
15 June 2001
Science
Vol. 292 No. 5524
Pages 1953–2204 $9
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Vitality™ hrGFP

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New data show that EGFP alters the expression profile of cells!

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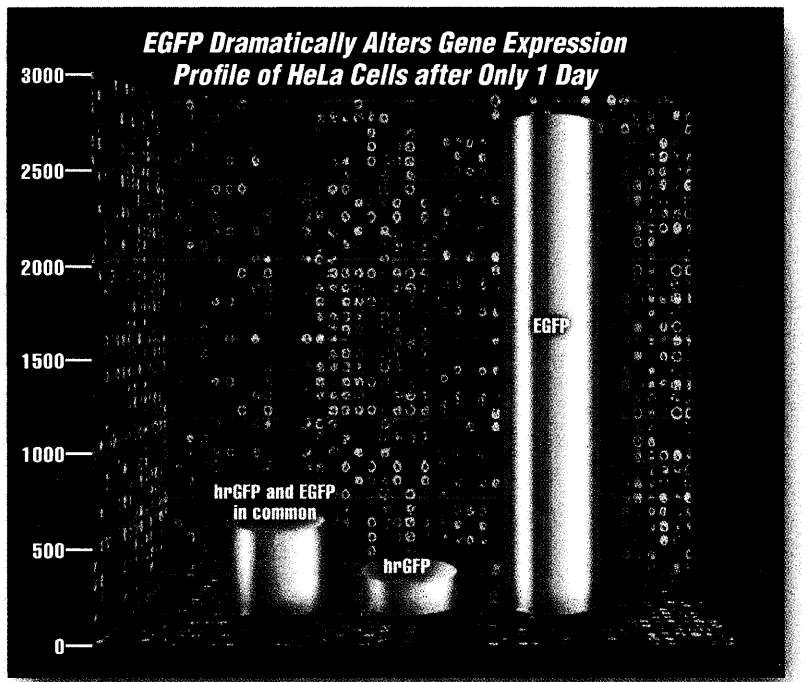
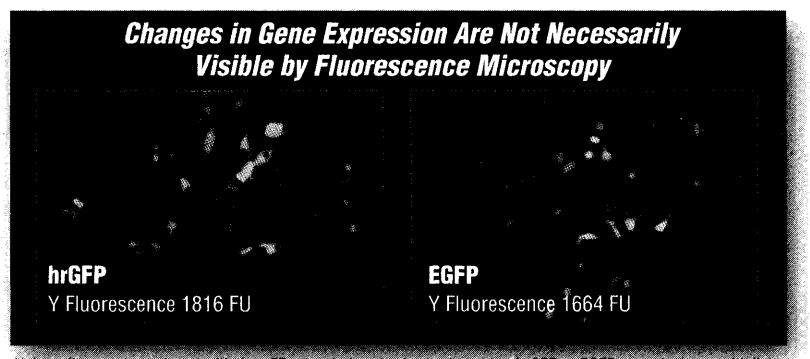
of unstable expression from toxic GFPs. Stratagene created this new high-level fluorescence, low-toxicity GFP by cloning GFP from a new organism, *Renilla reniformis*. The low toxicity enables you to study expression of your gene without altering the normal expression profile of the host.

To demonstrate the dramatic difference in performance, we used microarray analysis and fluorescence microscopy to compare the gene expression profile of HeLa cells expressing either hrGFP or EGFP. The microarray results reveal the underlying changes in gene expression caused by EGFP, even though these changes are not visible by fluorescence microscopy.



Data sets represent the number of genes either up- or down-regulated ≥ 2-fold from normal expression due to the presence of Vitality™ hrGFP or EGFP. The data was generated by probing microarray slides containing 10,800 human genes with RNA from three populations of HeLa cells; hrGFP-expressing cells, EGFP-expressing cells and an untransduced control population. The RNA was harvested 1 day post-transduction.



HeLa cells were transduced with the pFB retroviral vector harboring either hrGFP or EGFP at equivalent multiplicity. Mean (Y) fluorescence is an indirect indicator of relative expression levels and indicates the GFP expression levels were comparable. Photographs were taken using fluorescence microscopy 3 days post-transduction.

