4 May 2001

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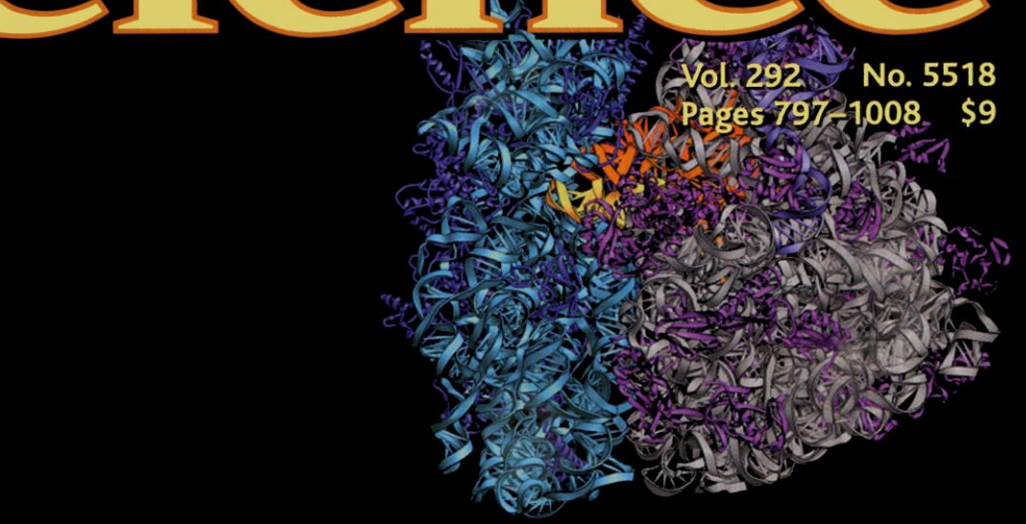

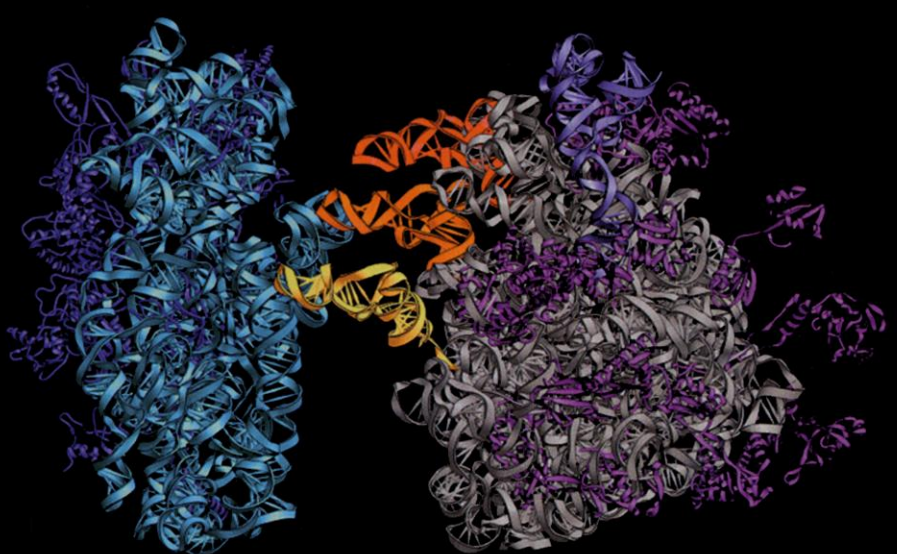

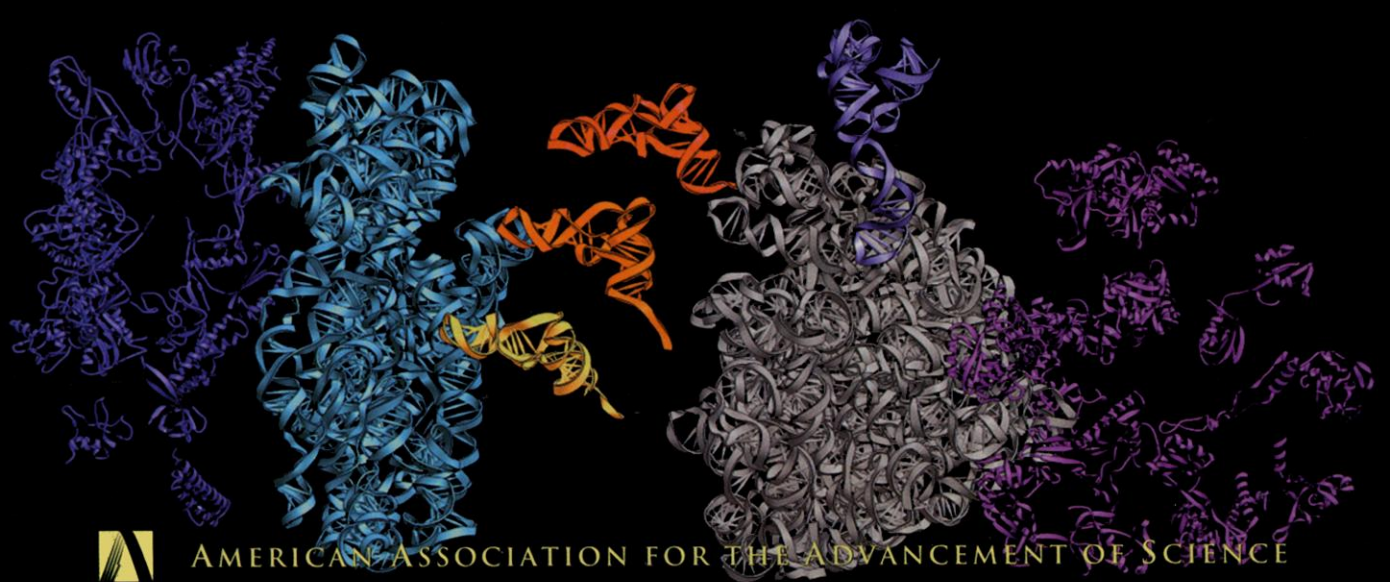

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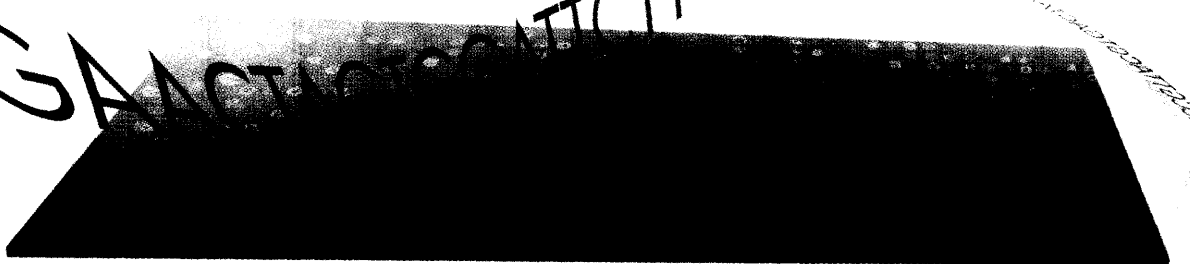

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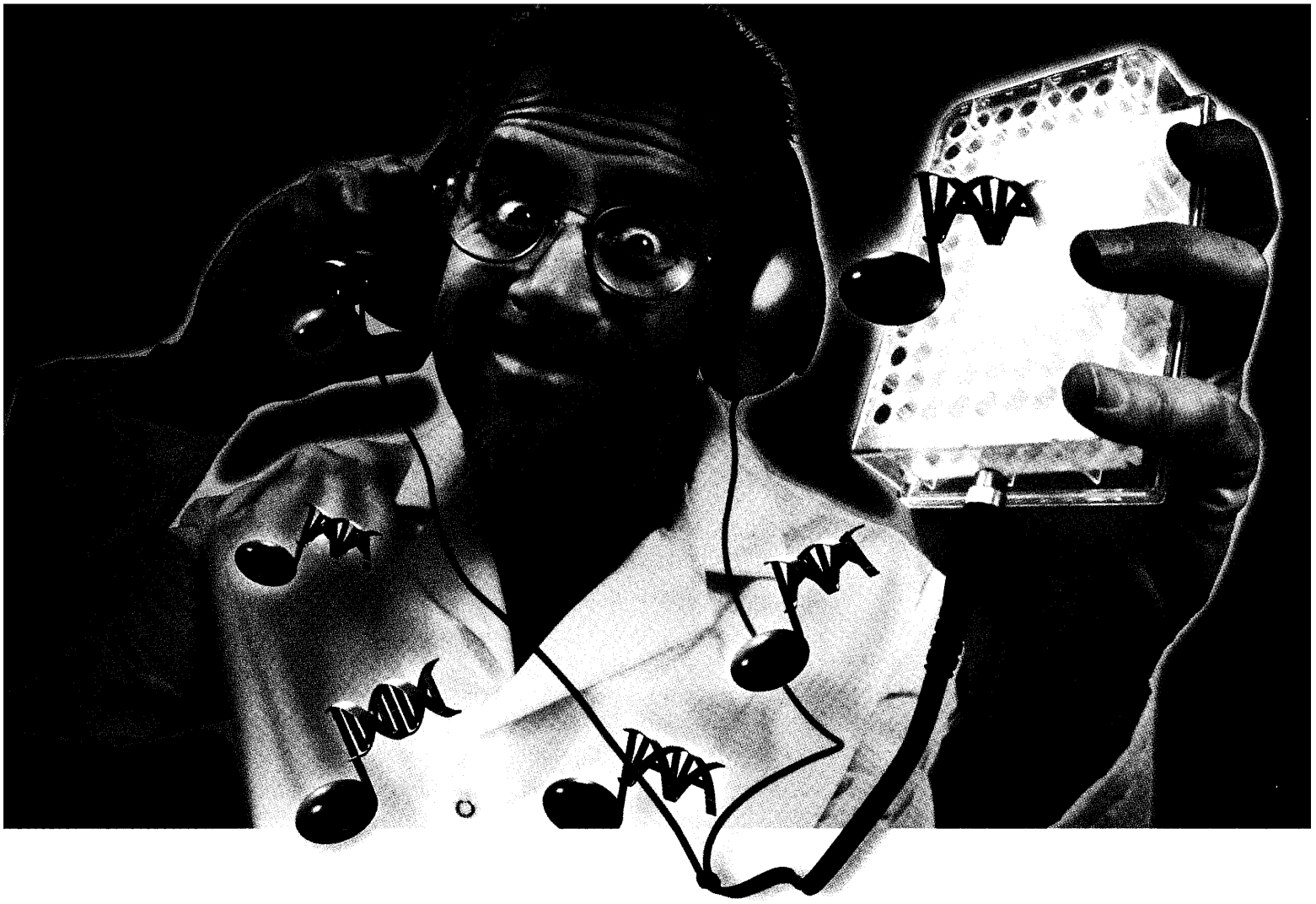

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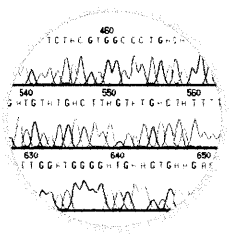

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Science

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NEWS

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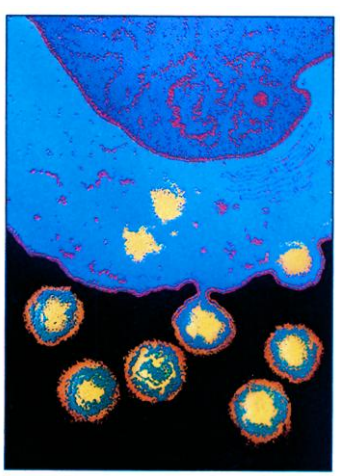
Talking up science

