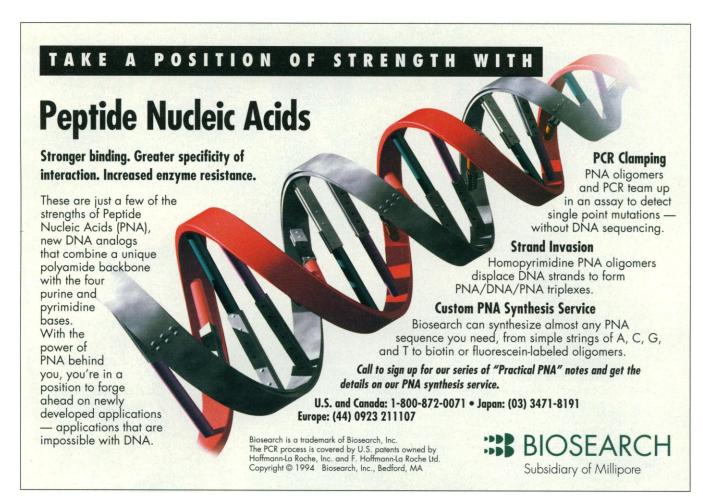
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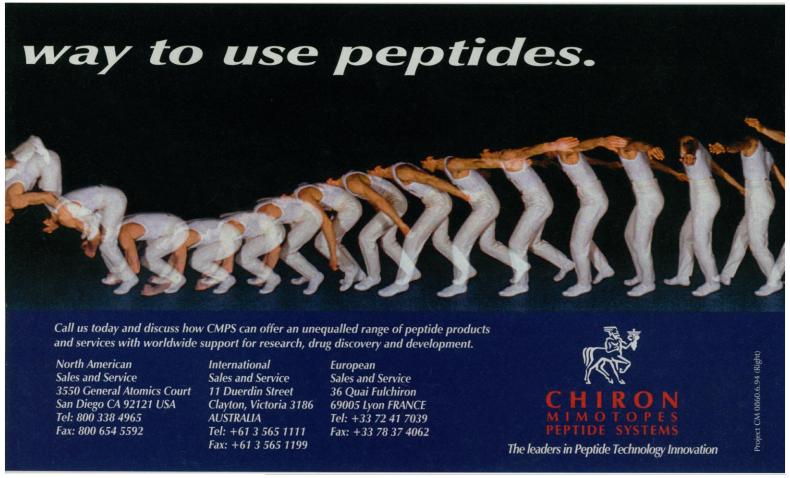


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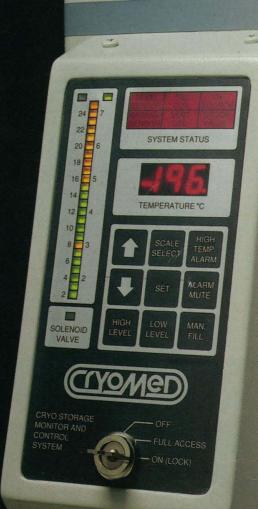
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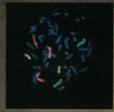
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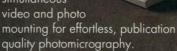
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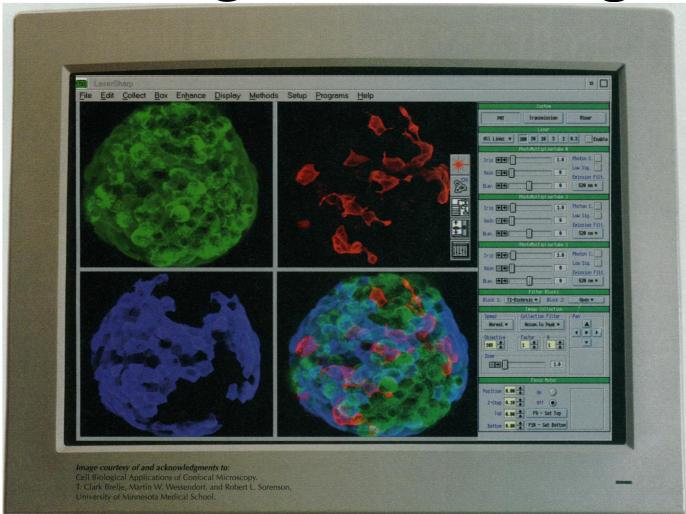
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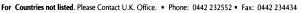