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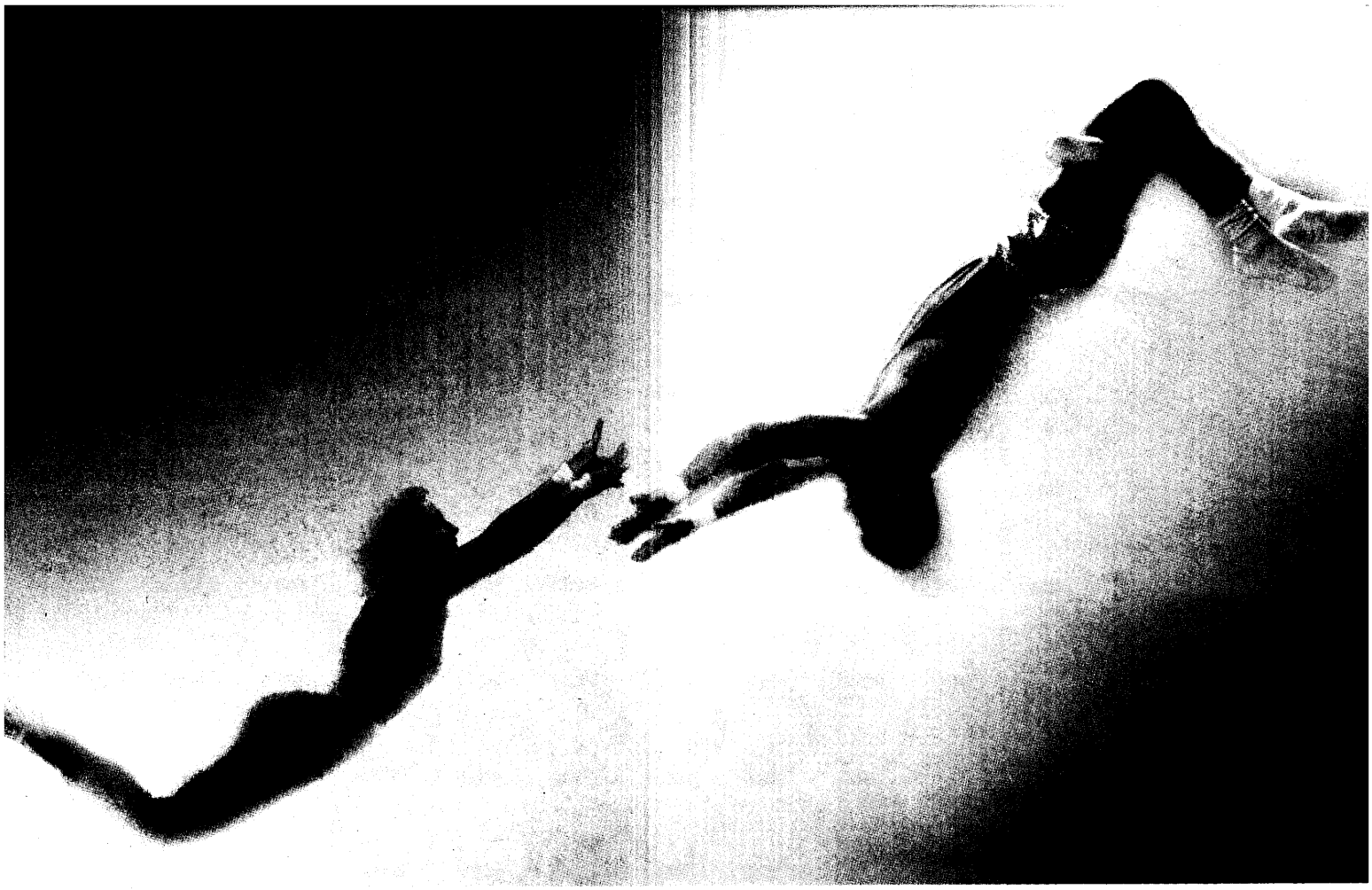

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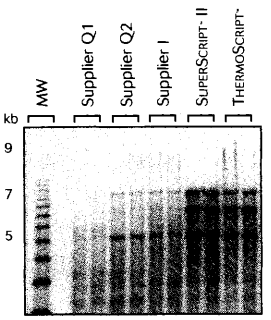

Comparison of yields of cDNA synthesis using various reverse transcriptases.