SCIENCE'S COMPASS



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Paths toward prevention

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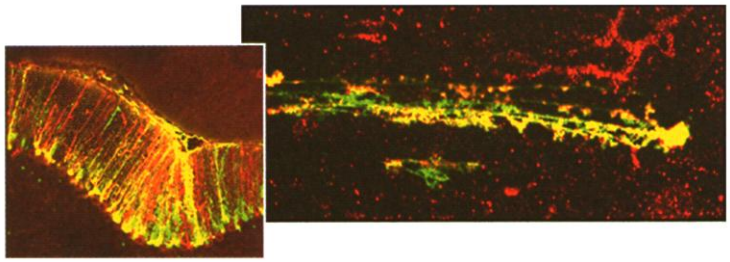

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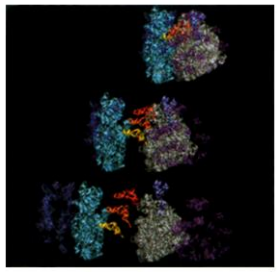
Converting glial AMPA receptors



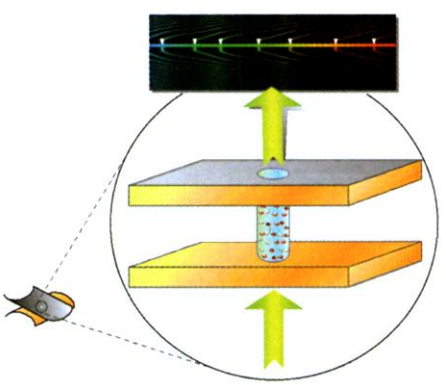
RESEARCH

RESEARCH ARTICLES

REPORTS

COVER 883

Crystal structure of a *Thermus thermophilus* 70*S* ribosome containing three bound transfer RNAs (top) and exploded views showing its different molecular components (middle and bottom). The 16*S*, 23*S*, and 5*S* ribosomal RNAs are cyan, gray, and light purple, respectively; the A-, P-, and E-site transfer RNAs are shown in yellow, orange, and red, respectively. The 30*S* subunit proteins are dark purple, and the 50*S* proteins are magenta. [Image: M. M. Yusupov, G. Zh. Yusupova, A. Baucom, K. Lieberman, T. N. Earnest, J. H. D. Cate, H. F. Noller]



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Density fluctuations in molecularly thin films

Signalosome controls photomorphogenesis **803**

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Linearly Polarized Emission from Colloidal Semiconductor Quantum Rods J. Hu *et al.*

Linearly polarized emission from quantum dots should open up new applications in biological imaging and in display technology.

Evidence for Substantial Variations of Atmospheric Hydroxyl Radicals in the Past Two Decades R. G. Prinn *et al.*

After rising steadily through most of the 1980s, the global concentration of atmospheric OH is shown to have fallen over the past decade to below its 1978 level.

Promotion of NEDD8-CUL1 Conjugate Cleavage by COP9 Signalosome S. Lyapina *et al.*

Interactions of the COP9 Signalosome with the E3 Ubiquitin Ligase SCF^{TIR1} in Mediating Auxin Response C. Schwechheimer *et al.*

838 Photomorphogenesis in plants likely involves interactions between the signalosome and one of the SCF ubiquitin ligases to promote reversible modification of target proteins with the ubiquitin-like molecule NEDD8.

TECHNICAL COMMENTS

Bacterial Genomic Reorganization upon DNA Replication

Myllykallio *et al.* (Reports, 23 June 2000, p. 2212), studying the circular genome of the archaeal species *Pyrococcus abyssi*, found evidence for a single origin of bidirectional replication and for a region of termination of replication opposite it; further, they reported that "as in Bacteria, the chromosomal region containing the replication terminus was a hot spot of genome shuffling." Makino and Suzuki comment that their study of a closely related *Pyrococcus* species "suggests that the orientation of a 350-kb region was reversed relative to the rest of the genome at some point" in the evolution of the two species—with the center of the inversion close to the replication origin—and outline "a general model of genomic reorganization" to explain these observations. Zivanovic *et al.*, in their response, discuss some of the implications of their own data and the Makino and Suzuki model for studies of archaeal genomic rearrangements.

The full text of these comments can be seen at www.sciencemag.org/cgi/content/full/292/5518/803a

science's stke www.stke.org

Perspective: New Functions for DNA Binding Domains A. Rao

How interactions with subtly different DNA sequences can influence gene activation or repression by the same transcription factor.

science's next wave www.nextwave.org

Global: Careers in Clinical Writing

This month's special feature correspondents reveal their own diverse, and informative, experiences in a writing career that can keep you connected with scientific research.

US: Job Search Bias—Is It Real or Am I Just Imagining It? S. Summerour Clemmons

Our guest "Tooling Up" columnist helps you to spot the differences between real and perceived bias during the job search process.

Germany: Wanted—500 Patent Lawyers! E. von Ruschkowski

To keep up with the continuing flood of biotech patent applications, the European Patent Office is hiring . . . and how!

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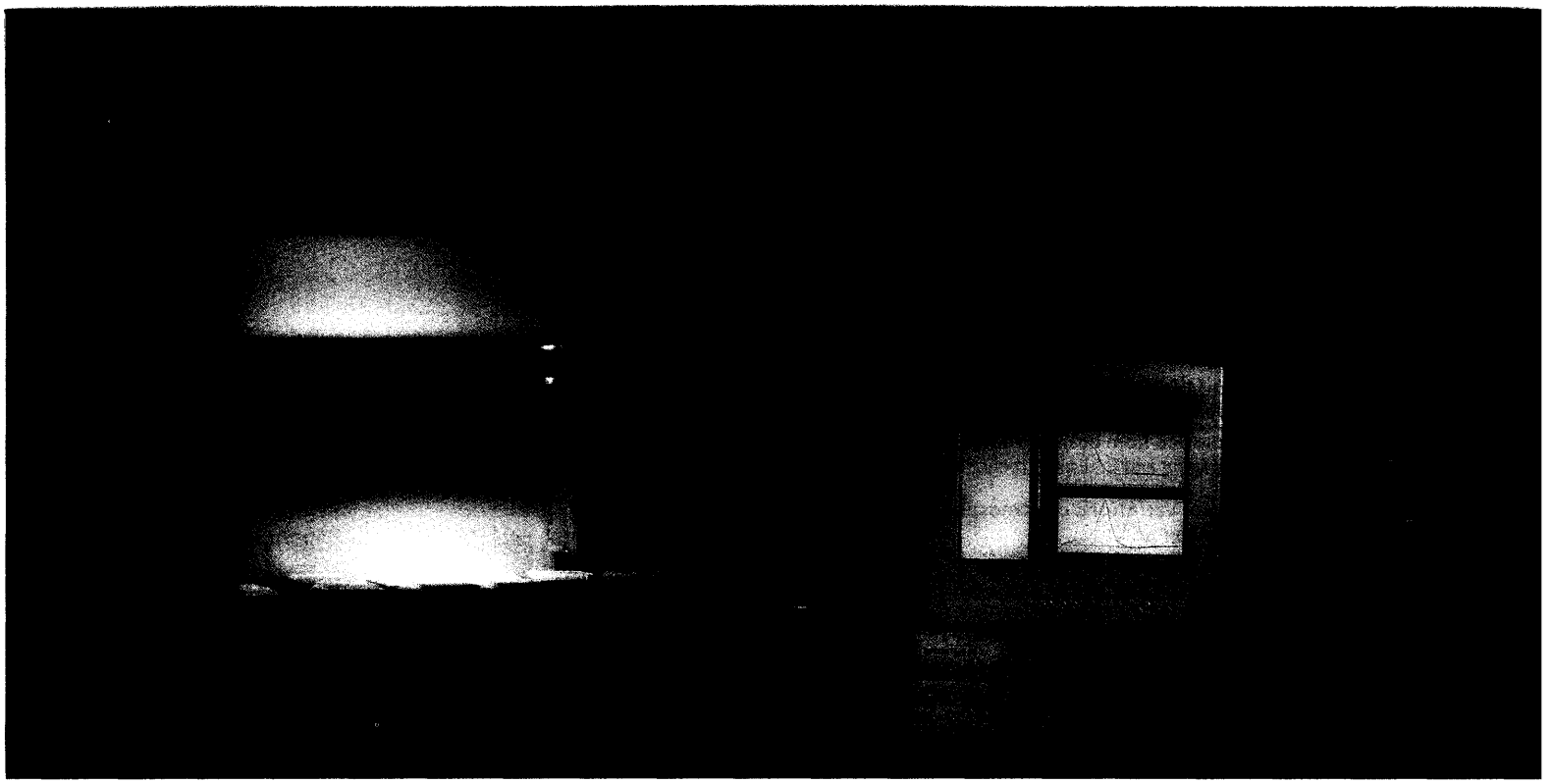

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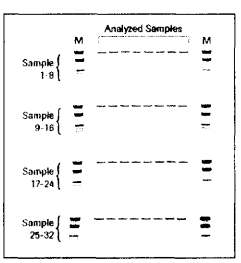
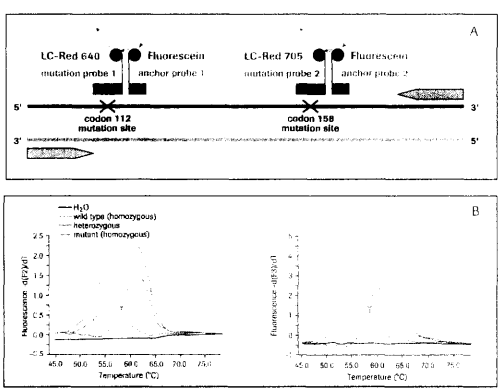


Genomic DNA isolation from 32 samples of human whole blood using MagNA Pure LC. *No visible intra-assay variance.*



Dual-color genotyping using the LightCycler Instrument. ***A.*** *Schematic of the PCR.* ***B.*** *Melting curve analysis was performed on different genotypes at codons 112 and 158 of the Apo E sequence (in channels 2 and 3) to discriminate wild type, heterozygous, and mutant genotypes.*

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THIS WEEK IN Science

edited by Phil Szuromi

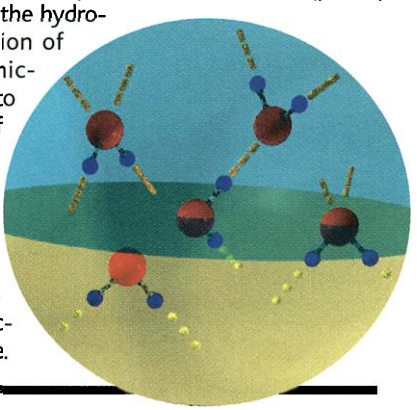
Monitoring Electron Paths in Atoms

When an intense laser field interacts with an atom, the excited electrons driven by the laser field can be pulled from the nucleus, perform some complex orbits, and then be driven back to the nucleus, where they can scatter or recombine. Although it is often possible to calculate quantum-mechanical descriptions of such processes, they often can be difficult to appreciate. Feynman's approach to quantum mechanics, which involves summing over all possible paths, or quantum trajectories, provides a somewhat more intuitive description of the processes involved, but many experiments have been difficult to describe in this fashion because of the shear number of paths involved. Salières *et al.* (p. 902; see the news story by Seife) used a polarized laser field to limit the number of possible paths and show that the quantum orbit approach can describe the processes.