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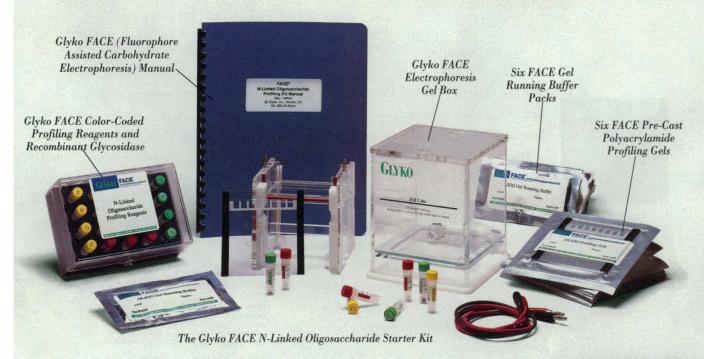
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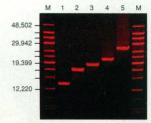
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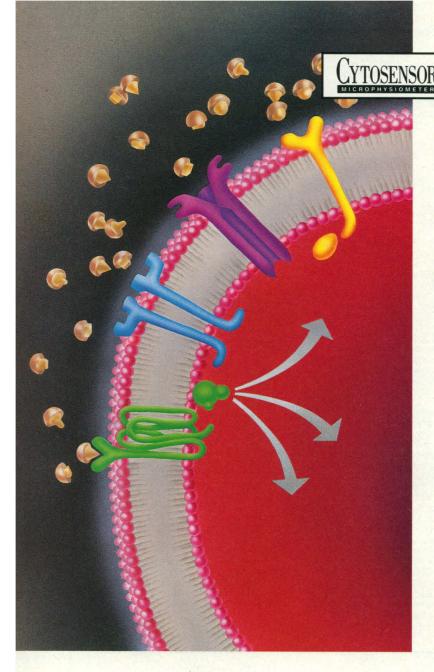
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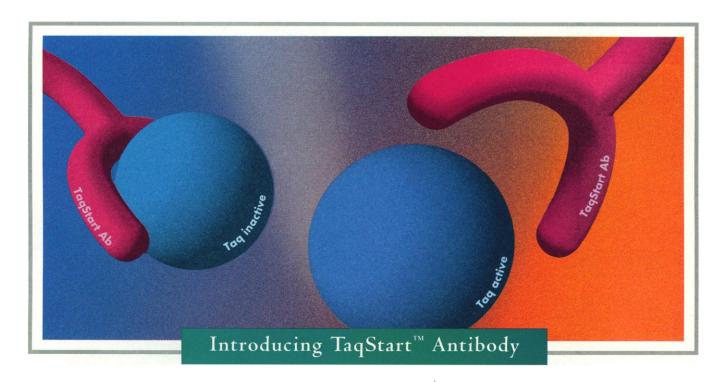
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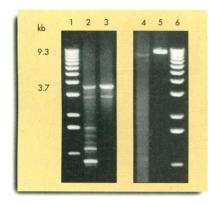
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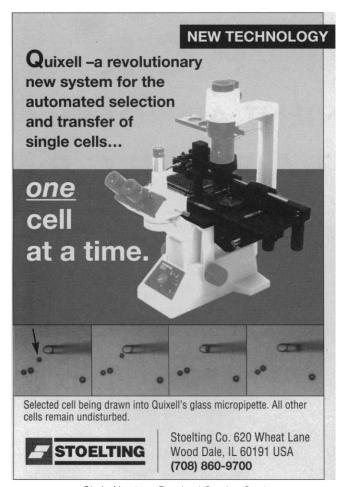
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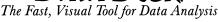
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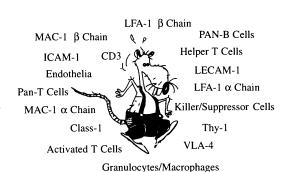




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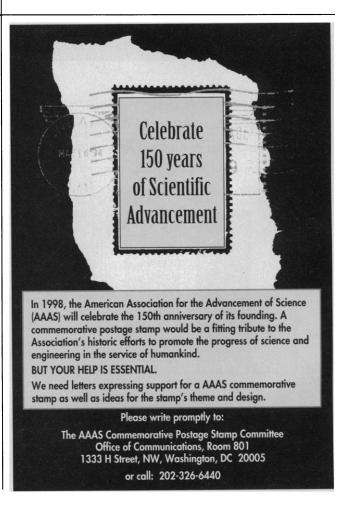
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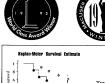
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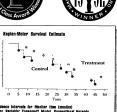
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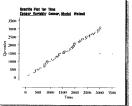
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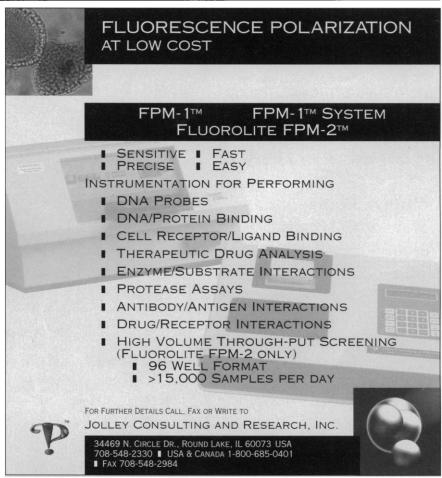






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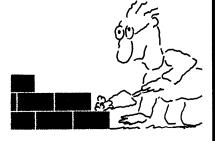
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