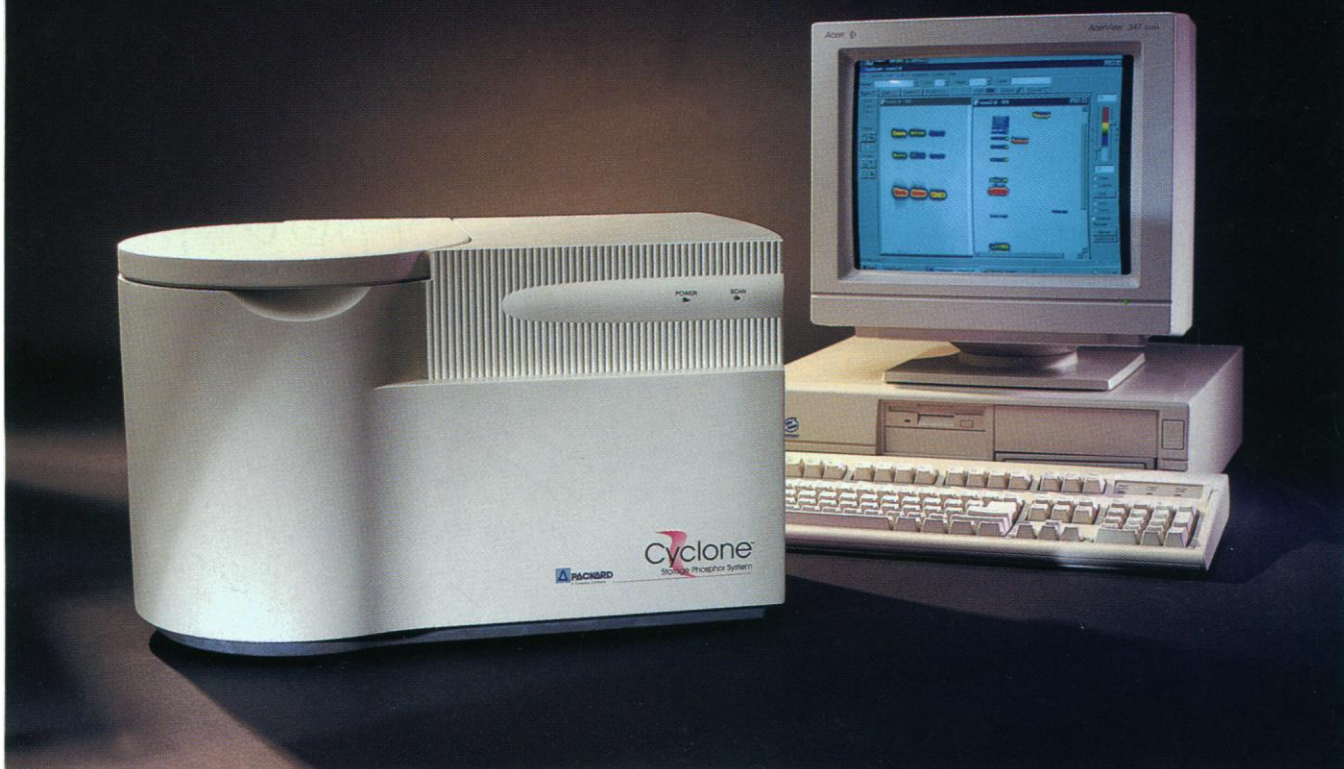


VOL. 274 • PAGES 465-688

GENOME ISSUE



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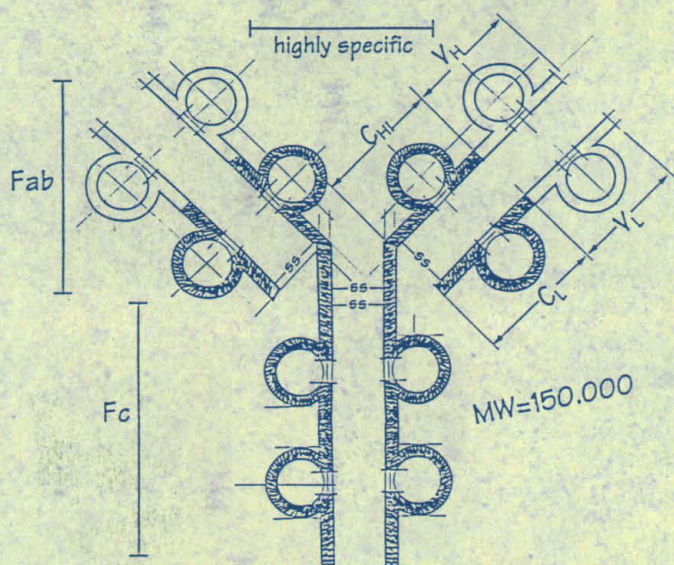
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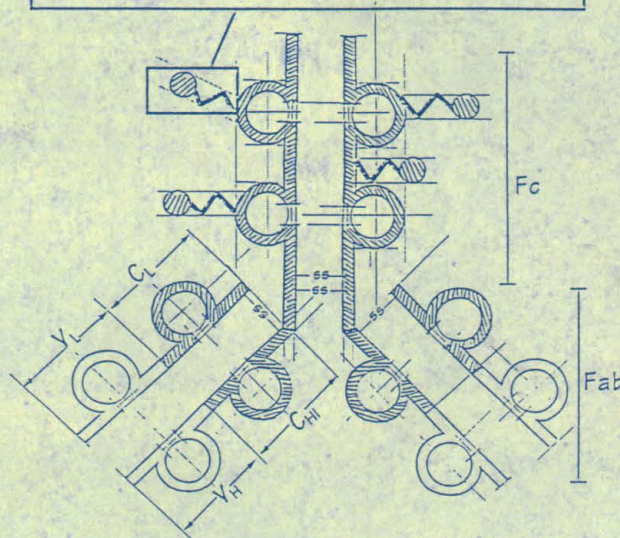
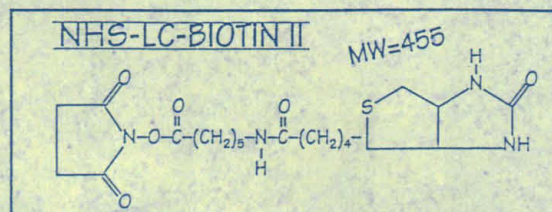
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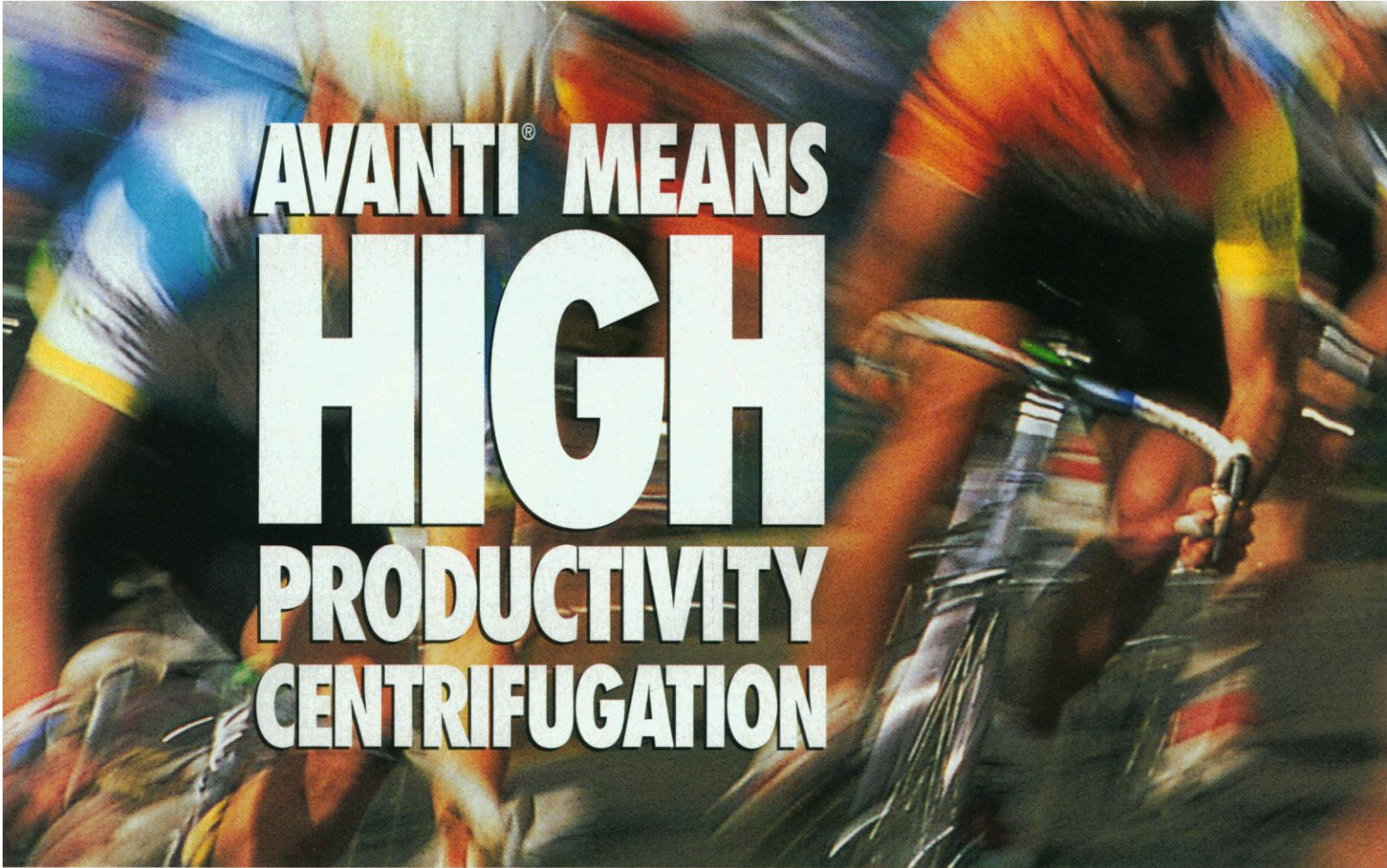
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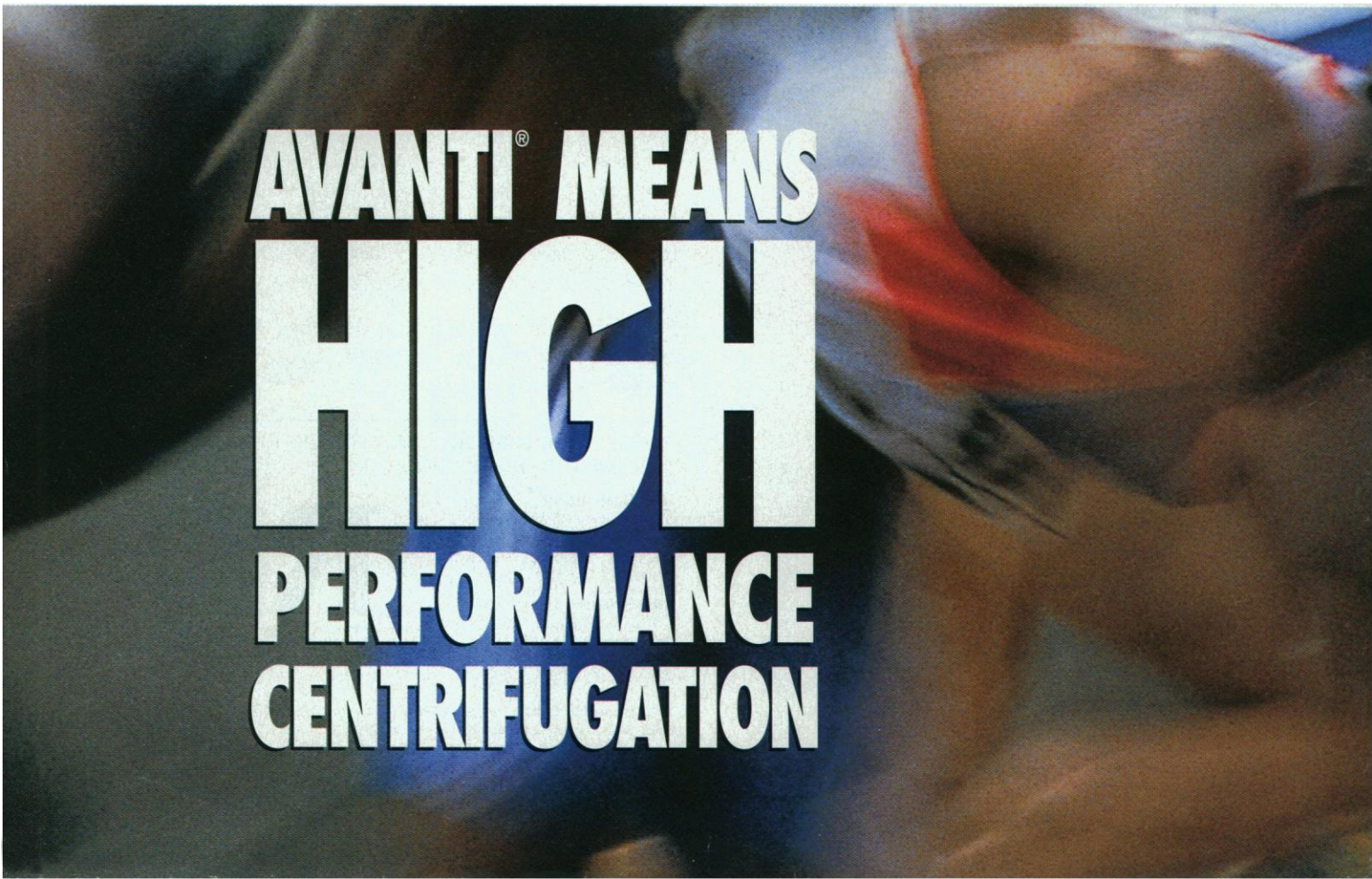
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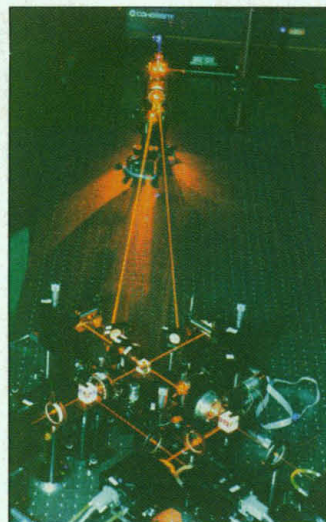
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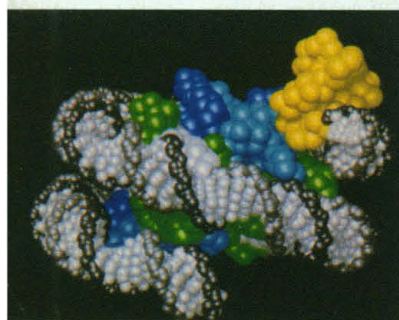
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COVER