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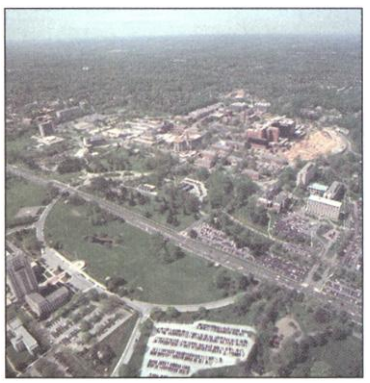
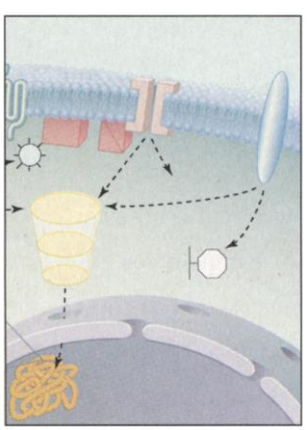
Volume 292 15 June 2001 Number 5524

NEWS

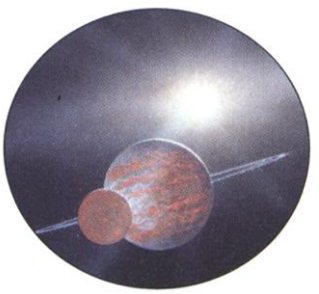


1988
NIH: Beyond doubling

SCIENCE'S COMPASS



2022
Multitalented plant G proteins

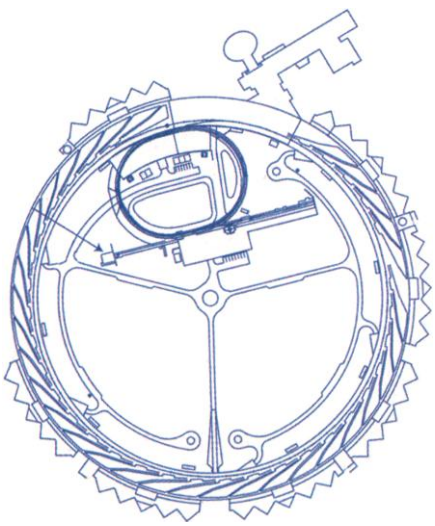


2016
Earth and Moon: the early years

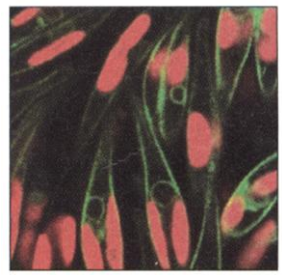
RESEARCH

RESEARCH ARTICLE

REPORTS

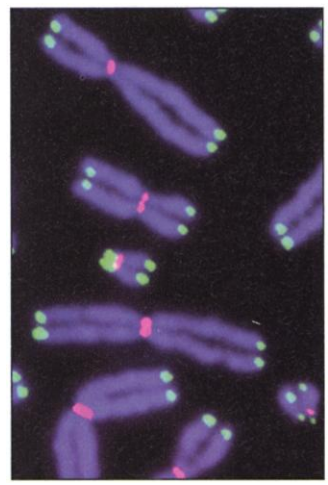


2037
Ultraviolet free-electron laser



COVER 2073

In algal cells, the chloroplast (red fluorescence) produces photosynthetically derived energy for cell growth. Through the introduction of a glucose transporter (green fluorescence), algal cells are able to import and then metabolize glucose for energy. These cells can now thrive without light, permitting culture by efficient fermentation technology. [Image: D. Ehrhardt]



2075
All quiet near human telomeres

New on *Science* Express

A fruit fly model of tauopathy

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Deterministic Delivery of a Single Atom S. Kuhr *et al.*

The ability to trap and manipulate single atoms over macroscopic distances is demonstrated using a combination of magneto-optical and optical atom traps.

Loss of Huntingtin-Mediated BDNF Gene Transcription in Huntington's Disease C. Zuccato *et al.*

Huntingtin boosts transcription of BDNF, a survival factor for striatal neurons in the brain, which die in Huntington's disease.