908

Oil and Water Try to Mix

The interfaces between different liquids are notoriously difficult to probe experimentally because the signature of the molecules at the interface must be separated from that of the surrounding bulk liquid. Vibrational sum frequency spectroscopy is one of the few techniques that can overcome this problem. Scatena *et al.* (p. 908) used this method to probe the hydrogen bonding and orientation of water molecules at organic-water interfaces. Contrary to the conventional picture of strong hydrogen bonding between water molecules near fluid hydrophobic surfaces, they find that the water molecules are weakly bonded and are partially oriented by interactions with the organic phase.



CREDIT: L. F. SCATENA *ET AL.*

A Tight Squeeze

What happens to fluids when they are confined to very small spaces? The surface force apparatus, in which molecules are confined between cylindrical mica surfaces whose separation is carefully controlled, has been used to determine forces as a function of separation distance. Heuberger *et al.* (p. 905; see the Perspective by Israelachvili and Gourdon) have built an extended surface force apparatus in which fast spectral correlation improves the instrument resolution by a factor of 10 to 30. They probed the behavior of liquids for separation distances much smaller than molecular dimensions and could measure both the pressure and specific volume of the samples. They present data both for the case where the sample coexists as a liquid and a gas and for the case where only one fluid phase is present and continuum behavior no longer applies.

Rotating with Light

Microscale objects can be readily translated with light beams ("optical tweezers"), but the methods that have been developed to add rotational control have been limited by the properties of the materials to be manipulated. Paterson *et al.* (p. 912) introduce a generalized technique that allows rotational control over arbitrarily shaped objects. The interference of a laser beam with a helical component in the electric field and a plane wave results in the formation of a light beam with a corkscrew-shaped intensity profile. By varying the path-difference between the two beams, any object trapped within the optical field is also rotated as the corkscrew is turned.

Ironing Out Vibrations

Phonons are elementary vibrations of solids that influence many of their fundamental properties, but describing their variations with pressure and temperature, especially at extreme conditions, can be difficult to do in practice. For example, for iron, which primarily makes up Earth's core, many of these properties have yet to match seismic measurements or heat-flow data accurately. Mao *et al.* (p. 914) now combine both an experimental approach using a modified diamond-anvil press and nuclear-resonant inelastic x-ray scattering measurements with ab initio calculations to infer the phonon density of states at pressures up to those of Earth's core. These results indicate that the mean atomic number for the core is higher than that for pure iron and suggest the presence of some nickel in the core.

Organics in the Oceans

Much of the organic carbon in the ocean is actually not in organisms themselves but is present as dissolved organic matter (DOM) in seawater. It has been difficult to characterize DOM, yet understanding its origin, dynamics, and its further availability to organisms is critical to quantifying the role of the oceans in the global carbon budget. In a laboratory study, Ogawa *et al.* (p. 917) examined and traced organic matter as it was processed by naturally occurring marine bacteria. Ocean bacteria play a critical role in processing most of the oceans' DOM as well as producing the large amount of DOM that is refractory or that has low bioavailability.

Helping with Her Coat

Phospholipase C (PLC) hydrolyzes a membrane phospholipid to produce two second messengers that can in turn function in cellular signaling pathways. Fukami *et al.* (p. 920) examined the PLCδ4 isoform, which is localized to the acrosomal region of sperm. Upon disrupting the *PLCδ4* gene in mice, the resultant males were either sterile or produced very few progeny. The sperm were unable to interact appropriately with the egg coat, the zona pellucida. Because PLCδ4 is essential for an early step in mammalian fertilization, greater insight into the function of this protein may aid in understanding human fertility and have application to contraception.

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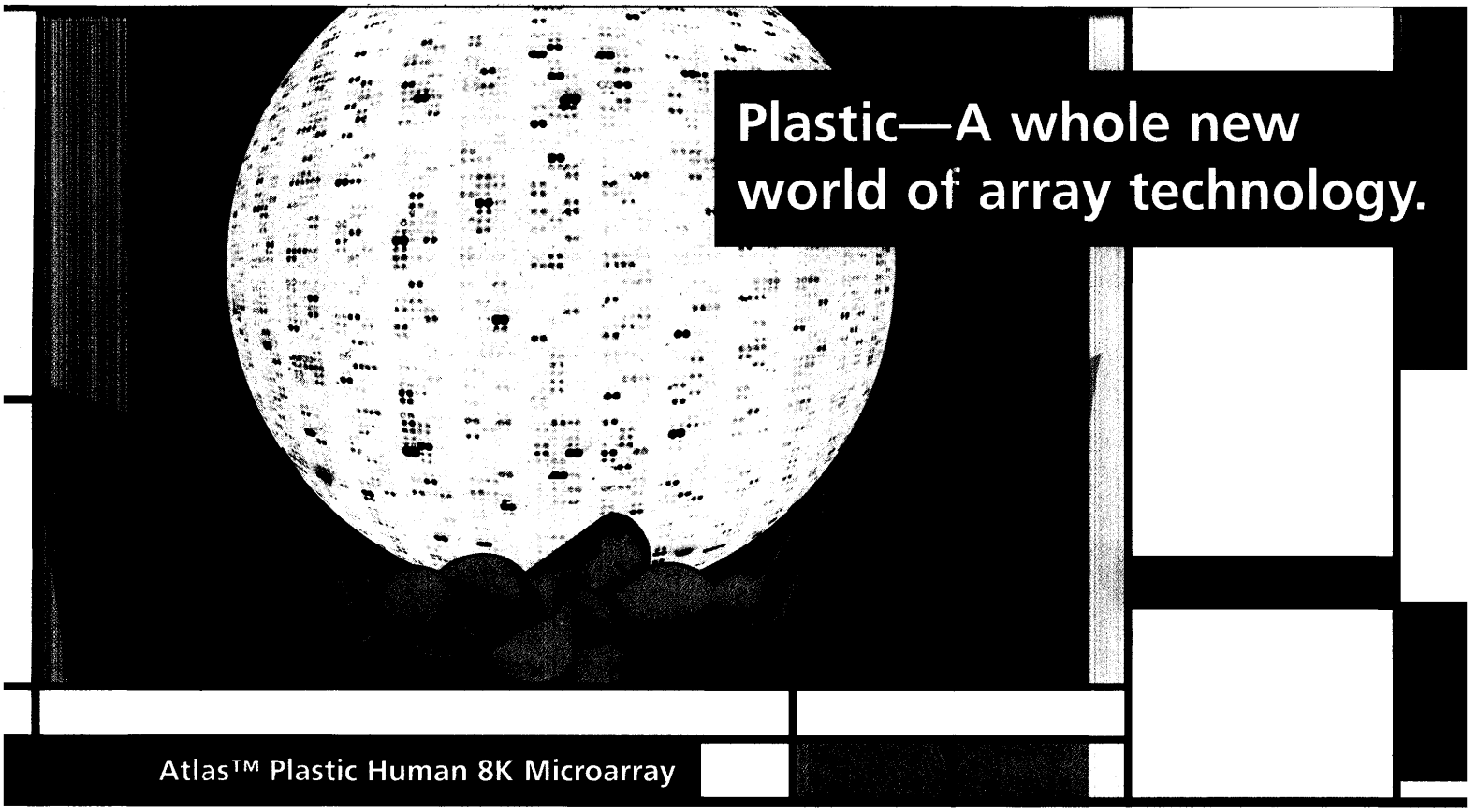
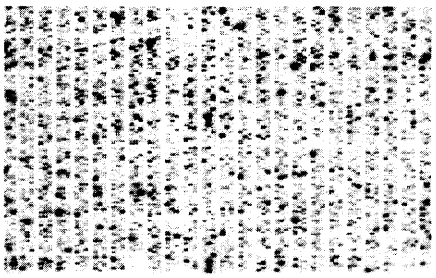


Illustration inspired by the art of Juan Gris (1887–1929) & Piet Mondrian (1872-1944).



Expression profiling of diabetic human skeletal muscle using the Atlas™ Plastic Human 8K Microarray. 10 µg of total RNA from diabetic human skeletal muscle tissue was isolated and labeled with ^{33}P using the Atlas™ Pure Total RNA Labeling System (#K1038-1). The probe was hybridized to the Atlas Plastic Human Array according to the User Manual.

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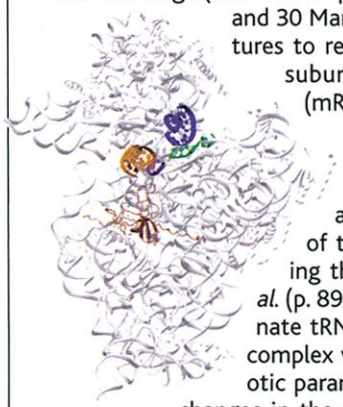
Putting the Pieces Together

Atomic-resolution snapshots of the large and small subunits of the ribosome have now been used to determine the structure of whole ribosome and to tease out some of its inner workings (see the Perspective by Dahlberg). Yusupov *et al.* (p. 883; see the cover and 30 March news story by Pennisi) have used the subunit structures to resolve a complex of the whole ribosome (large and small subunits) at 5.5 angstroms (Å) with bound messenger RNA (mRNA) and transfer RNAs (tRNAs) that enter at the A or acceptor site, move into the P or peptidyl transferase site, and exit via the E site. What is now visible are the precise physical relations between the three tRNA binding sites and the interfacial bridges that underlie relative movement of the subunits, which is the postulated means of coordinating the in-register translocation of tRNAs and mRNA. Ogle *et al.* (p. 897) show how the ribosome discriminates against near-cognate tRNA from their structures of the small ribosomal subunit in complex with A-site tRNA and mRNA, with and without the antibiotic paramomycin, at resolutions between 3.1 and 3.3 Å. Structural changes in the small subunit allow specific interactions with the codon-anticodon double helix that require Watson-Crick base pairing of the first two base pairs. The antibiotic paromomycin partially induces these structural changes and so disrupts decoding by facilitating binding of near-cognate tRNAs.