Map makers during the Age of Exploration opened up new worlds, literally and in terms of new ideas and possibilities. Similarly, mapping of the human genome and the genomes of model organisms is opening up new dimensions in our understanding and potentiating

a global view of life. A special section beginning on page 533, related News, Editorial, and Reports, a transcript map (page 547) of the human genome, and a Web feature highlight views of this rapidly developing area. [Illustration: Tracy Keaton-Drew, Washington, DC]



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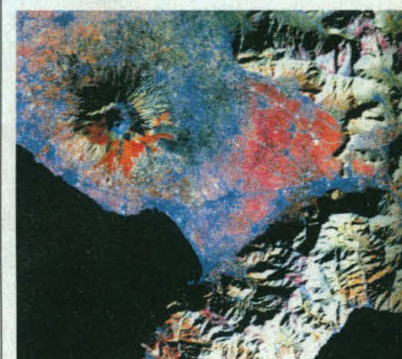
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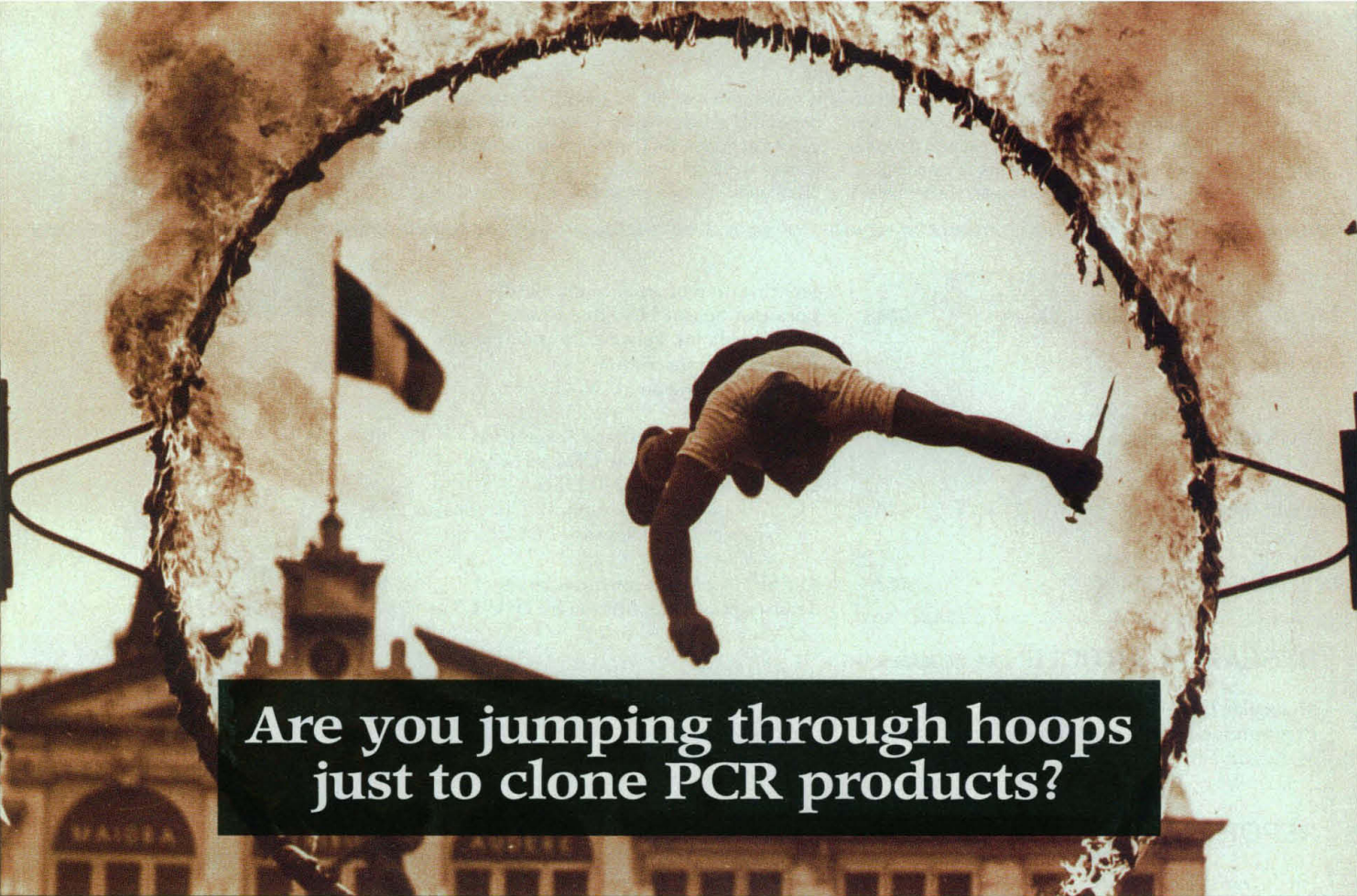
On the Web

Have you ever wanted to play with a genome?
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<http://www.sciencemag.org/science/feature/data/genomebase.shl>



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Magma beneath Vesuvius



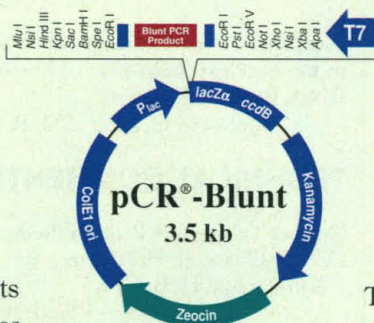
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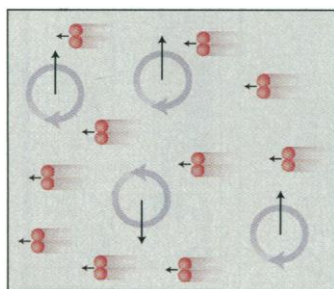
edited by PHIL SZUROMI

Series switches

One possible route for the development of electronic devices functioning on a molecular length scale is the utilization of photoinduced electron transfer in donor-acceptor (D-A) systems. Debreczeny *et al.* (p. 584) devised a molecule consisting of covalently bound units in a D-A-A-D sequence and show that photogeneration of ion pairs in the first unit leads to the generation of local electric fields that can be used to control a second photoinduced electron transfer reaction within the same molecule.

Upon reflection

In theoretical physics, it is sometimes the case that the solution to one problem can be used to solve another by the proper transformation of the system, such as switching the role of electrical fields and charges with their magnetic analogs in electromagnetism (see the Perspective by Girvin, p. 524). Shahar *et al.* (p. 589)



measured the current-voltage characteristics of a fractional quantum Hall effect fluid and its nearby insulating state and found that the results are essentially identical for the two states when current and voltage are interchanged. The existence of this duality symmetry for charge and magnetic flux may lead to new theoretical insights into the quantum Hall effect.

An older first appearance of animals

Animals suddenly first appear in the fossil record at the dawn of the Cambrian, about 565 million years ago. The fossil record has generally been interpreted as evidence of a rapid evolution over a few tens of millions of years. Wray *et al.* (p. 568; see the Perspective by Vermeij, p. 525) examined this notion by determining rates of molecular sequence divergence among metazoan phyla. The data suggest that animals may have instead arisen about 1 billion years ago and that the radiation was prolonged.

Volcanic hazard

Mount Vesuvius, responsible for the famous volcanic eruption in 79 A.D. that destroyed Pompeii, has had many less dramatic and more recent eruptions that have occurred as recently as the middle of this century. In an effort to assess its potential hazard to the nearby Naples metropolitan area, Zollo *et al.* (p. 592) report results of an active seismic study of Mount Vesuvius aimed at understanding the internal geometry of its magma system. The results suggest that a melt zone may be present at depths of about 10 kilometers beneath the volcano.

Fusin and CD4 in HIV-1 entry

Entry of the human immunodeficiency virus-type 1 (HIV-1) into human cells requires the presence of chemokine coreceptors such as fusin. Lapham *et al.* (p. 602; see the news story by Cohen, p. 502) show that when human cells are treated with the HIV-1 envelope glycoprotein gp120, the complex formed between gp120 and CD4 associated with fusin. No similar complex could be isolated from nonhuman cells. The design of molecules that can block this association without interrupting the normal functions of CD4 and chemo-

kine receptors may provide another strategy against HIV-1 infection.

DNA data arrays

An important goal in genome analysis is the rapid determination of variations and mutations in specific sequences for individuals. Chee *et al.* (p. 610) have developed a high-density DNA array chip and used it to analyze the human mitochondrial genome (16.6 kilobases). A sequence of length L was probed by hybridization to an array containing $4L$ probes of 15-nucleotide oligomers. Sequences can be read out in minutes by comparing the sample (tagged with one dye color) to the reference sequence (tagged with another color). Rapid analysis of sequence polymorphisms should be possible with this approach.