Tauopathy in *Drosophila*: Neurodegeneration Without Neurofibrillary Tangles ▼C. W. Wittmann *et al.*

1983 A fruit fly model may offer new insights into a group of human neurodegenerative diseases called "tauopathies."

The Human Nuclear Xenobiotic Receptor PXR: Structural Determinants of Directed Promiscuity R. E. Watkins *et al.*

The crystal structure of PXR provides insights into how the receptor recognizes various drugs and xenobiotics, and may offer avenues for predicting and preventing dangerous drug-drug interactions in humans.

TECHNICAL COMMENTS

Questions Regarding Precambrian Sulfur Isotope Fractionation

Farquhar *et al.* (Reports, 4 August 2000, p. 756), examining isotopic ratios in rocks spanning much of the Precambrian, presented data to show that mass-independent gas-phase processes controlled sulfur isotope fractionation before about 2.4 billion years ago, whereas mass-dependent processes dominated later. Ohmoto *et al.*, in a comment, suggest that the patterns observed by Farquhar *et al.* "may have been created by analytical rather than natural processes"—in particular, by processes related to "the presence of impurity gases (C-F-S-O-H compounds)" that can cause measured sulfur isotope values to "differ considerably from the true values" and thus, "create an apparent mass-independent fractionation." Farquhar *et al.* respond by presenting data from analyses using both the technique employed in the original study and an independent technique. The combined results, they assert, "attest to the robustness and accuracy of the isotopic data" reported in the original study.

The full text of these comments can be seen at www.sciencemag.org/cgi/content/full/1959a

science's stke www.stke.org

Book Review: Manipulating Programmed Cell Death for Better Living! S. C. Manolagas

A review and commentary on the book *Apoptosis in Health and Disease*.

science's next wave www.nextwave.org

Germany: Career Services in Germany—Supporting Students' Needs A. Eimer

With activities such as lectures, excursions, and counseling, career services organizations at German universities have a fundamental and increasingly important goal: supporting students on their way from the university to the working world.

UK: Personal Development for Ph.D.'s J. Wood

The winner of our recent "Hindsight" competition tells us about his prize—5 days in a Research Councils' Graduate Schools Programme course on personal and career development.

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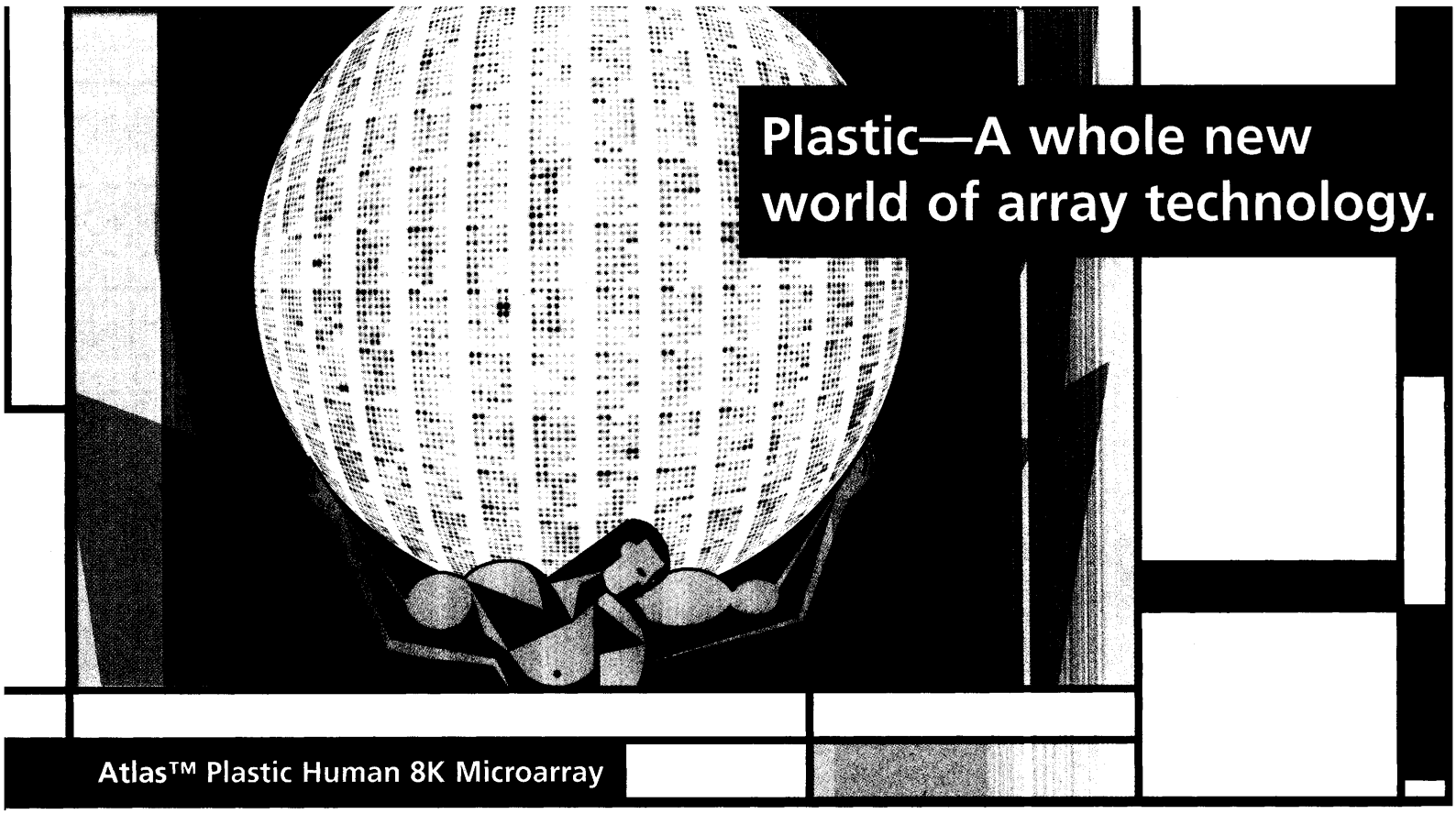
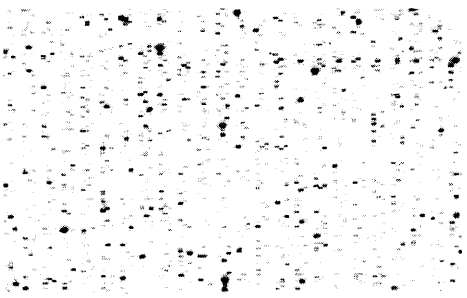