CREDIT: J. M. OGLE *ET AL.*

Glial Cells and the Synapse

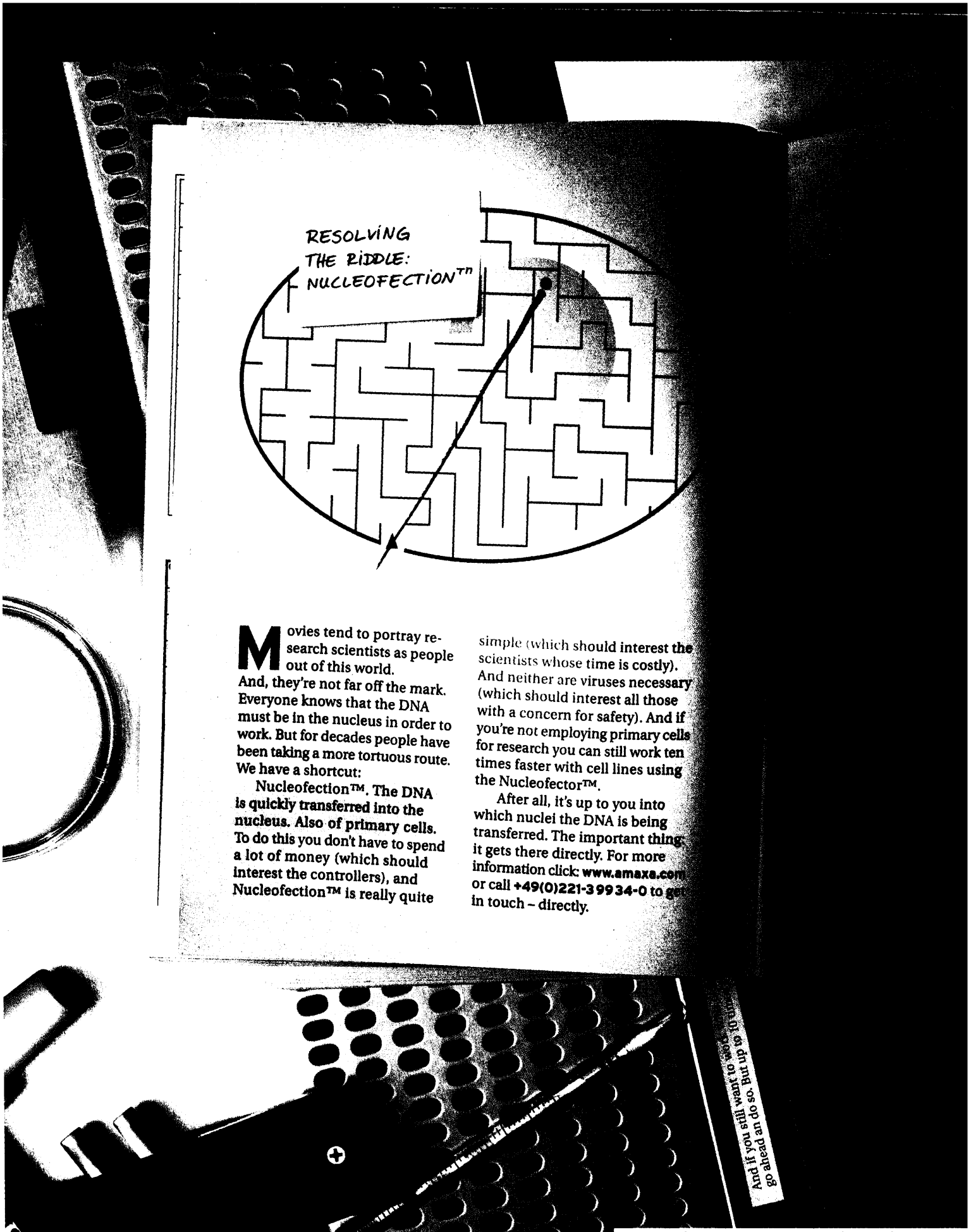
Glial cells participate in the regulation of synaptic transmission by controlling neurotransmitter diffusion and concentration in the synaptic cleft. Two reports explore the role of glutamate and its receptors in this control (see the Perspective by Gallo and Chittajallu). To investigate the role of glial glutamate uptake, Oliet *et al.* (p. 923) took advantage of a substantial anatomical rearrangement between astrocytes and neurons in the hypothalamus during changes in reproductive state. During lactation, astrocyte ensheathment of synapses and hence glutamate removal are reduced, which leads to changes in the amplitude of evoked excitatory postsynaptic currents caused by activation of presynaptic metabotropic glutamate receptors. Glial cells express AMPA glutamate receptor subunits, the physiological role of which has been poorly understood. Iino *et al.* (p. 926) altered the function of Bergmann glial cells in the cerebellum by adding an AMPA receptor subunit that renders the channels calcium impermeable. They observed major changes in the morphology of the glial cell specializations around the synapses: The glial envelopment around Purkinje cell dendritic spines became retracted. Removal of released glutamate was impaired and led to an unusual multiple climbing fiber innervation of Purkinje cells.

Managing Metabolic Models

Understanding gene function in complete genomes will require the integration of different kinds of information in a way that will facilitate analysis and the generation of testable hypotheses. Ideker *et al.* (p. 929) have developed a systems approach to DNA microarrays, proteomics, and physical interactions for probing the yeast galactose utilization pathway. The pathway was perturbed by mutation and growth in selective media, and the changes were assayed in approximately 6200 yeast genes. They generated and tested hypotheses regarding the regulatory capabilities of Gal-1-P and galactose transporter.

A Natural Take on Preservation

Natural killer (NK) cells provide a vital link between innate and adaptive immunity and play a specific role in protection against tumors and viruses. Several lines of study have shown that NK cells operate through signals delivered by inhibitory and activation receptors, yet direct evidence for activation receptors in protection against pathogens has been lacking. Brown *et al.* (p. 934) show that the NK activation receptor—LY-49H—plays a critical role in the resistance of mice to infection with cytomegalovirus. LY-49 contains immunoreceptor tyrosine activation motif signaling motifs normally found in lymphocyte membrane receptors, which suggests that NK cells may operate through similar pathways of cell signaling as the cells of the adaptive immune response.



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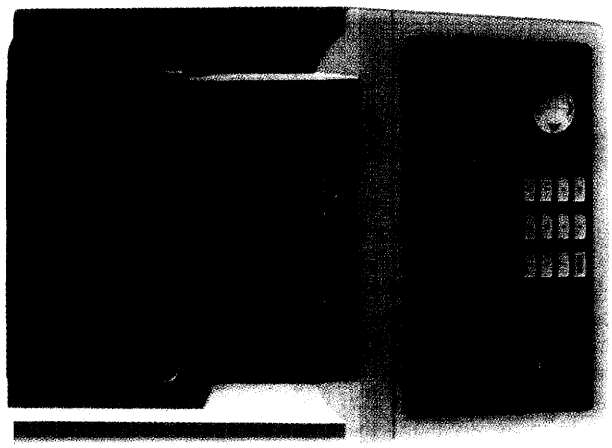
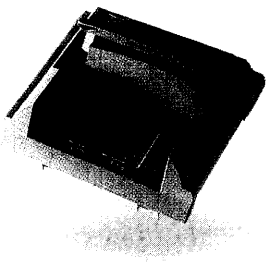
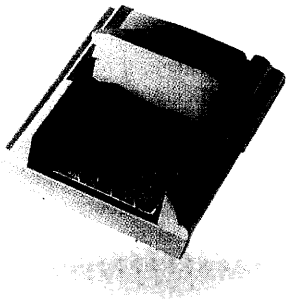
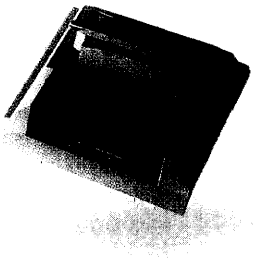




96-well 0.2 mL block



Dual 384-well 0.02 mL block



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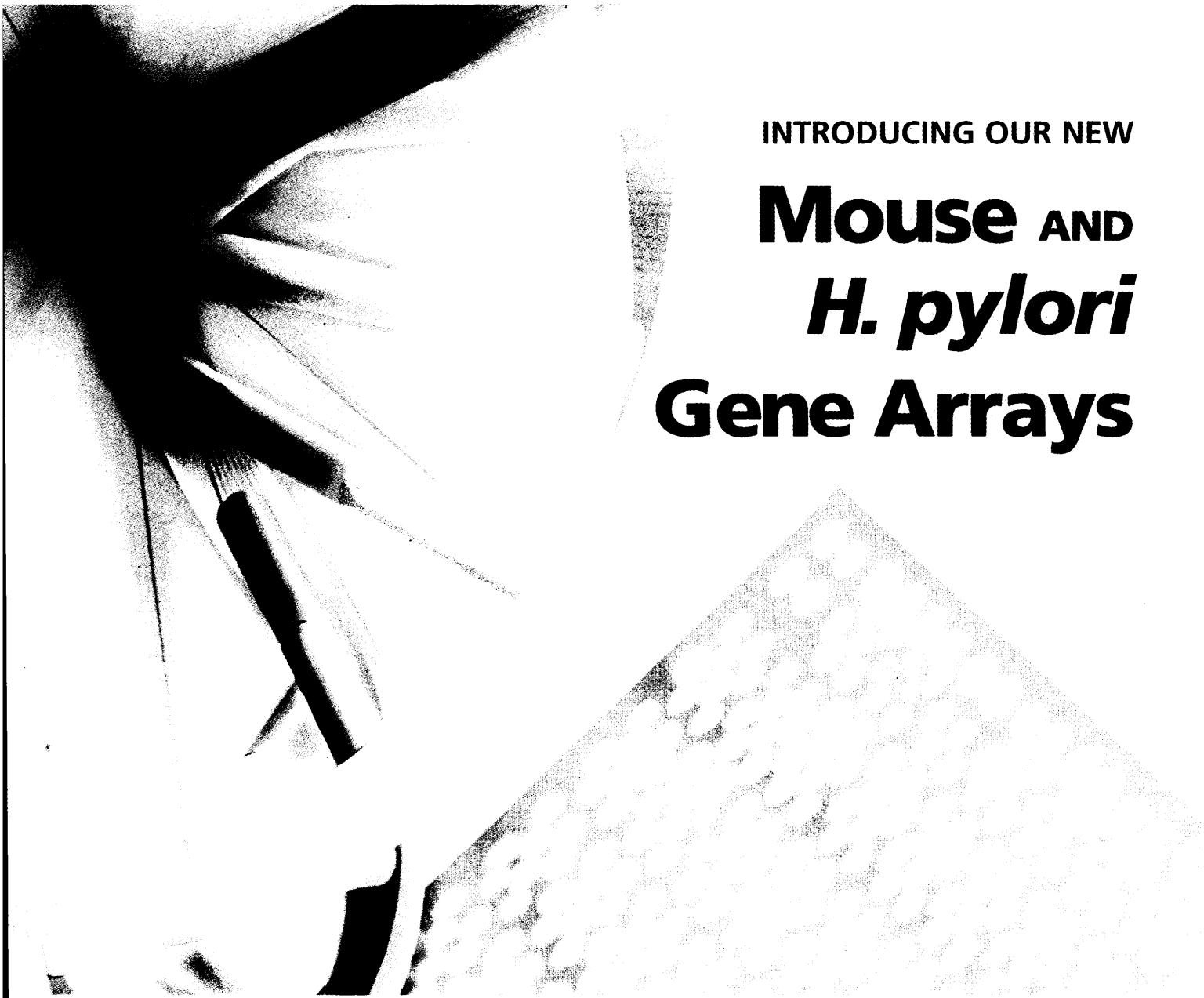