Inside and off-center

In chromosomes, DNA is wrapped around complexes of proteins called histones to form structures called nucleosomes that not only condense the DNA but also play a role in gene regulation. Pruss *et al.* (p. 614; see the news story by Pennisi, p. 503) have analyzed the structure of the nucleosome by attaching photoactivated

probes along major groove sites of a DNA sequence. The location of contacts to the globular domain of the histone that links nucleosomes (GH5) suggests that the linker histone is well within the nucleosome core and is located in an asymmetric, off-center position inside the strands of DNA that wrap the nucleosome.

Reducing transmitter release

Metabotropic glutamate receptors regulate neurotransmitter release in the brain, but the mechanisms by which the receptors inhibit transmitter release have been difficult to ascertain. Takahashi *et al.* (p. 594) measured electrical activity in individual synapses and demonstrate that activation of the glutamate receptors reduces calcium conductance in the presynaptic cell and thus reduces calcium-triggered neurotransmitter release.

Nuclear recycling

The paradigm by which proteins are imported to the nucleus from the cytosol is well established—a cytosolic receptor binds to a nuclear localization signal on the nuclear protein after its synthesis in the cytosol and directs the receptor-nuclear protein complex to the nuclear pore for import. Aitchison *et al.* (p. 624) describe a new nuclear import receptor with a role in returning an unusual class of nuclear proteins to the nucleus—the proteins that help to export messenger RNA from the nucleus and which must be recycled to the nucleus for further rounds of transport.

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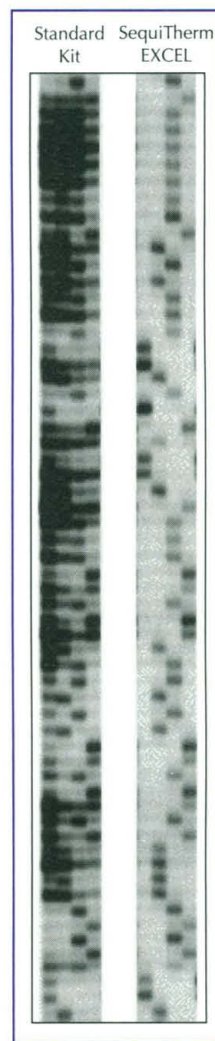
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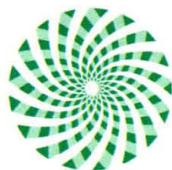
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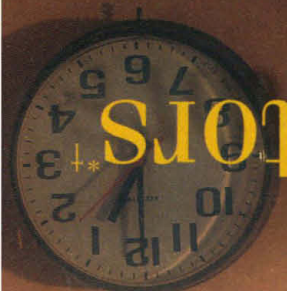
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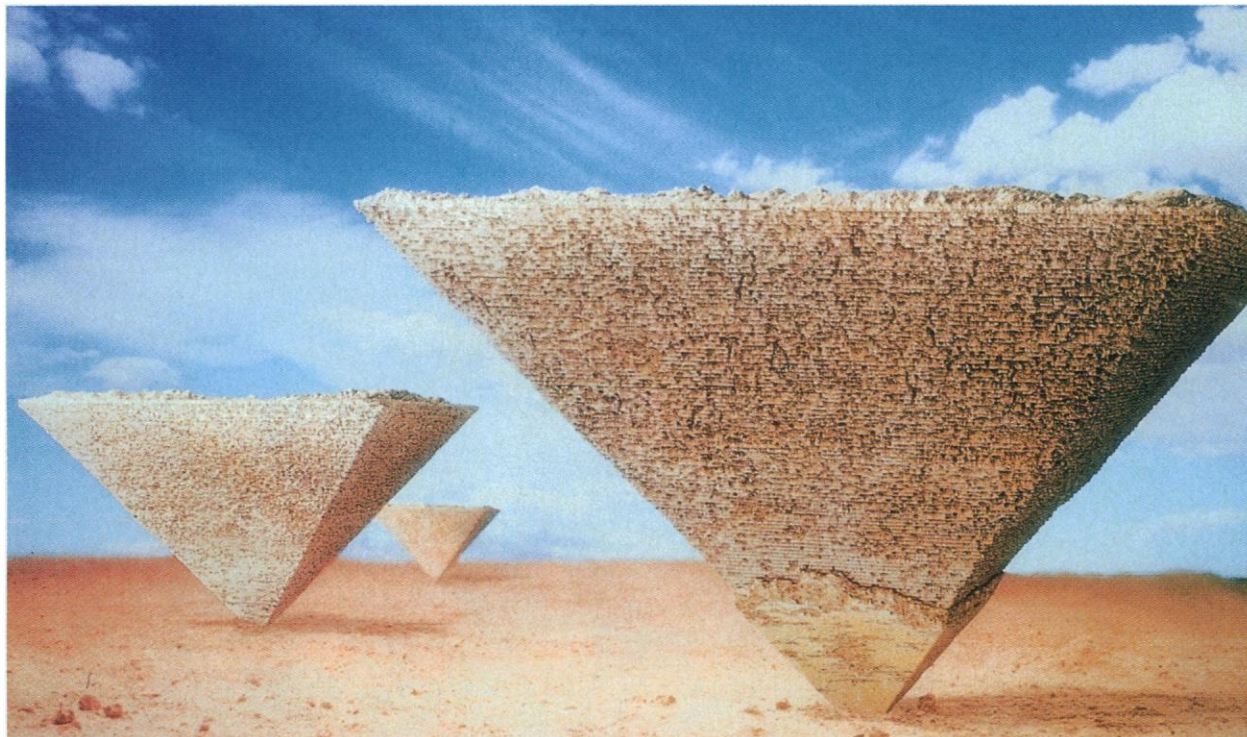
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Radiation Hybrid Mapping



Radiation hybrid mapping based on somatic cells was proposed as a general method for constructing whole genome maps in 1990¹. This method is based on panels of radiation fragmented genomic DNA, with each panel containing a random fraction of the complete genome. Three such panels are offered by Research Genetics and consist of individual hamster cell lines with each cell line harboring a random fraction of the human genome. Each of the three panels contains human DNA which has been fragmented by different levels of radiation and therefore contain different average fragment lengths. Panels with longer average fragment lengths require fewer markers to build a map but the resolution of the resulting map will not be as great as one constructed from a

panel containing fragments with a smaller average length. Mapping is accomplished by determining the presence or absence of an unmapped sequence in each member of a panel and comparing this information with that of previously mapped sequences. Once a large number of sequences have been scored, the pattern for an unmapped sequence will be very similar or identical to previously mapped sequences thus allowing its placement on the map. Today these panels allow anyone to map their human DNA sequence in relation to over 20,000 previously mapped loci including over 12,000 human transcripts.

Maps have been developed for two of the three human panels offered by Research Genetics. The map for the GENEBRIDGE4 panel contains over 20,000 loci and offers the greatest chance of success in mapping a sequence. Developed at Oxford in the Goodfellow lab, this panel has an average fragment length of about 10 million base pairs.

The Stanford map for the G3 panels contains over 6,000 framework loci and has a 75% chance of successfully mapping an unknown sequence and a 90% chance of success if the chromosome is known. Developed at Stanford in the Cox and Myers labs this panel has an average fragment length of about 5 million base pairs.

The TNG panel, also developed at Stanford, has the shortest fragments with an average length of 800 thousand base pairs. No comprehensive map is yet available for this panel but it is very useful for high resolution mapping of loci in a specific region of interest.