Illustration inspired by the art of Juan Gris (1887–1929) & Piet Mondrian (1872–1944).



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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THIS WEEK IN Science

edited by Phil Szuromi

Trailing Blazars

The high-energy sources of emission creating the extreme brightness of quasars are thought to be supermassive black holes with an accretion disk and two polar jets. About 10% of quasars show high emissions at radio wavelengths, and a fraction of these radio-loud quasars display large outbursts of radiation. The outbursts of these "blazars" have been attributed to relativistic beaming from the jets. A unified scheme suggests that all radio-loud quasars have relativistic jetting—blazars are different only because we are viewing them down the axis of the jet. Ma and Wills (p. 2050; see the news story by Schilling) found indirect evidence of blazars, independent of the viewing geometry, in a survey of radio-loud quasars. They conclude that all radio-loud quasars are produced by similar physical processes.

2037 Saturation of Ultraviolet Free-Electron Lasers

Free-electron lasers (FELs) combine the ability to tune the wavelength of emitted radiation with the potential to achieve an x-ray brightness that could be 10 orders of magnitude higher than that of existing synchrotrons. In a FEL, an electron beam is passed through the undulator, a region of modulated magnetic field, and radiation is emitted that interacts with the electron beam. Saturation of this interaction, a key issue in the development of FELs, has so far been limited to millimeter wavelengths. Milton *et al.* (p. 2037) demonstrate saturation for shorter visible and ultraviolet wavelengths, a critical step on the road to even shorter x-ray wavelengths. ✗

Linearly Polarized Light from Out-of-Shape Particles

Spherical semiconductor quantum dots can emit light at optical wavelengths when charge carriers in the dot recombine, and by changing the size of the particle, the emission strength and wavelength can be tuned. This emission is plane polarized, but it would be even more useful in applications such as displays if the emission were linearly polarized. Hu *et al.* (p. 2060) now show experimentally that elongating CdSe dots even to only an aspect ratio of 2 results in linearly polarized emission. ✗