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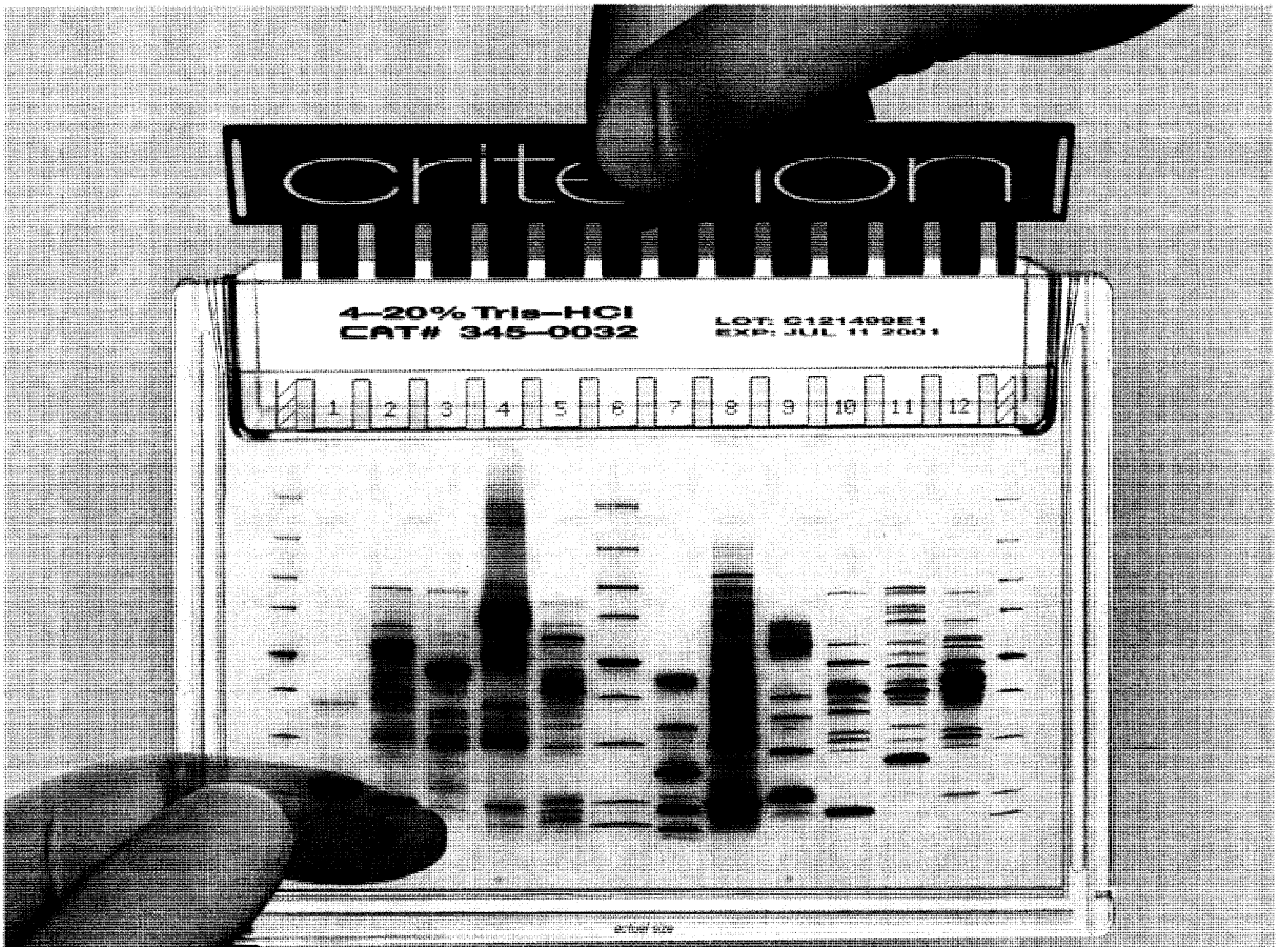

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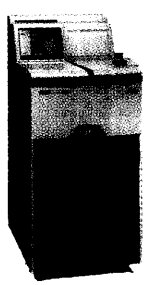

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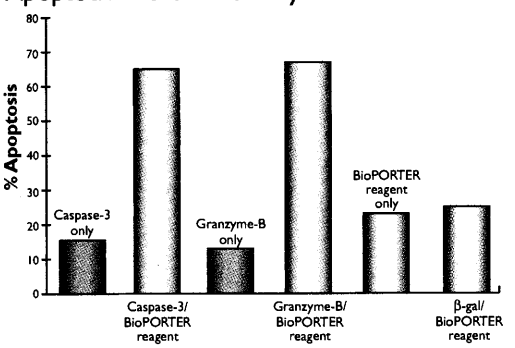

Granzyme B (450 ng), Caspase-3 (3.3 ng) or β-galactosidase (2 µg) were added to Ki-Ras 267 cells (prostate cancer) with or without BioPORTER reagent. 24 hours after protein delivery, cells were directly analyzed for apoptosis by flow cytomerty using an Annexin V-FITC assay kit.

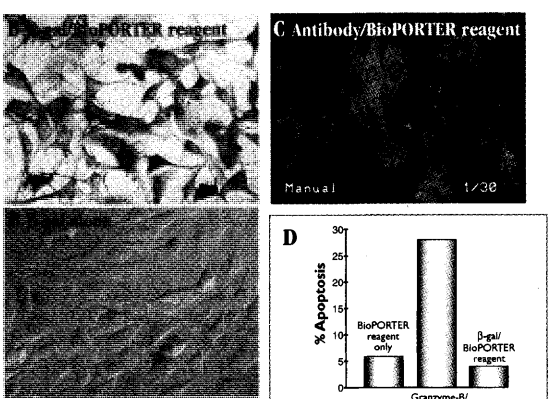

Fig. B/C FITC-Antibody or β-gal Delivery Into Mouse Fibroblasts
FITC-labeled antibody (2 µg) or β-gal (0.5 µg) were delivered with 2.5 µl of BioPORTER reagent into NIH/3T3 cells grown on coverslips in serum free conditions. Cells were examined 4 hours after protein delivery.

Fig. D Functional Granzyme-B Delivery Into Jurkat Cells
Granzyme-B (450 ng) or β-gal (1 µg) were delivered into cells growing in serum-free medium. 24 hours after protein delivery, cells were directly analyzed for apoptosis by flow cytomerty using Annexin V-FITC.

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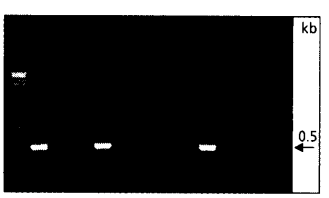
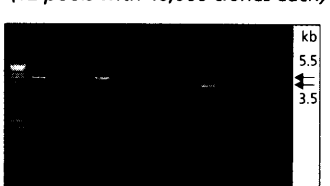


Master-Plate PCR screen using a pair of gene - specific primers to identify the positive pools *(12 pools with 40,000 clones each)*



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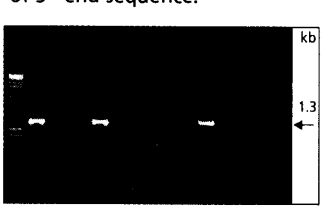
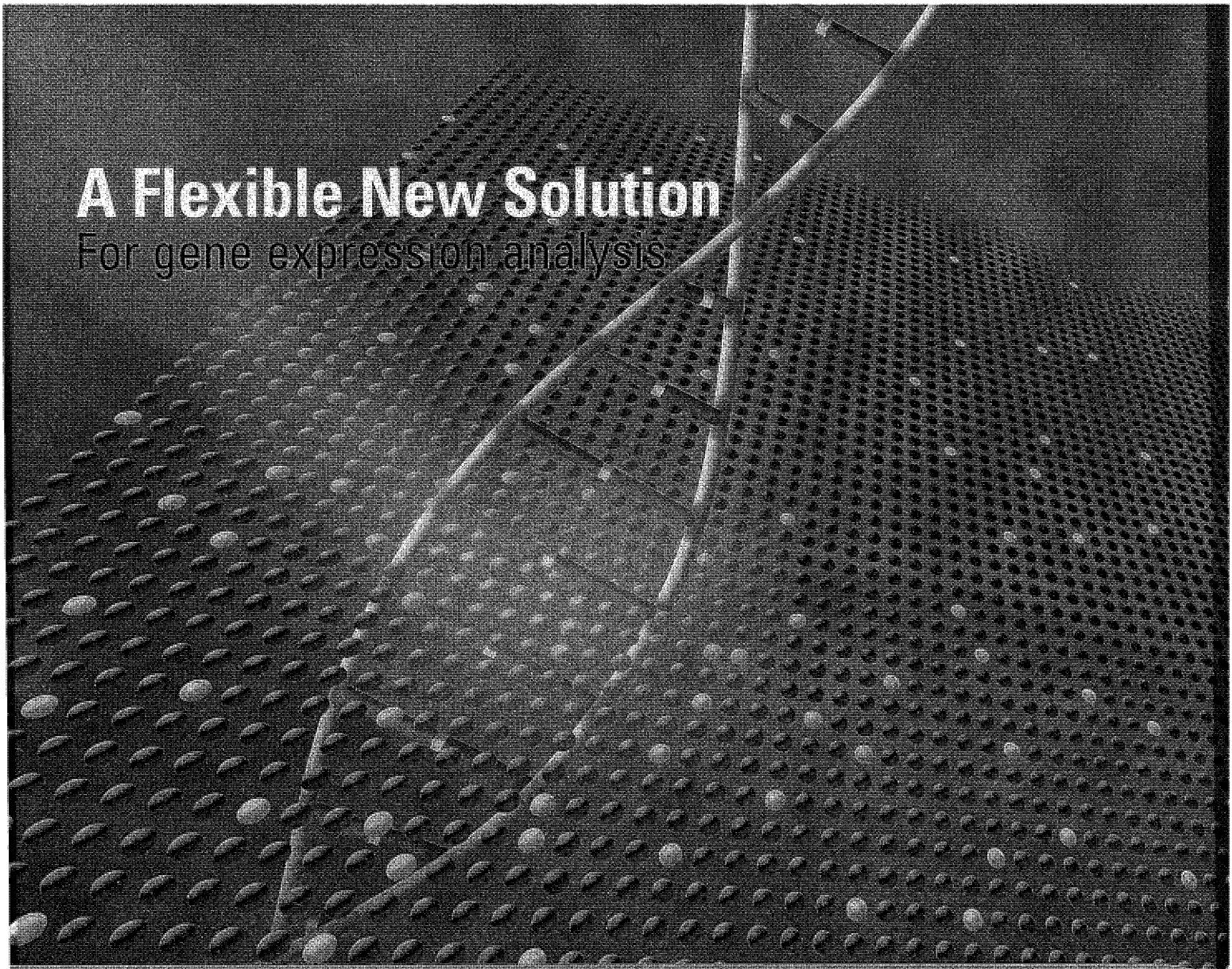


Alternate screen using the 3' vector-derived primer and a gene-specific forward primer to determine extent of 3' sequence



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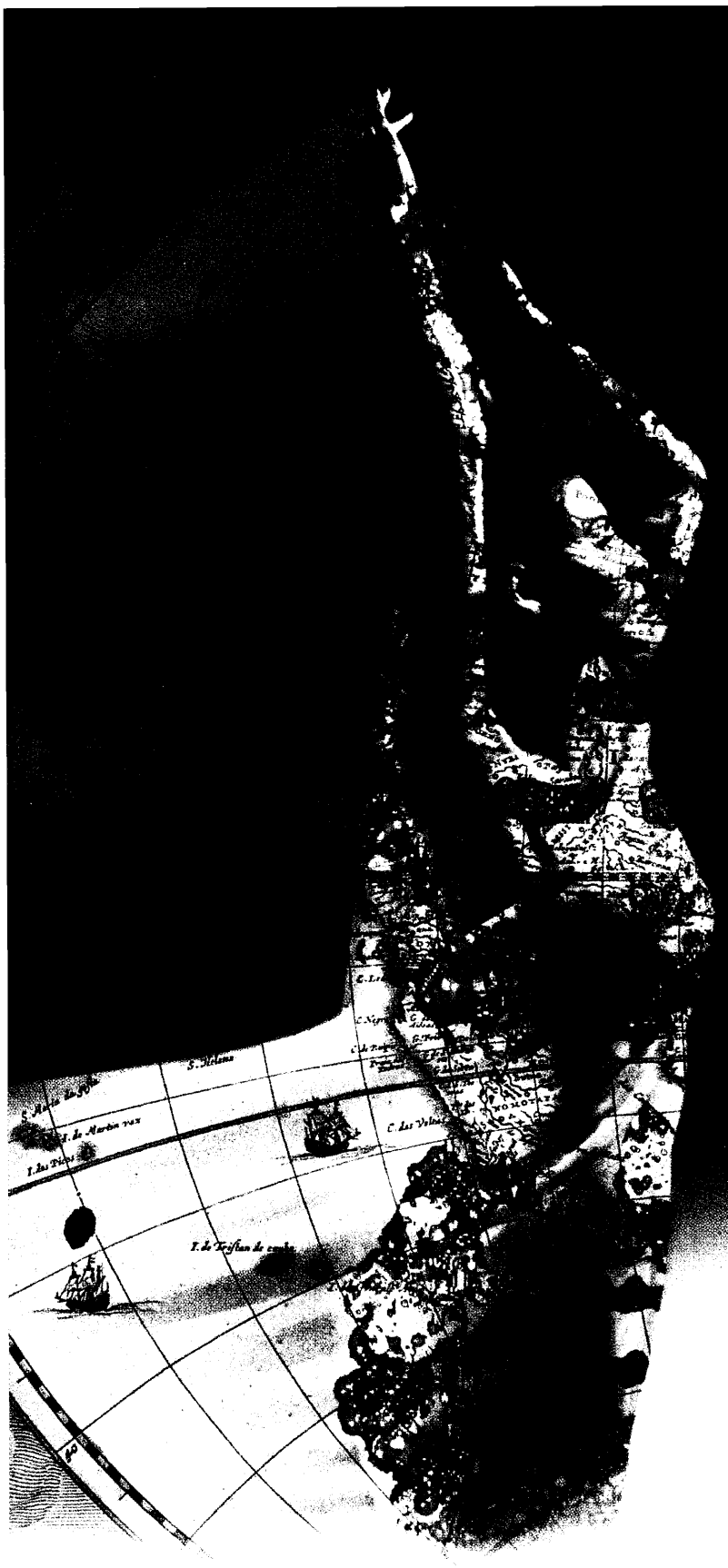