1. Cox, D.R., M. Burmeister, E.R. Price, S. Kim, R.M. Myers (1990) *Science* 250 (4978) 245-250.

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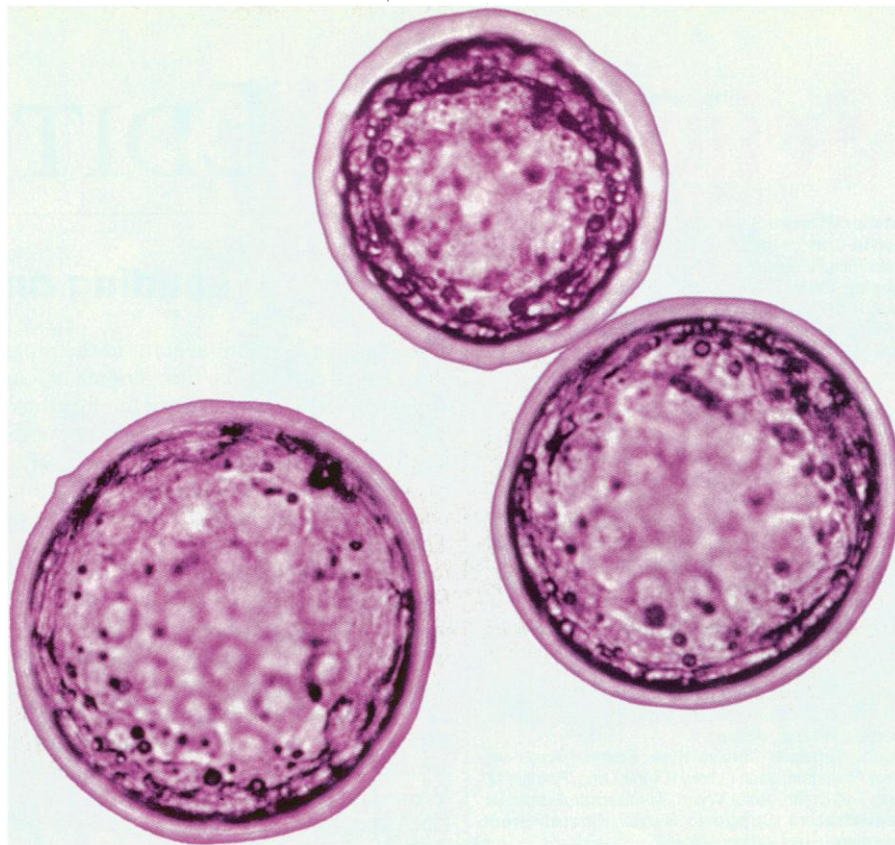
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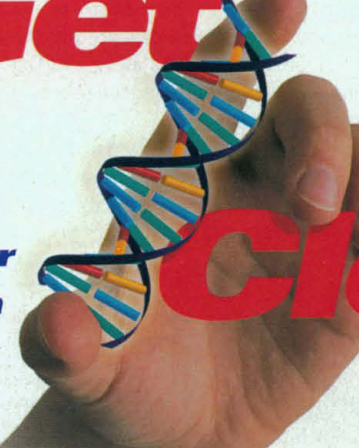
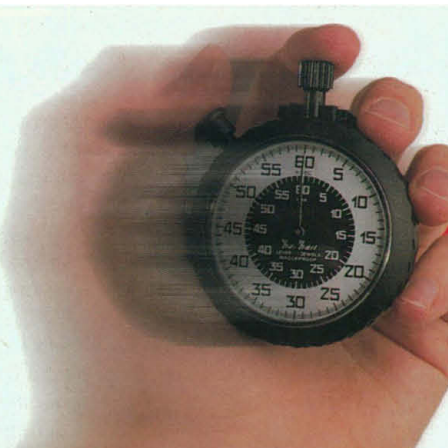
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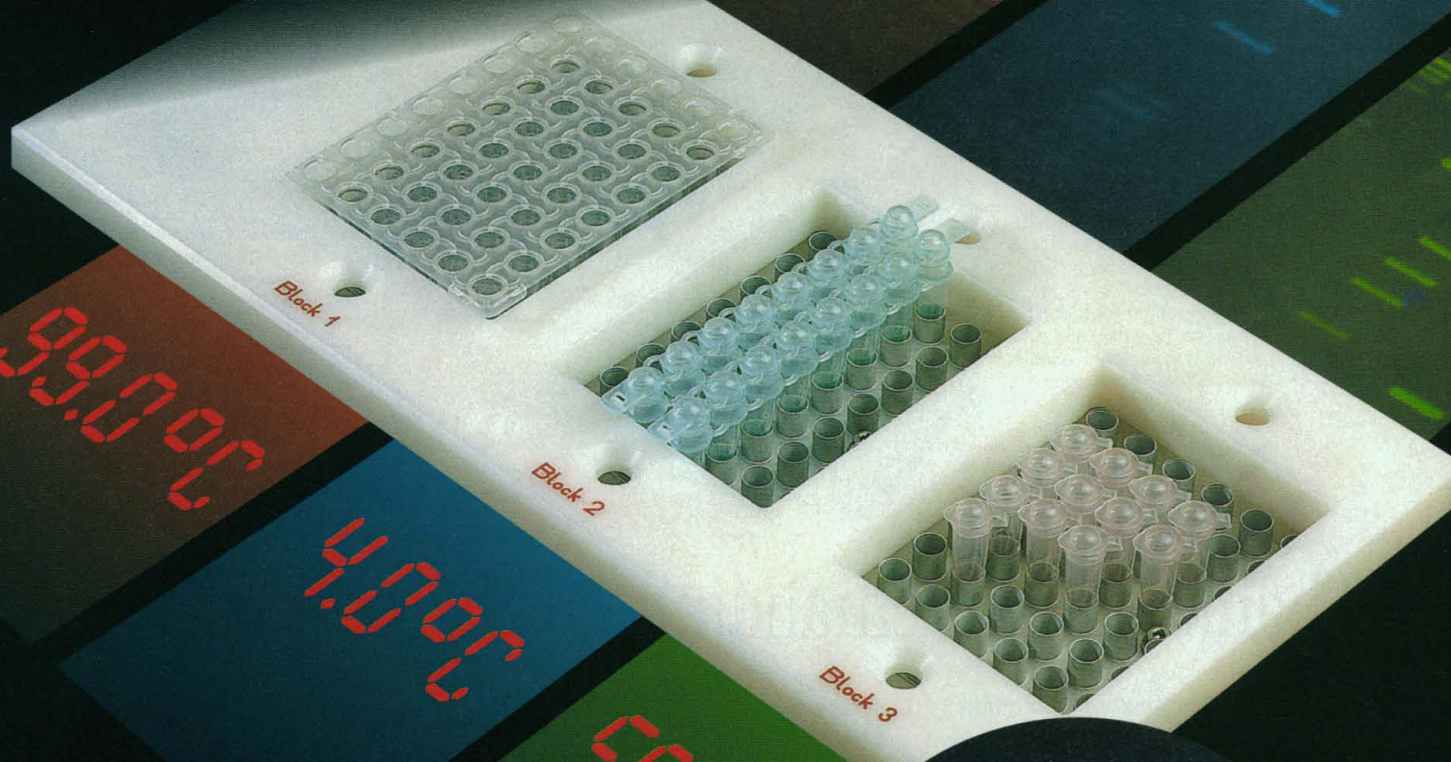
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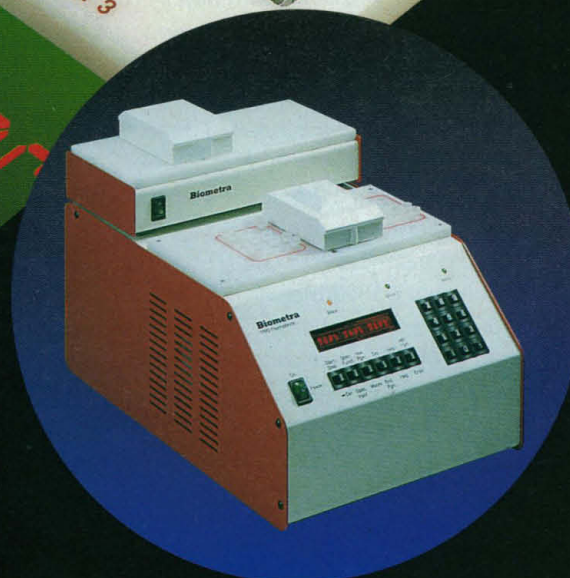
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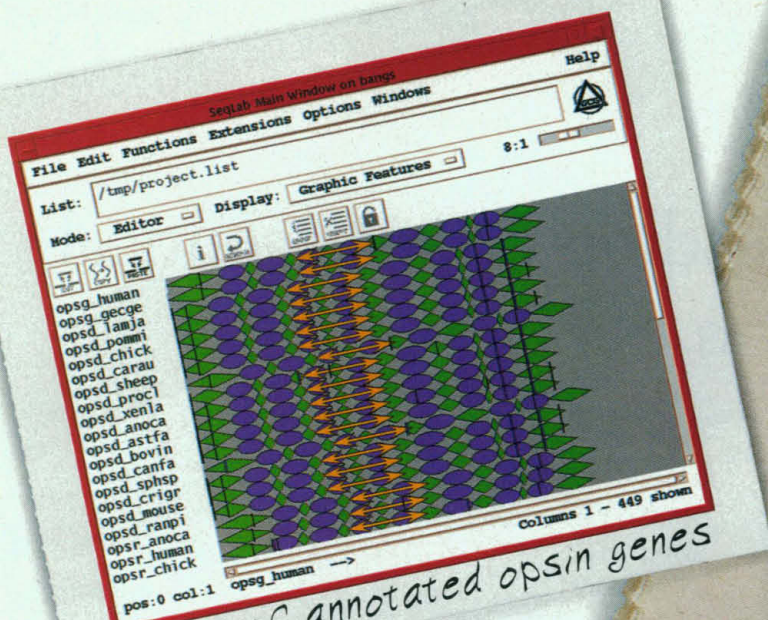
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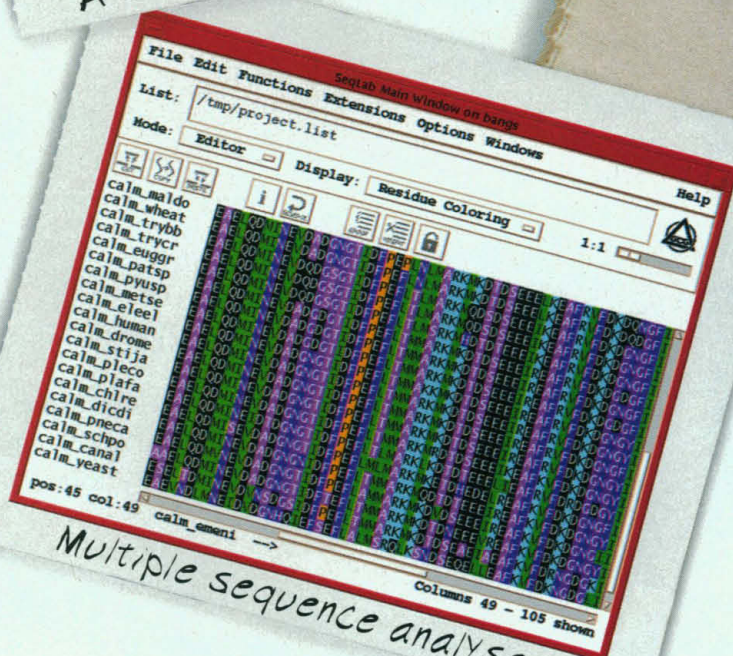
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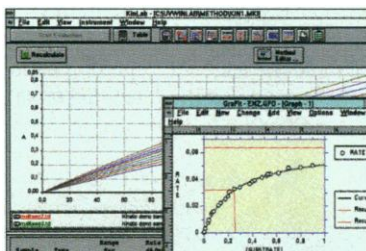
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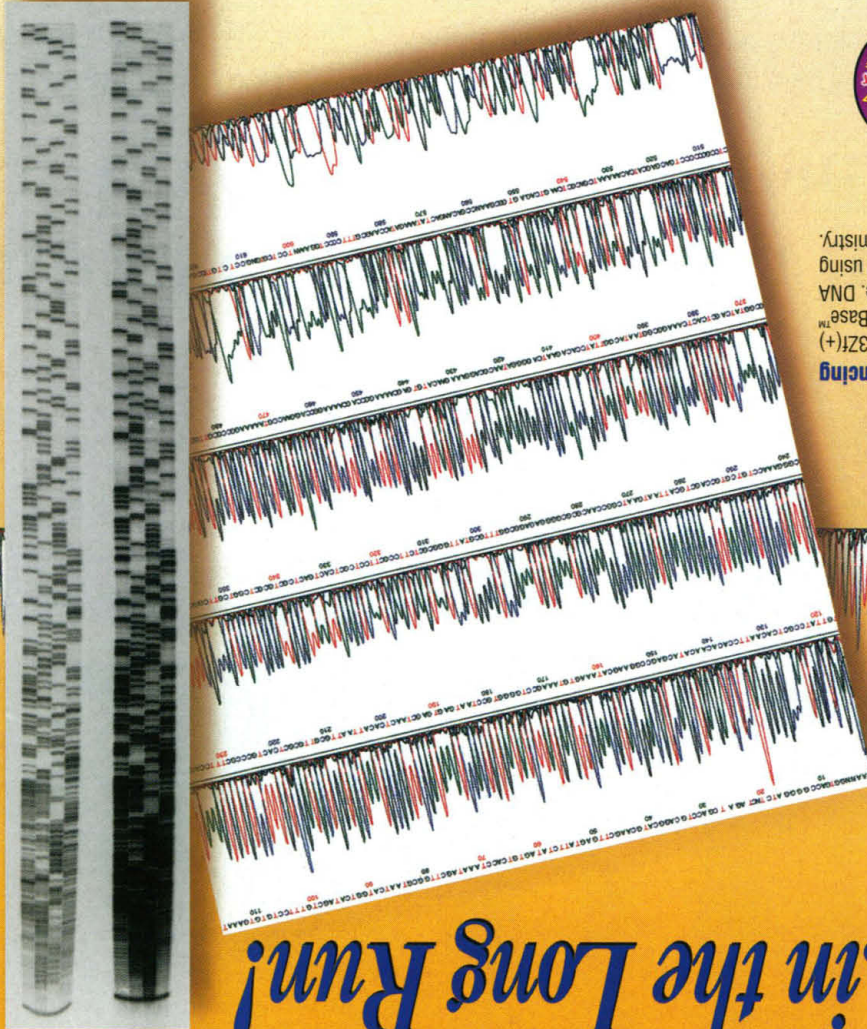
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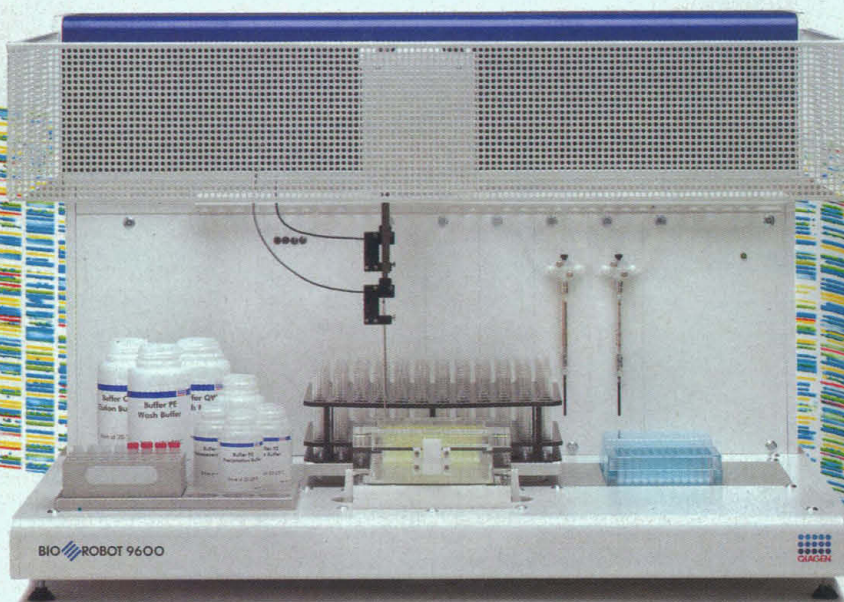
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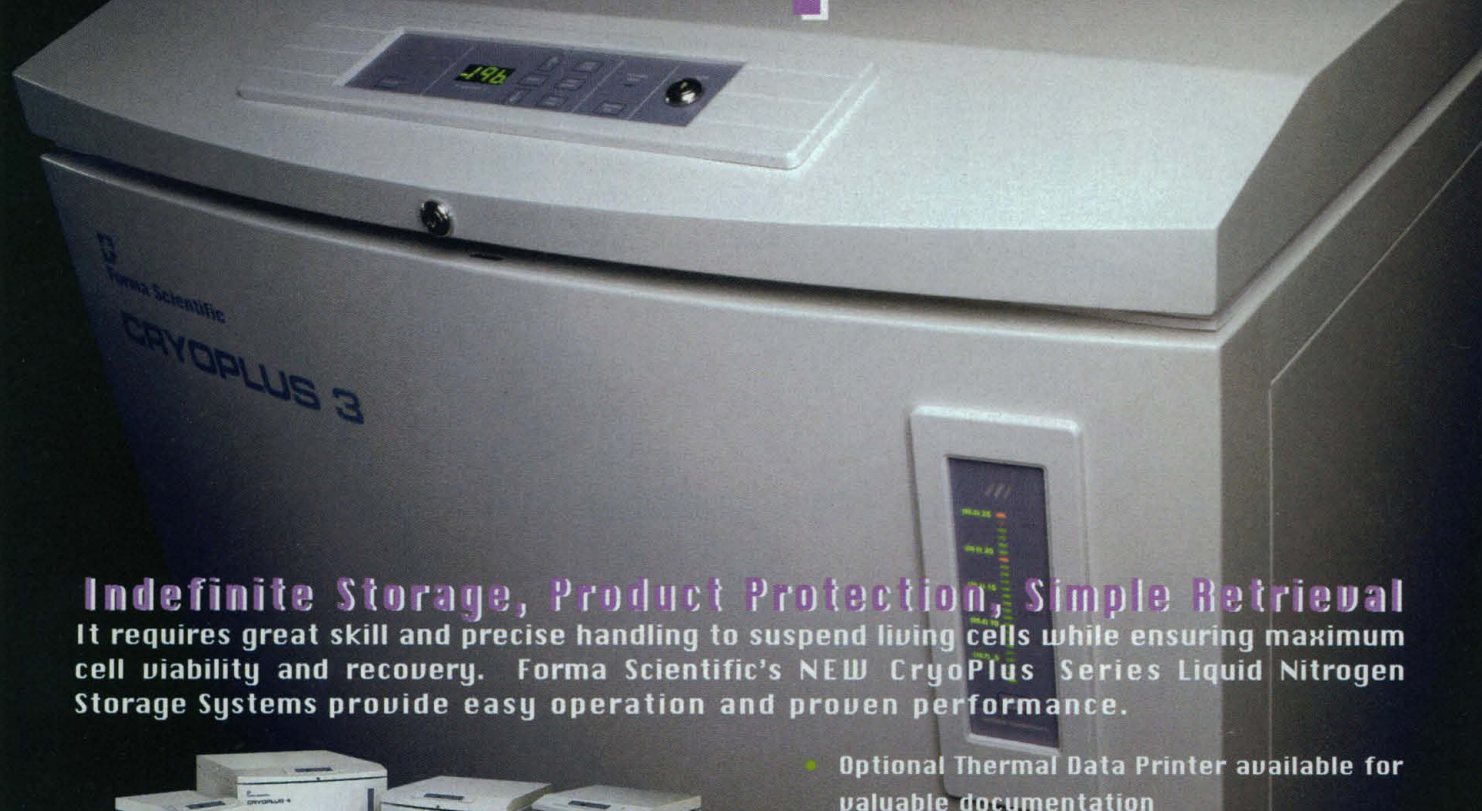
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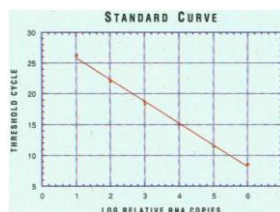
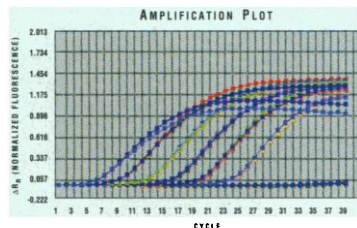


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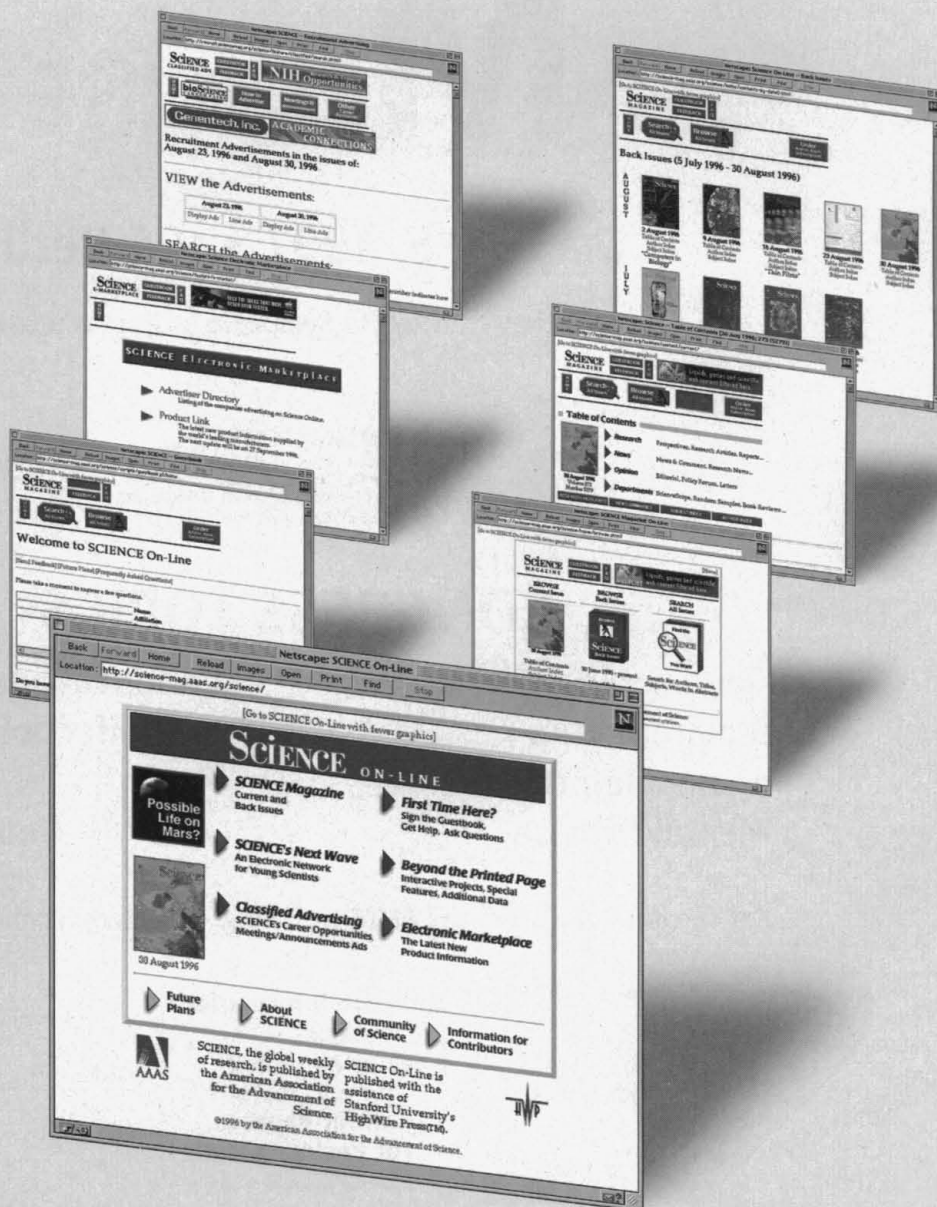
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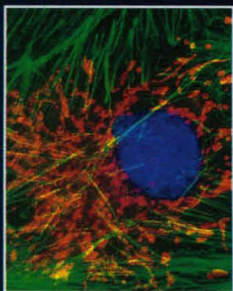
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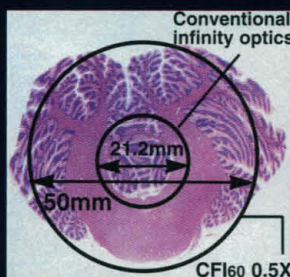
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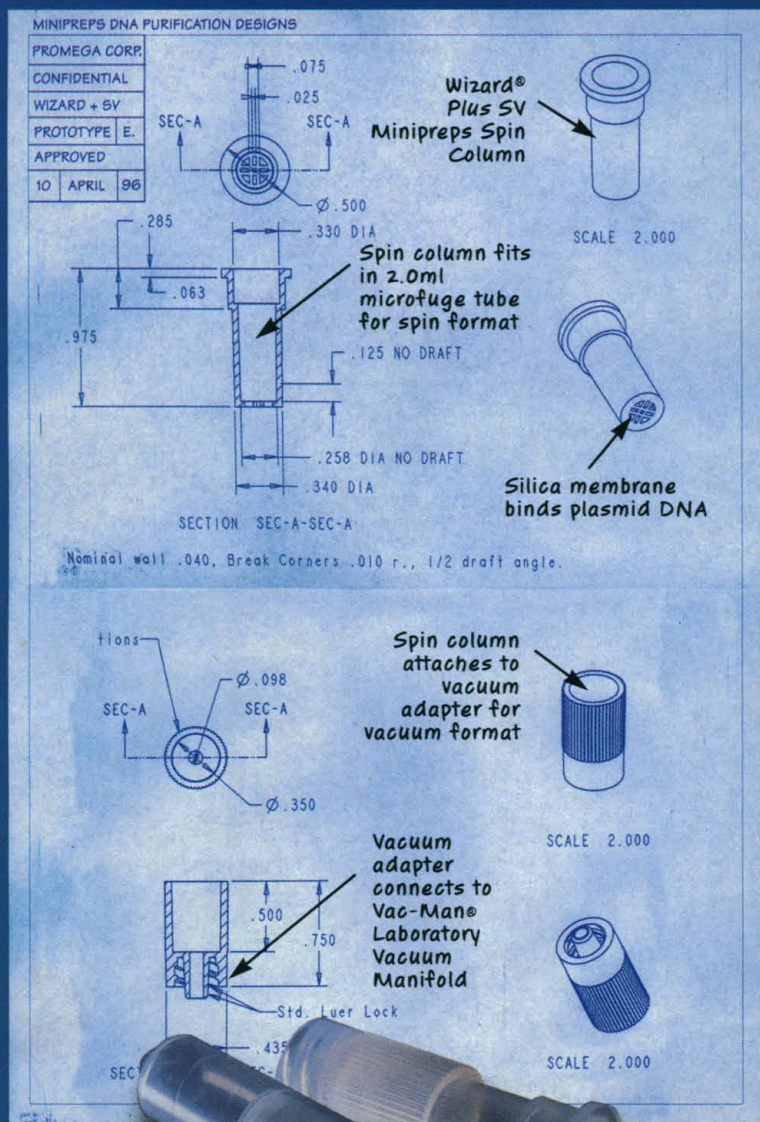
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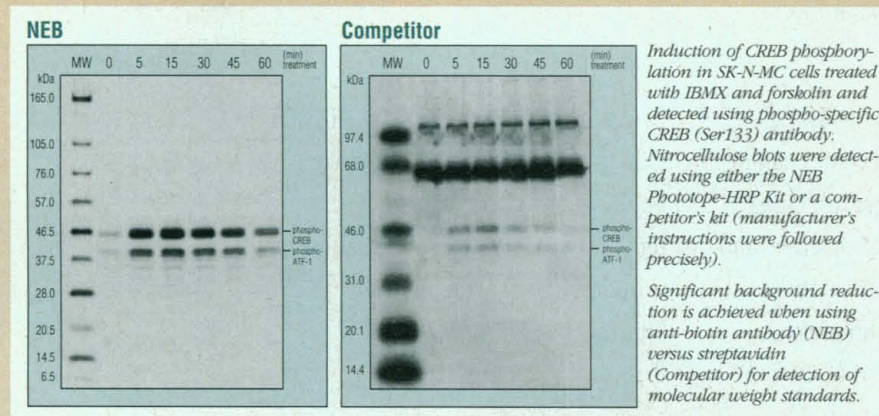
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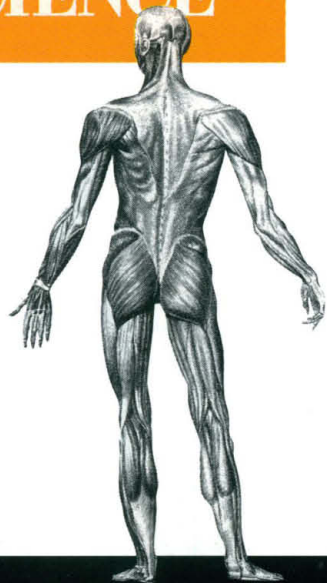
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The Human Transcript Map