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R Sfi I	GGCCN4/NGGCC	100-60%	R0123
Sbf I	CCTGCA/GG	75%	V0101
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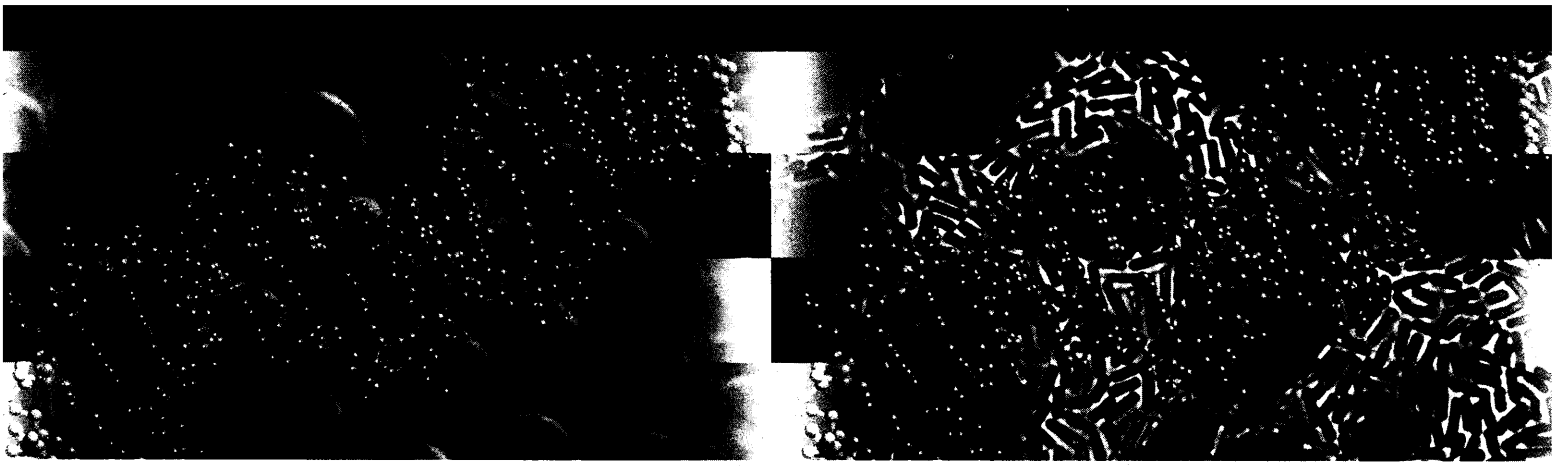

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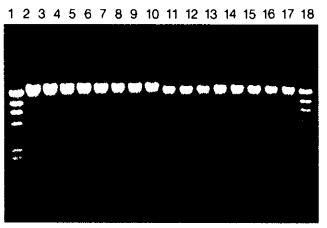
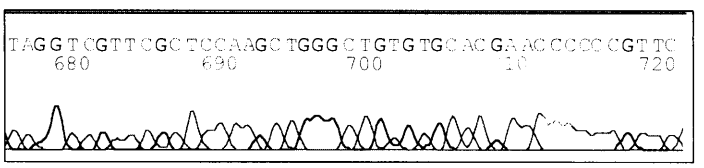


● Yields obtained using the Eppendorf Perfect gDNA Blood Kit and from a competing product. 200 µl fresh human whole blood, 5 µl eluate volume applied.
Lanes 1 and 18: λ *Hind* III Marker
Lanes 2 to 9: Eppendorf gDNA Blood Kit
Lanes 10 to 17: Competing kit

Perfectprep Plasmid 96 Vac Direct Bind



● Part of an ABI Model 3700 automated fluorescent DNA cycle sequencing of pUC19 prepared by the Perfectprep Plasmid 96 Vac, Direct Bind method. Phred Q > 20 = 701.

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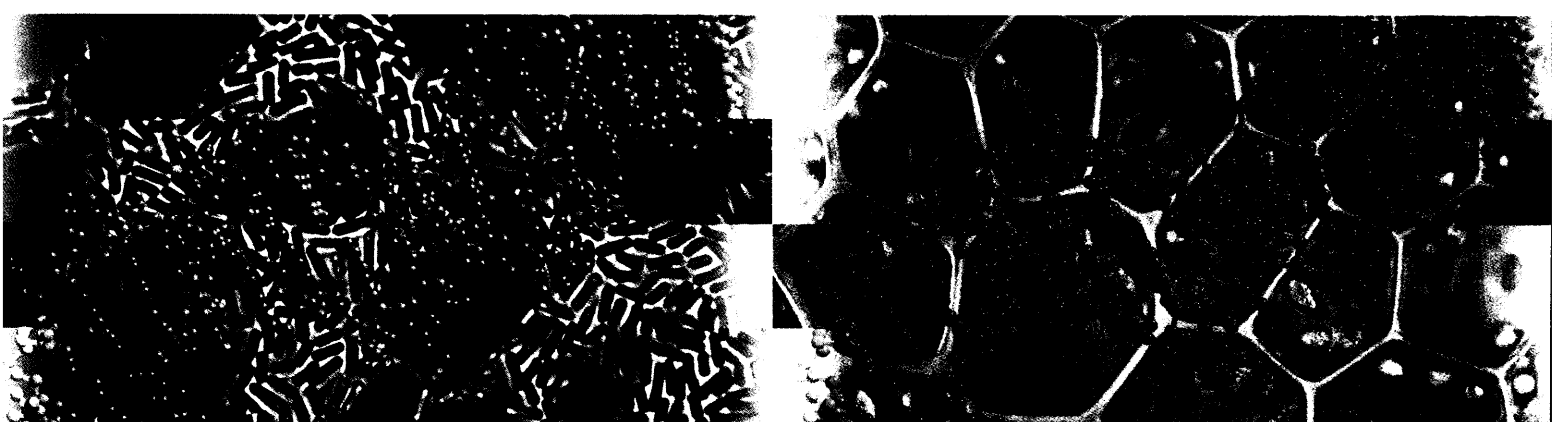

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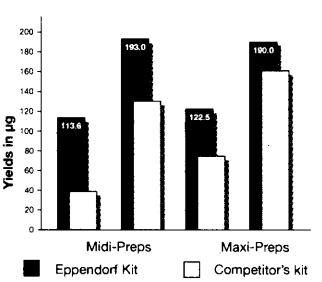

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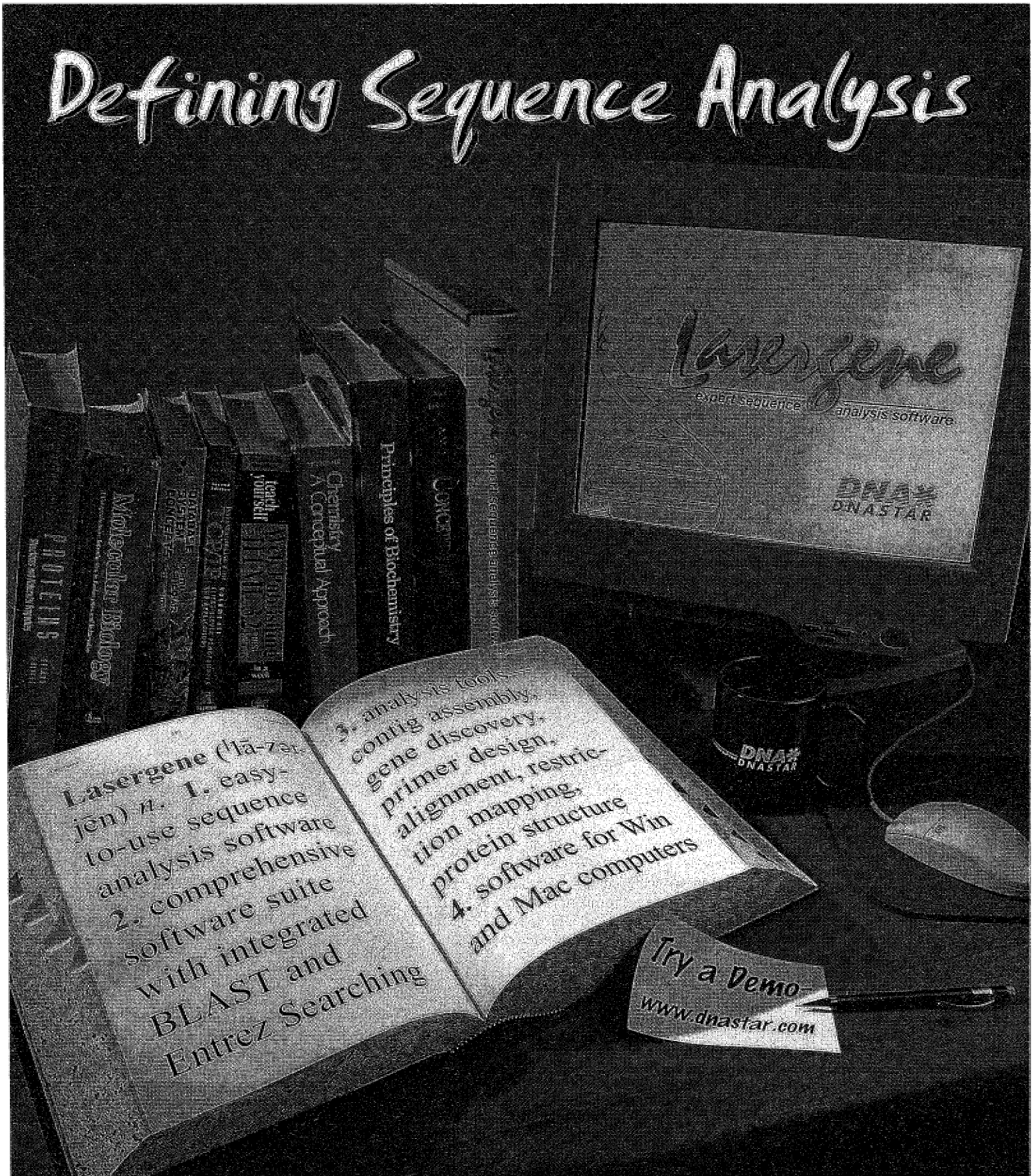