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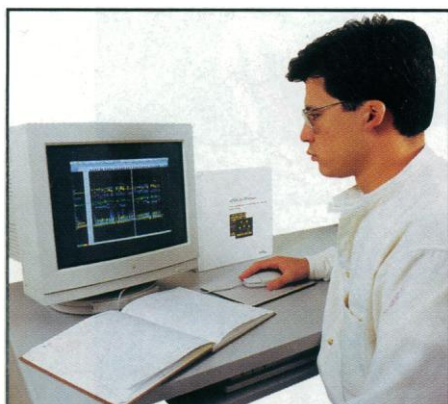


Using the Human Transcript Map

This human transcript map is a visual representation of a powerful new resource which is constantly being updated and released over the world wide web by Mark Boguski, Greg Schuler, and the staff at NCBI.¹ Because of the considerable efforts of over one hundred authors from sixteen laboratories around the world, which have joined the Radiation Hybrid Consortium,² anyone can now place almost any human sequence in one of the 1,000 genetically anchored bins on this radiation hybrid based transcript map.

Armed with this knowledge, investigators can look up all of the transcripts that have been mapped to that bin and simply purchase transcript-specific STS primer pairs, cDNA clones, and YAC clones for most of the transcripts. BAC clones containing the transcripts can be determined by PCR or hybridization-based screening.

Over the course of the next year, tens of thousands of additional transcripts will be added to this resource. Most of these will be based on the invaluable cDNA resource made possible by the I.M.A.G.E. Consortium,³ the end-sequencing of which is being performed by Washington University and has been made possible by the generous support of Merck & Company.⁴ The construction of this transcript map has been accelerated by a significant donation from Sandoz to map 10,000 brain related transcripts.⁵ An article on transcript mapping is included in this issue of *Science*. All of the resources needed to make the maximum use of this new tool are available from Research Genetics. Visit our web site at <http://www.resgen.com> or call and ask for Kay Swanson or Jim Hudson for more information.



References:

1. <http://www.ncbi.nlm.nih.gov/SCIENCE96>
2. An international collaborative project, the Radiation Hybrid Consortium, has agreed to divide the work and share the data. Patricia Rodriguez-Tome, at European Bioinformatics Institute, oversees the data and its distribution (Patricia.Rodriguez-Tome@ebi.ac.uk or URL <http://www.ebi.ac.uk/RHdb/>)
3. <http://www-bio.llnl.gov/bbrp/image/image.html>
4. <http://genome.wustl.edu/est/esthmpg.html>
5. Research Genetics *Resources*, Vol. 3, No. 2, pg.1

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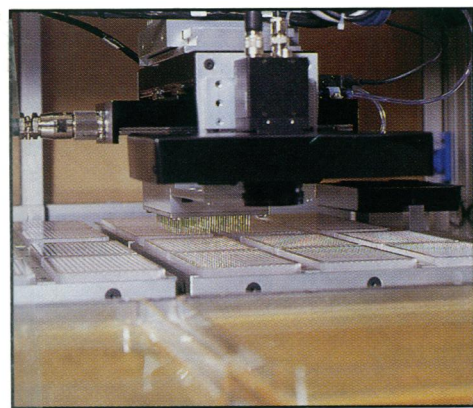
Resources for Radiation Hybrid Mapping

The GENEBRIDGE 4 and Stanford G3 Radiation Hybrid panels were used to create this human transcript map. By using DNA from these same panels, and using one of the e-mail servers, you can place your sequence on this map. With the map position you have access to the I.M.A.G.E. Consortium (LLNL) cDNA clones and CEPH YACs that have been mapped to the region, as well as GENEPAIRS™ designed to be transcript specific STSs. These can be used to screen our BAC DNA pools to find clones containing the genomic form of the transcript. The BAC and YAC clones may be used to screen

high density membranes for additional cDNA and/or genomic clones in the region.

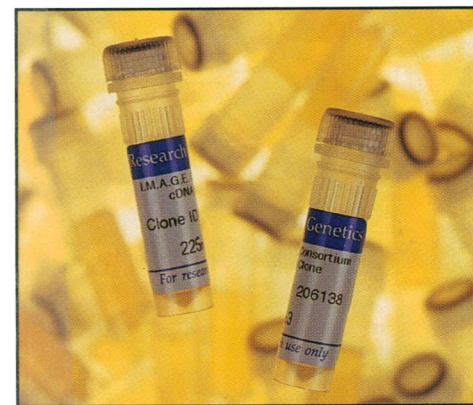
The Stanford G3 RH Panel is a medium resolution, 10,000 rad panel consisting of 83 clones and two controls. An e-mail server, operated by the Stanford Human Genome Center, will attempt to link your marker to framework markers they have already assayed. Current details may be obtained from <http://shgc-www.stanford.edu>.

The GENEBRIDGE 4 Panel is a lower resolution, 3,000 rad panel consisting of 93 clones and two controls. An experimental e-mail server, operated by the Whitehead Institute/MIT Center for Genome Research will attempt to link your marker to their recently released Whitehead RH framework map. Linkage to this map will often identify putative YAC clones containing and/or surrounding your marker as well as position your marker relative to thousands of ESTs and other PCR based markers. Current details may be obtained from <http://www-genome.wi.mit.edu>.



BAC DNA Pools

Now the row and column pools for the Human BAC library are included with the DNA pools. These row and column pools add twenty-four 96-well plates to the existing set of DNA pool plates. The screening of the superpools will total 48 reactions, and the screening of the plate, row and column pools will total another 48 reactions per positive superpool. After only 96 total reactions, the researcher will have a complete clone address! Sufficient DNA is provided for 50 screenings.



Individual cDNA Clones

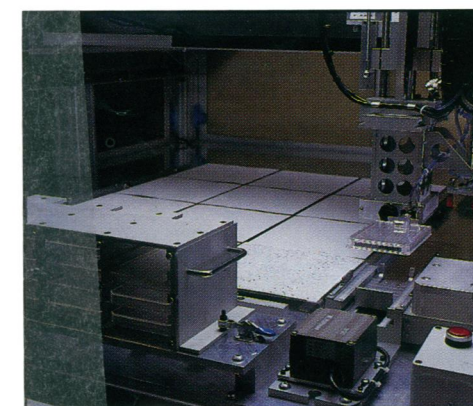
Each bin in the transcript map contains the IDs of transcripts that have been mapped to that bin. This information can be used to order individual clones. Currently, over 300,000 5' and 3' sequences are deposited into public databases and are searchable for any information such as putative gene function using the dBest/GenBank query (<http://www.ncbi.nlm.nih.gov/Web/Search/index.html>). When ordering cDNA clones, please keep in mind that the clone ID # is the number assigned by I.M.A.G.E. Consortium, not the NCBI number or the Genbank accession number.



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Working with a number of investigators, we have produced tens of thousands of transcript-based STSs including those used to generate the transcript map on the front of this chart. These primer pairs, which we call GENEPAIRS™, are designed to amplify the 3' ends of cDNAs for known genes as well as the thousands of unknown transcripts found in the I.M.A.G.E. Consortium (LLNL) cDNA library.