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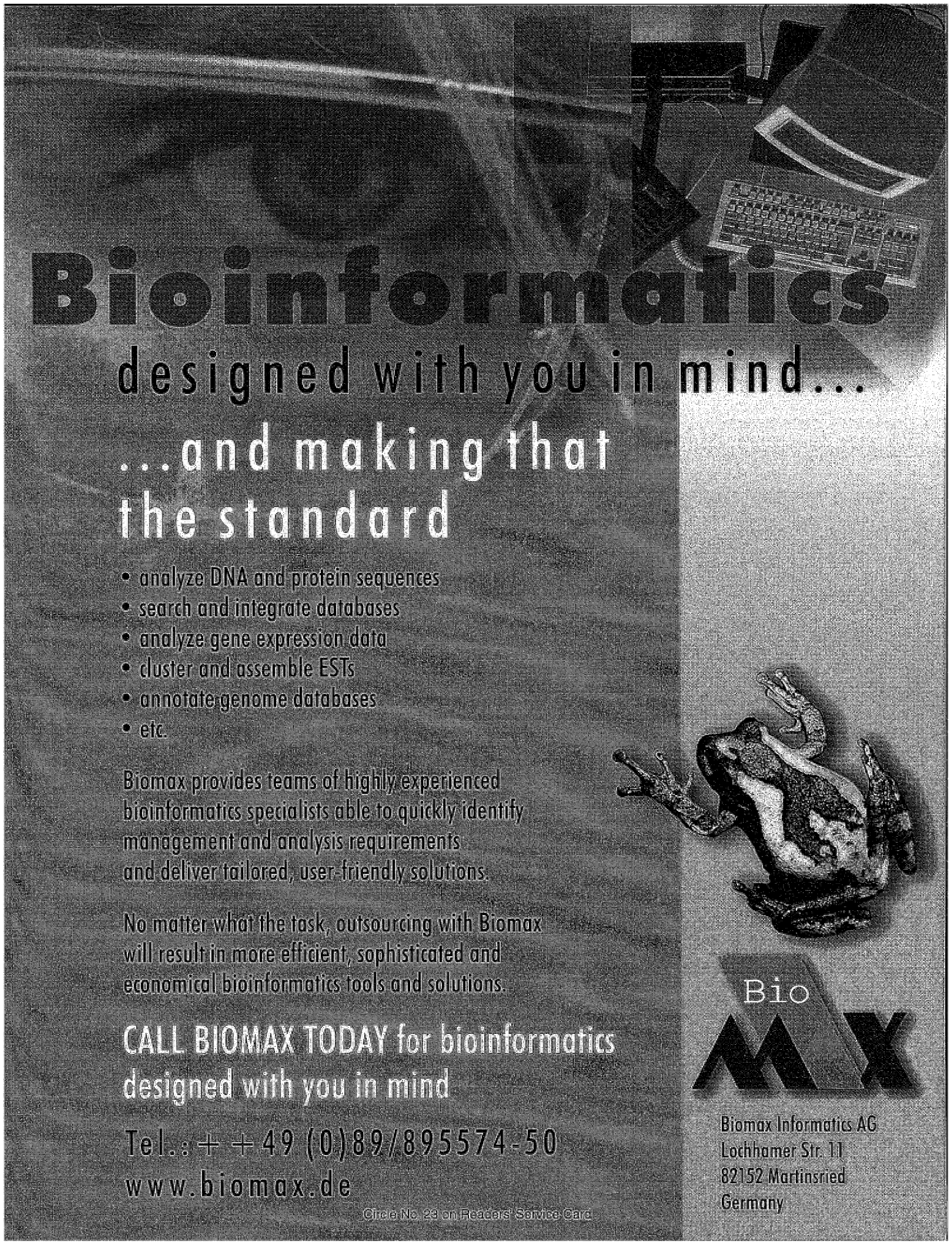
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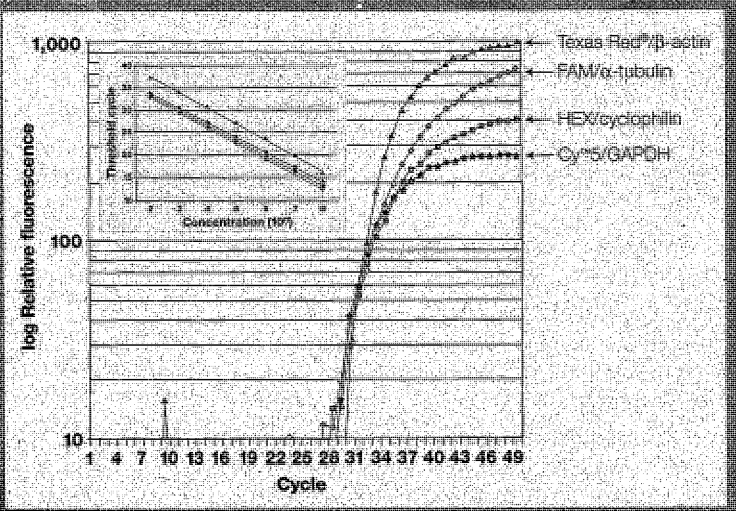

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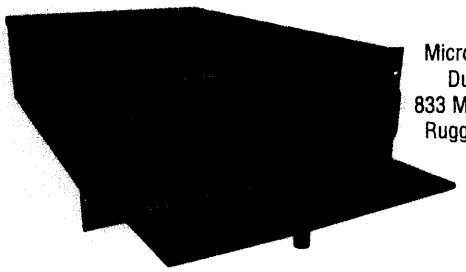

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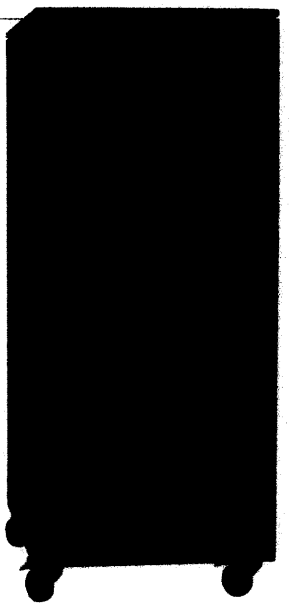

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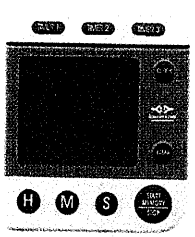

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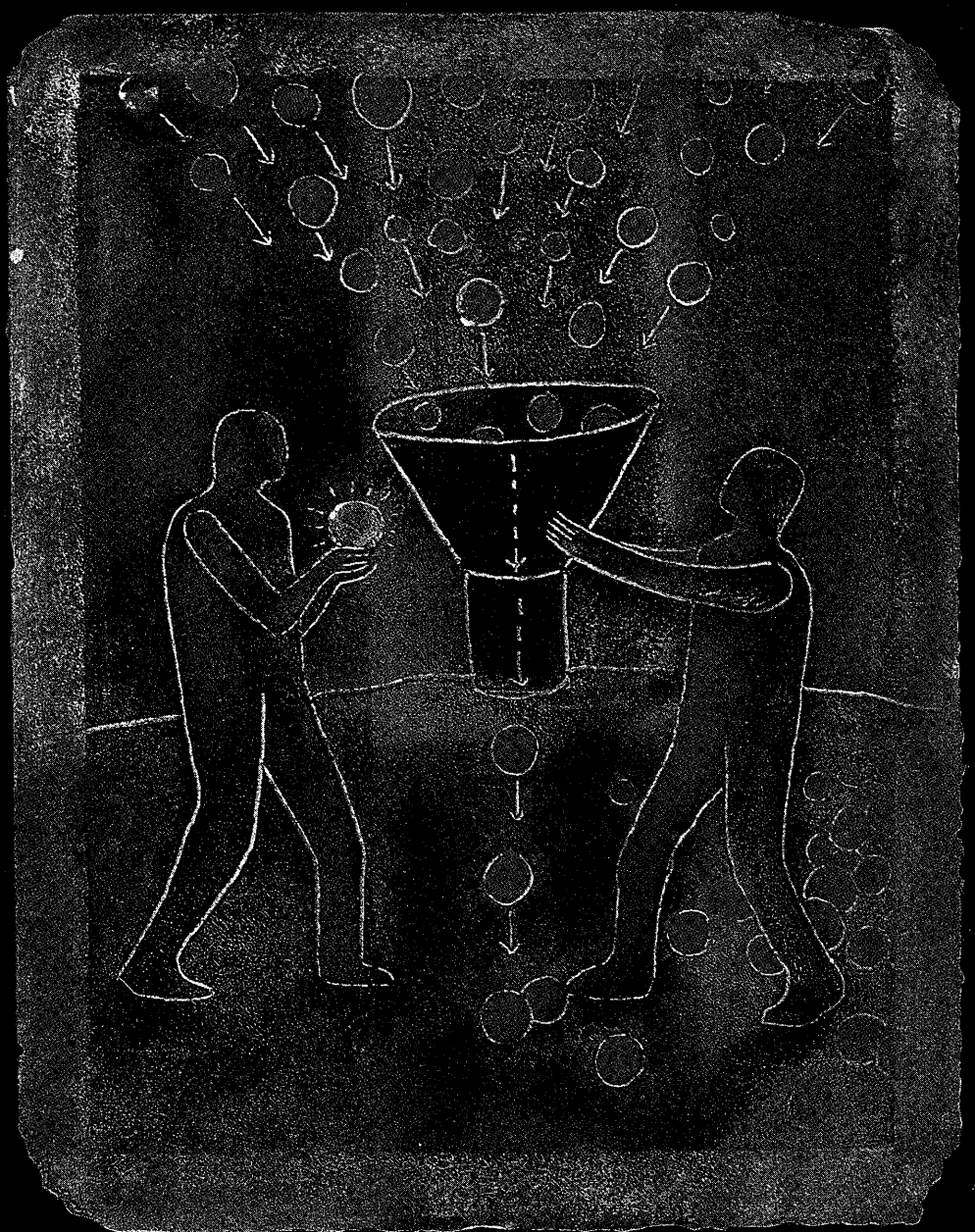

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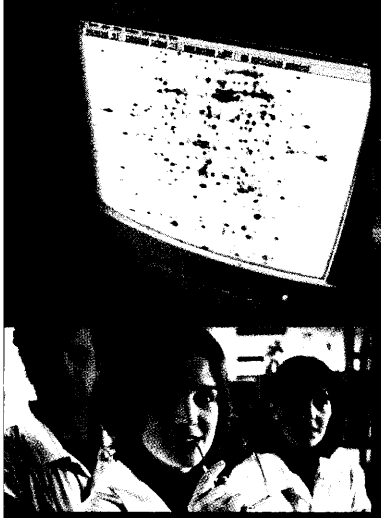

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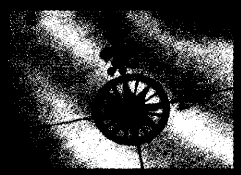

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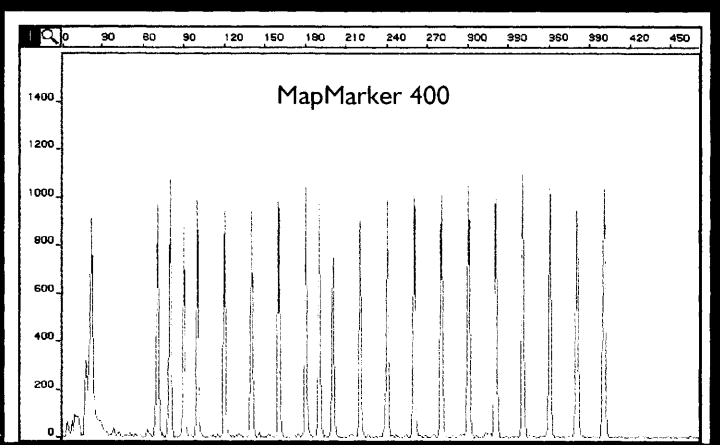

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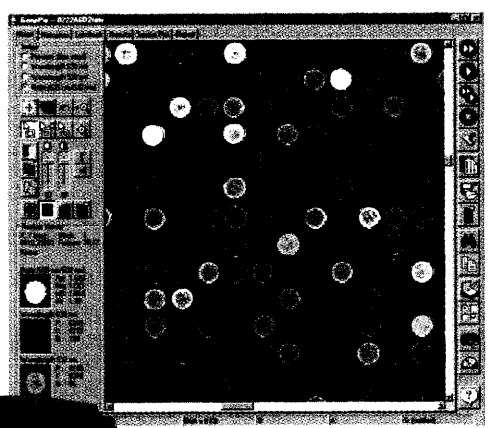

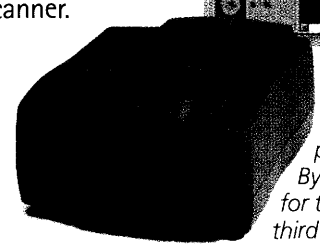

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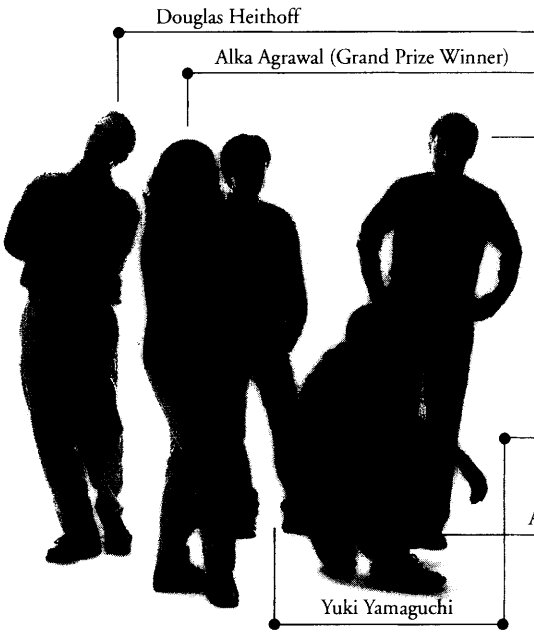

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* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell" (McGraw-Hill Dictionary of Scientific and Technical Terms, 4th Edition).

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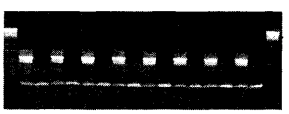

There is no cross-contamination between samples in the KingFisher genomic DNA purification process. Alternate wells in the KingFisher plate were loaded with blood and the others loaded with water, PCR-product was only amplified from the blood samples.

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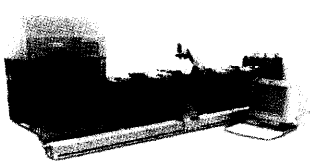

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[1] Living with Heart Failure. American Heart Association; 1999. http://www.americanheart.org

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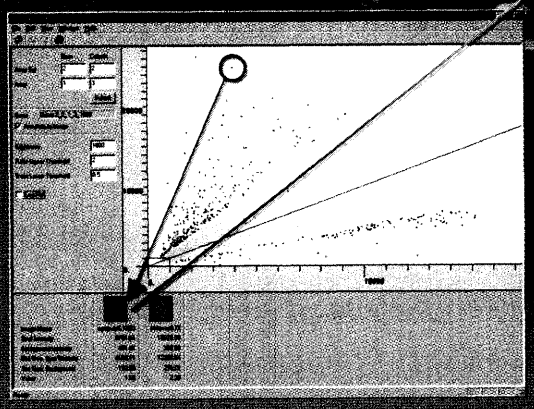

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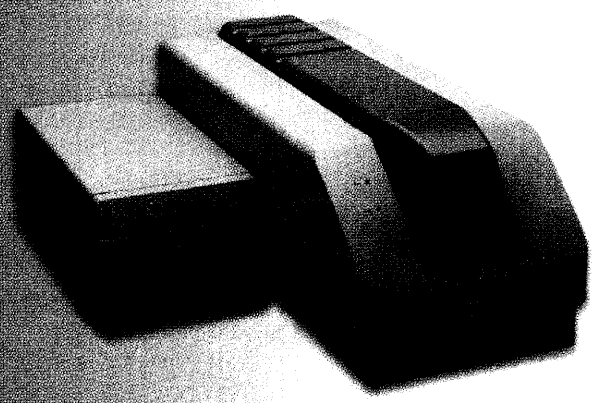

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CALL FOR APPLICATIONS FOR AWARD YEAR 2002

LONG-TERM FELLOWSHIPS

Long-Term Fellowships provide 3 years of support for postdoctoral research abroad in a laboratory of the fellow's choice. The final year of the Long-Term Fellowship may be used either in the host laboratory or in the fellow's home country. Third year funding in the home country can be postponed for up to two years. Fellows must be either nationals of one of the member countries, or applying to train in a member country.*

Applicants for the Long Term Fellowship program are expected to explore a **new area of research** since frontier life science research in the 21st century will require investigators able to span more than one scientific research field. Scientists trained in other disciplines such as chemistry, physics, mathematics, computer science, and engineering are encouraged to apply for training in life sciences.

Deadline for Long-Term Fellowship Applications: 3 SEPTEMBER 2001

(awards to be announced in April 2002)

SHORT-TERM FELLOWSHIPS

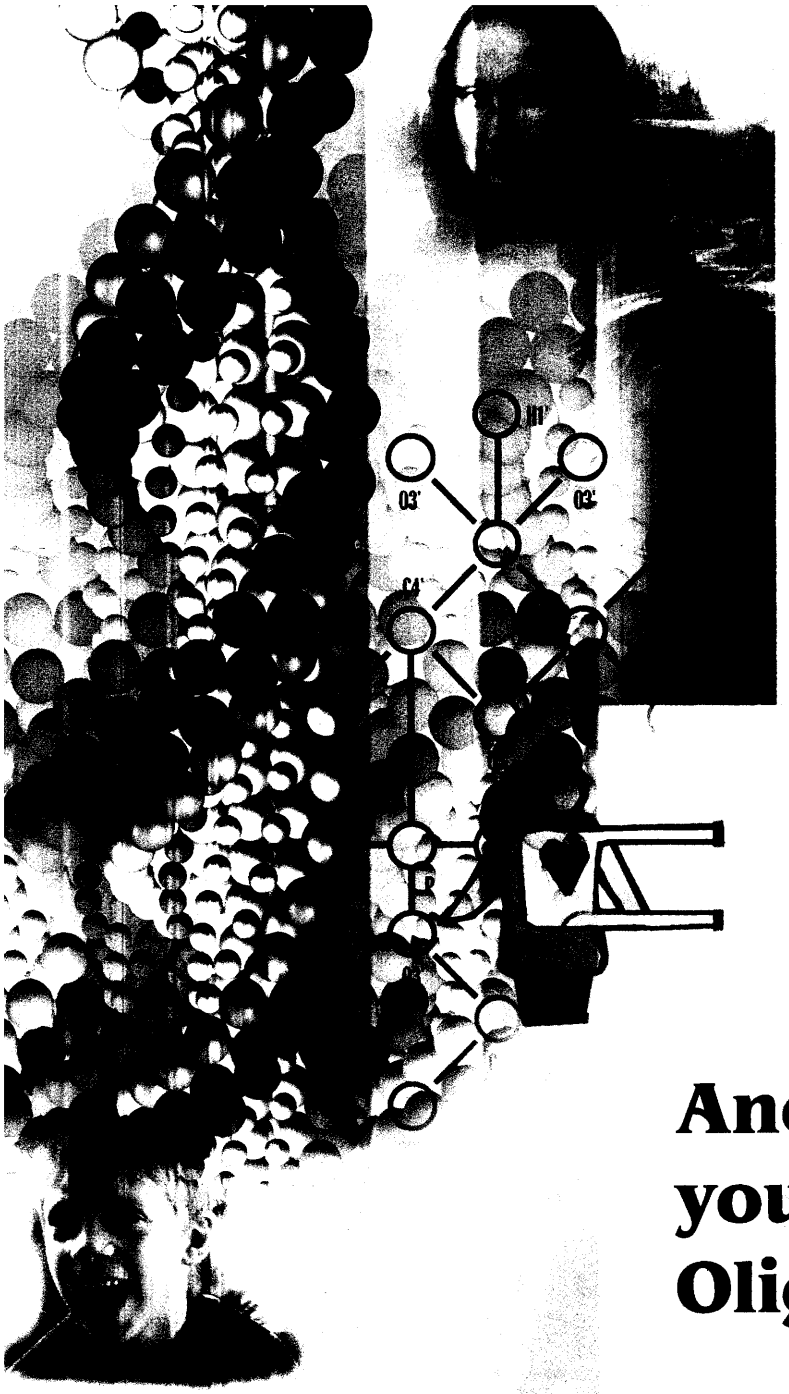
Provide up to three months of support to learn new techniques or establish new collaborations in another country. Applications are accepted throughout the year.

RESEARCH GRANTS

In 2001, the HFSP established a new procedure for the grant program requiring a letter of intent in March of each year and submission of complete applications **by invitation only** in mid-September. The next call for letters of intent for Award Year 2003 will appear in December 2001. Two grant schemes are supported. **Young Investigators' Grants** are for teams of scientists who are all within 5 years of establishing an independent laboratory. Successful teams will receive $250,000 per year for the entire team. **Program Grants** are awarded to independent scientists at all stages of their careers, and provide up to $500,000 per year for the entire team.

*Current member countries include Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Italy, Japan, Luxembourg, the Netherlands, Portugal, the Republic of Ireland, Spain, Sweden, Switzerland, the United Kingdom and the United States.

Guidelines and application forms are available on the HFSP web site (**www.hfsp.org**)



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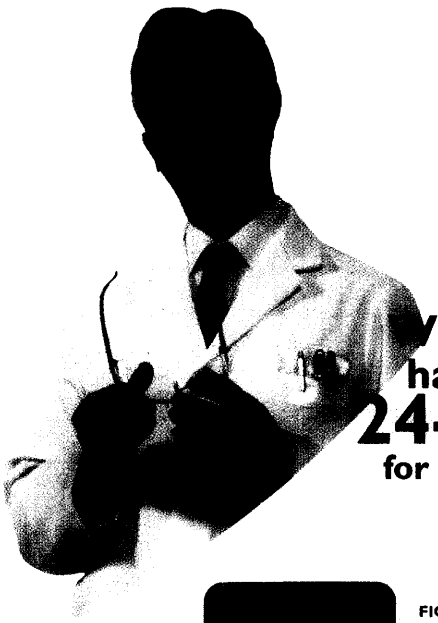

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- delivery of inhibitory peptides
- organelle labeling
- protein half-life studies
- transient complementation studies

FIG. 1 Cells are not fixed

Actin filament labeling of human fibroblast (HS68) cells using anti-actin antibody delivered by CHARIOT. (65% transfection efficiency)

FIG. 2 Cells are fixed

FIG. 3 CHARIOT delivery of a 10 kDa protein, labeled with lucifer yellow at the C-terminus, to the nucleus of human fibrobiast (HS68) cells. Cells are not fixed. (70% transfection efficiency after 30 min. incubation)

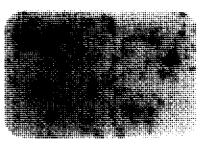

FIG. 4 CHARIOT delivery of a 119 kDa β-galactosidase protein into COS cells. Cells are fixed and stained 2 hours post-transfection. (60% transfection efficiency)

CHARIOT Advantages

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