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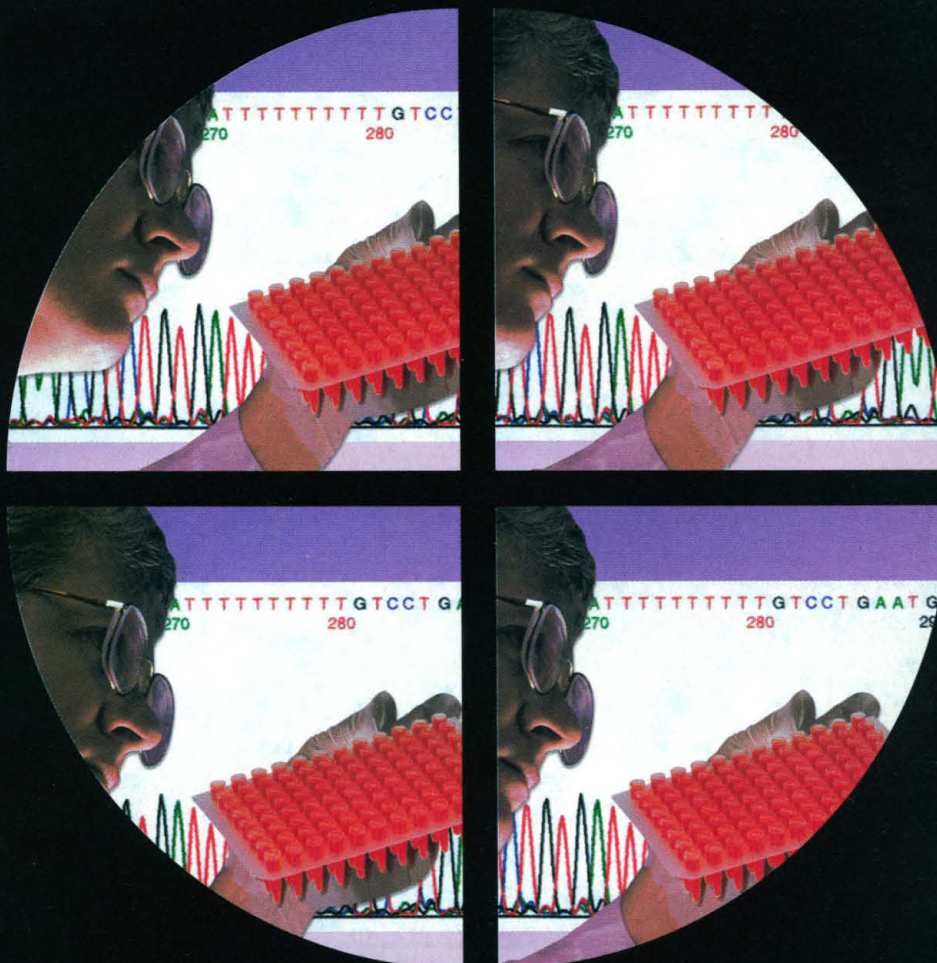
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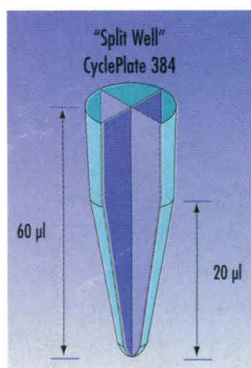
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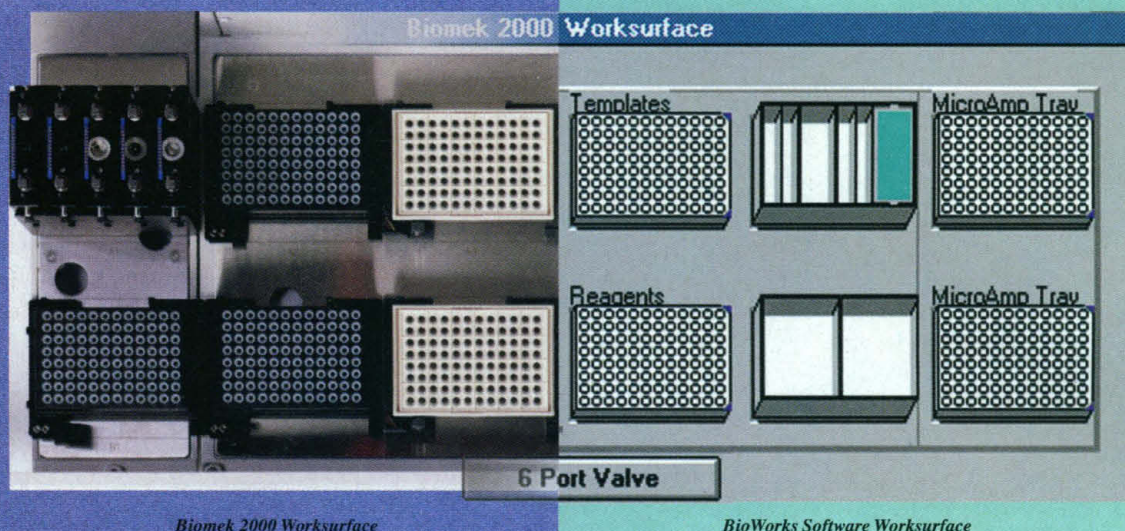
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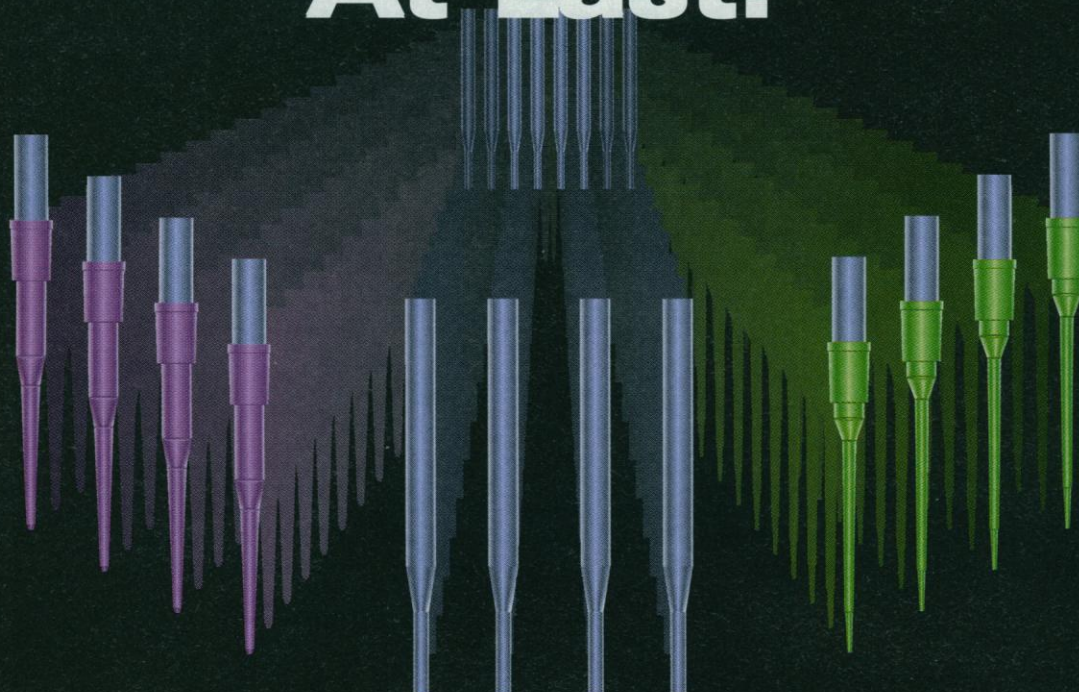
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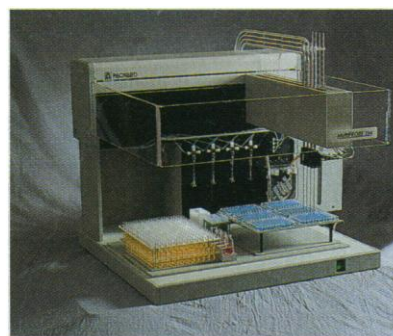
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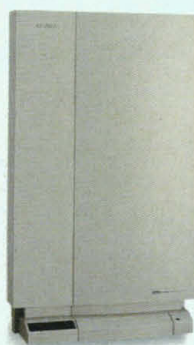
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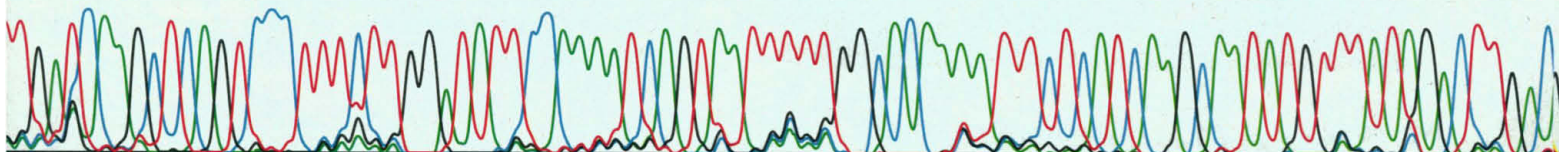
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Comparison for Long RT-PCR: cDNA Amplification from Dystrophin mRNA



RT reactions were carried out under optimum conditions for each enzyme, AMV RTase and MMLV RTase, in duplicate. One sample from each pair was followed by RNase H treatment. Then using two different primer pairs for 8 kb and 12 kb amplification, PCR was performed with *TaKaRa LA Taq* for AMV RTase products and Long distance DNA polymerase for MMLV RTase products respectively. No full length cDNA of 12 kb was detected with MMLV RTase.

mk λ Hind III marker
A+ AMV RTase reaction followed by RNase H treatment
M+ MMLV RTase reaction followed by RNase H treatment
A- AMV RTase reaction without following RNase H treatment
M- MMLV RTase reaction without following RNase H treatment



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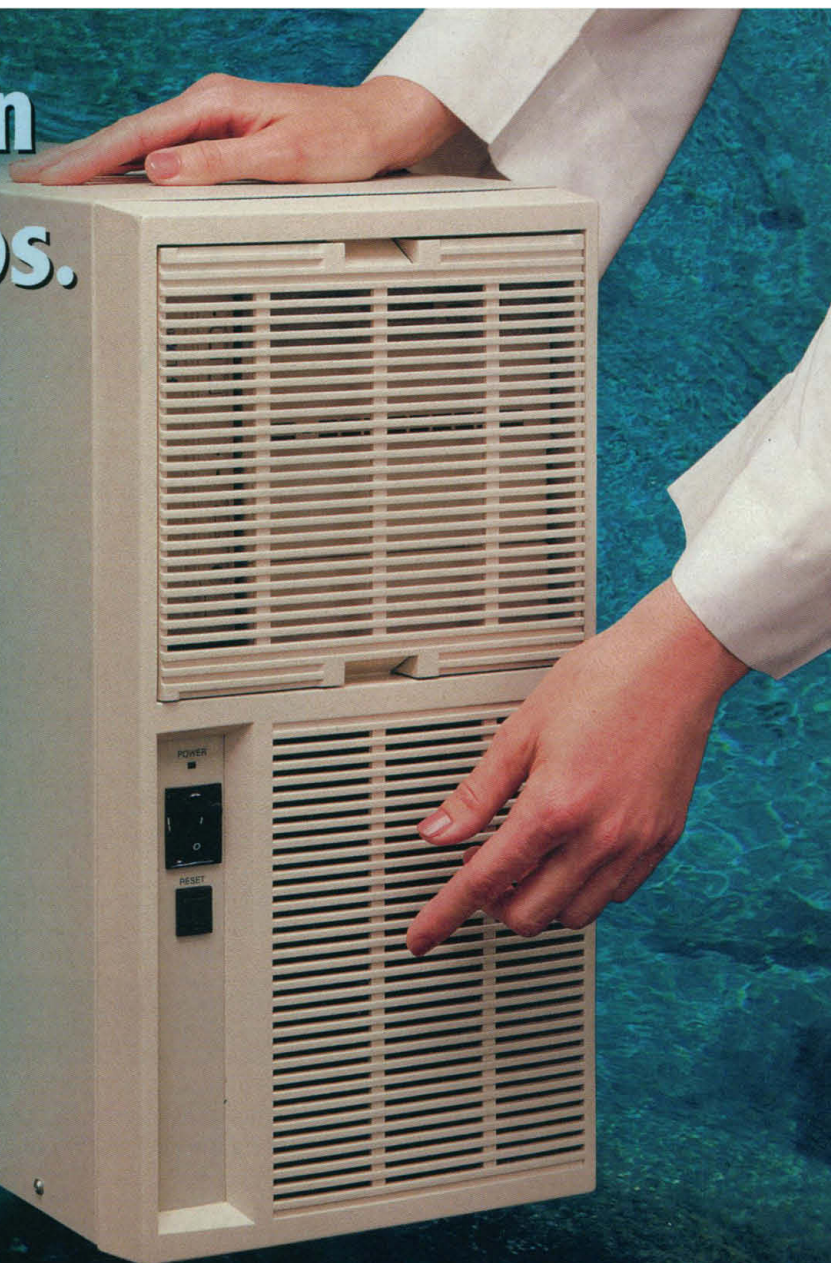
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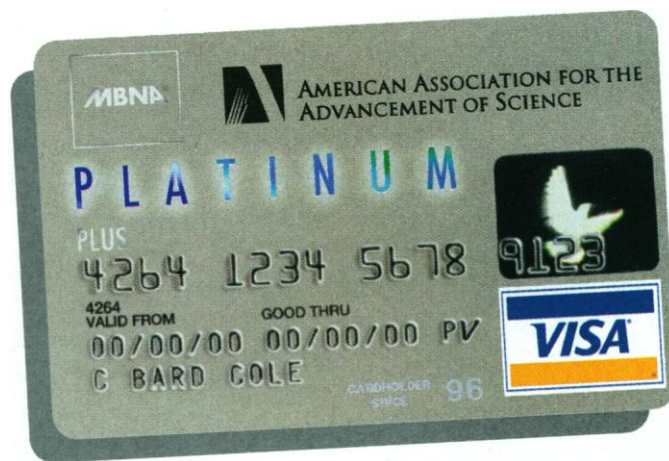
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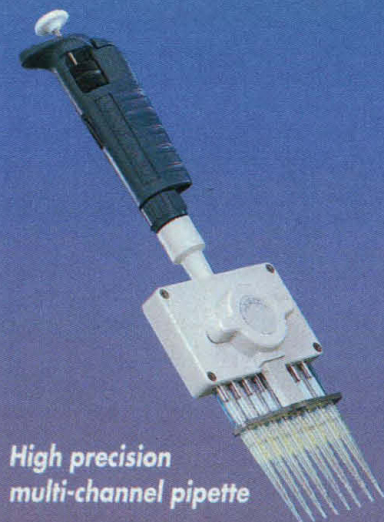


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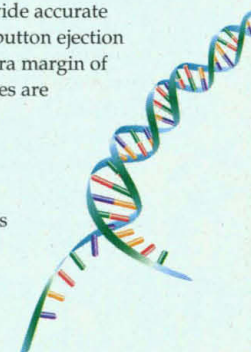
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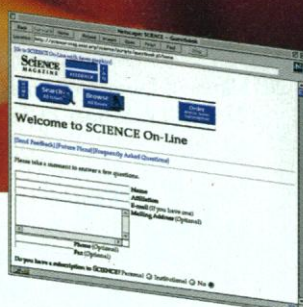
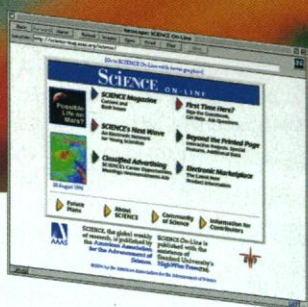


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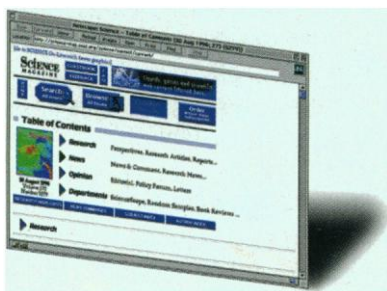
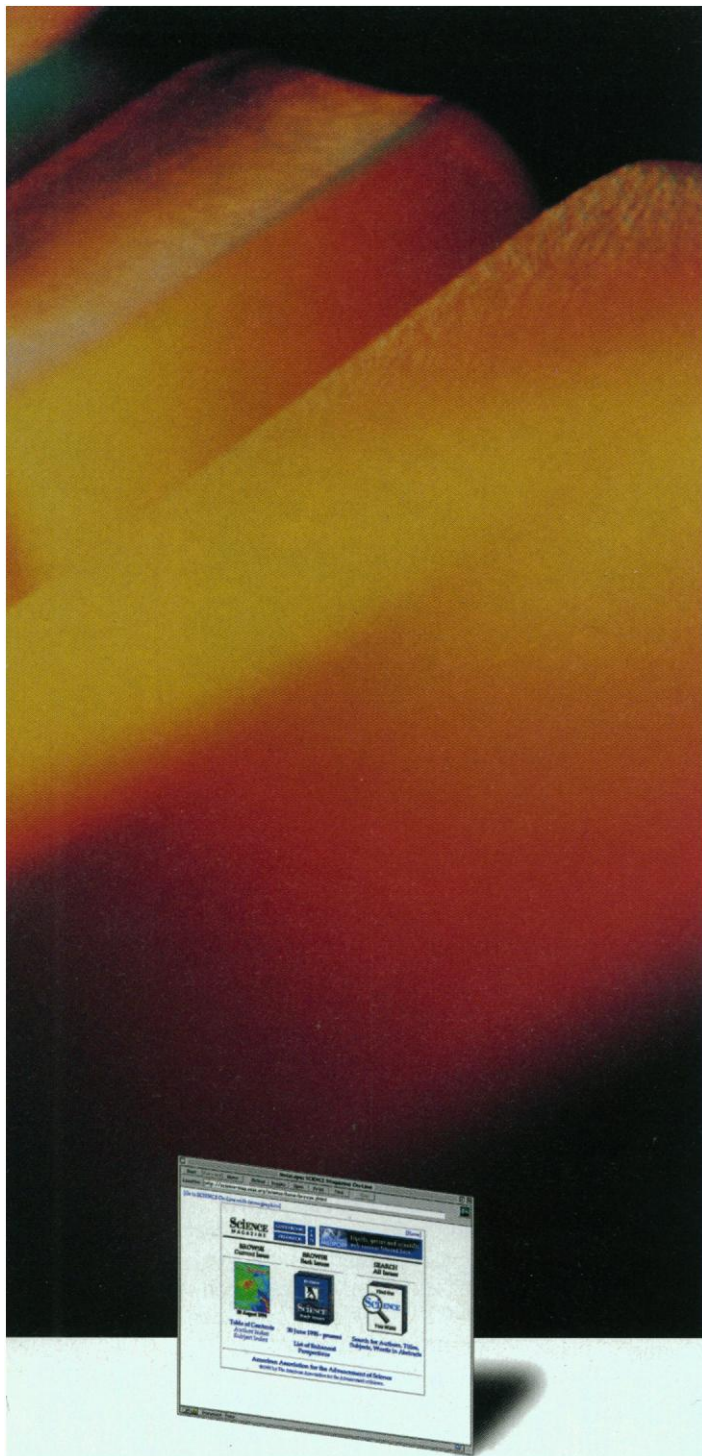
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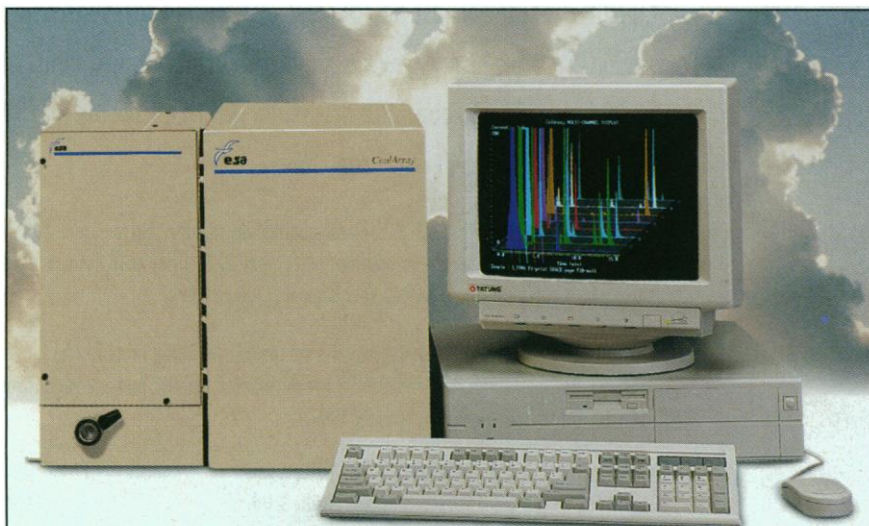
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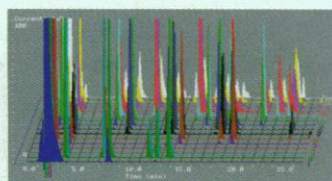


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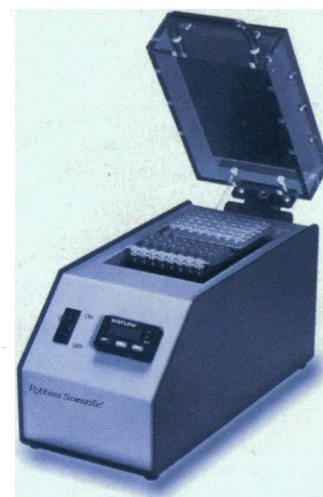
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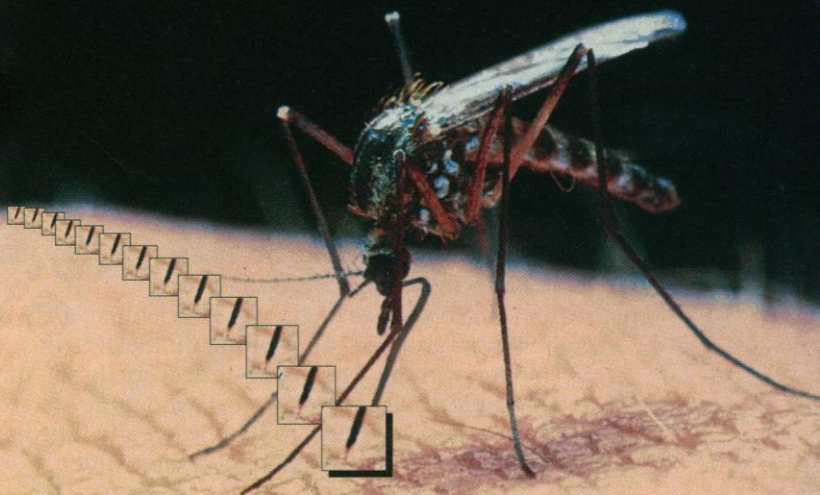
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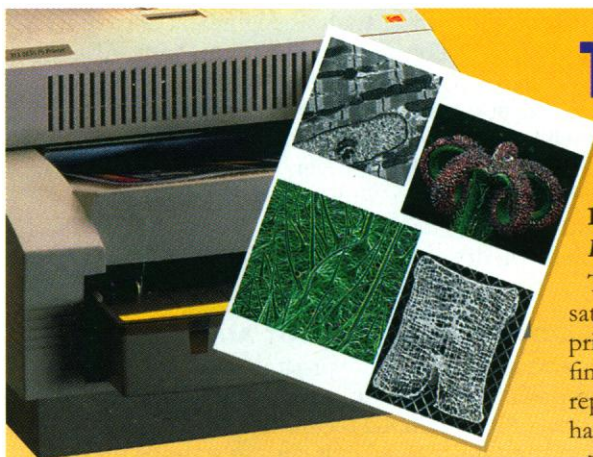
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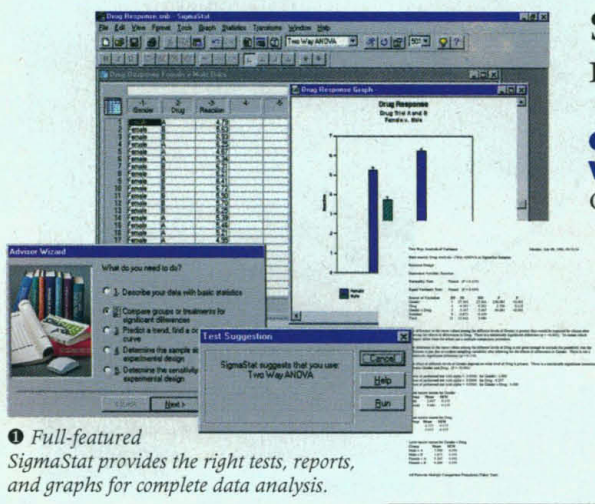
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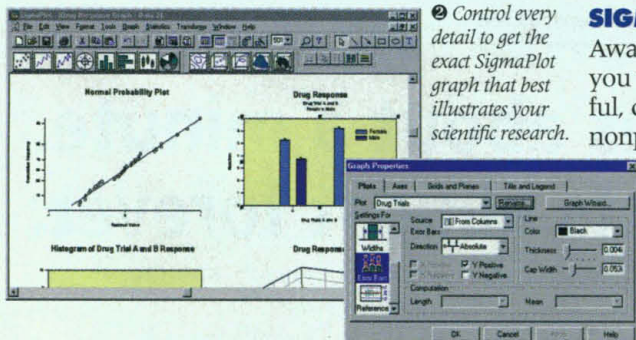
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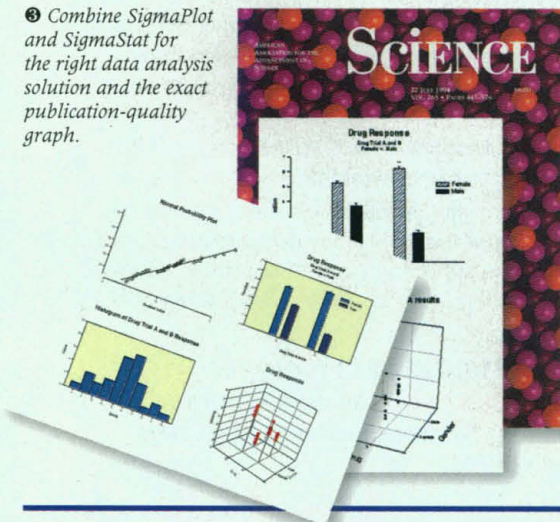


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