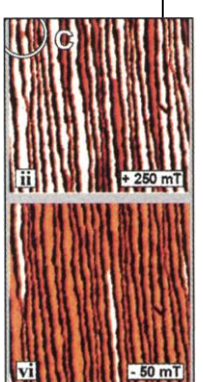
All Astir

Some achiral molecules can pack so that they form optically active crystals. Under quiescent conditions, equal populations of left- and right-handed crystals usually form, but in some cases, such as crystallization of sodium chlorate, stirring can cause an entire batch to form crystals all of one handedness (but with a equal chance of the batch being rights or lefts). Ribó *et al.* (p. 2063; see the Perspective by Feringa) studied the evaporative aggregation of achiral diprotonated porphyrin molecules, which form lyotropic liquid crystals at high concentrations. The aggregates formed are chiral, and the chirality depended on the direction of vortex motion of stirring during evaporation, apparently through the asymmetric influence of vortex motion on the specific binding sites between porphyrins.

From Receptor to Nucleus

The binding of extracellular ligands to their receptors on the cell membrane can evoke a variety of responses and, in some cases, these responses include a change in gene expression and transcription, which takes place in the nucleus. Santagata *et al.* (p. 2041; see the Perspective by Cantley) describe a new pathway in which occupancy of a plasma-membrane receptor results in mobilization of a transcription factor to the nucleus. In its inactive state, the tubby protein, which has been connected to adult-onset obesity in mice, binds to the phosphorylated head group of a plasma-membrane lipid. When the G protein–coupled serotonin receptor is activated, phospholipase C hydrolyzes the lipid and releases tubby, which then enters the nucleus, probably via its nuclear localization sequence. An intriguing suggestion is that this result may explain why serotonin-receptor knockout mice display symptoms of adult-onset obesity. ✗

Nanometer-Scale Imaging in a Magnetic Field



As magnetic devices continue to shrink in size toward to the superparamagnetic limit, there is a need to understand the basic physical behavior of these systems on the nanometer scale in the presence of a magnetic field. Most imaging techniques developed so far provide either high spatial resolution or compatibility with magnetic fields, but not both. Pietzsch *et al.* (p. 2053) report that spin-polarized scanning tunneling microscopy can be used to image magnetic switching and hysteresis in an array of iron nanowires.

Algae Aquaculture in the Dark

Diatoms and other sorts of algae, while unwelcome during a summer swim in a pond, are important sources of food and useful phytochemicals. However, for obligate photoautotrophic algae, their success in aquaculture is limited by the amount of light that can be reliably delivered. Zaslavskaia *et al.* (p. 2073; see the cover and the Perspective by Stephanopoulos and Kelleher) show that transforming a diatom with a gene encoding a human glucose transporter converts them into heterotrophs that can grow without light so long as they are supplied with glucose.

A Busy Gα Subunit

A typical animal genome contains several genes encoding G protein α subunits, but the *Arabidopsis* genome appears to encode just one. Two reports show that this one Gα is busy regulating physiological functions in plants (see the Perspective by Ellis and Miles). Wang *et al.* (p. 2070) found that Gα mediates abscisic acid regulation of stomatal pore size, thus affecting water conservation in the plant. Ullah *et al.* (p. 2066) show that the same protein is involved in regulation of cell proliferation.

CREDIT: PIETZSCH *ET AL.*

✗ Published online in *Science* Express

CONTINUED ON PAGE 1963

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Telomeres Silence in Human Cells

The reversible silencing of a gene near the end, or telomere, of a chromosome [the telomere position effect (TPE)] depends both on telomere length and the distance between telomere and the affected gene. This effect is well characterized in yeast. By seeding de novo telomere formation in human cells with DNA linked to a luminescent reporter gene, Baur *et al.* (p. 2075) show that reporters adjacent to the new telomeres produced 10 times less luminescence than those located at internal sites in the chromosomes. As in yeast, increasing the length of the telomeres further increased TPE. Because most human telomeres shorten with age, TPE might provide a mechanism for altering cell expression during the replicative life-span of the cell.

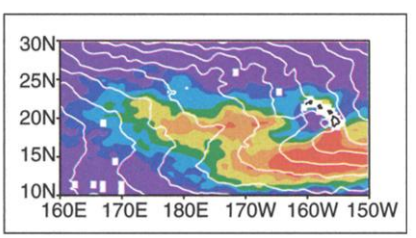
Gene Silencing and CpXpG Methylation

Methylation of DNA provides an important epigenetic "mark" involved in controlling gene expression and genomic stability and most often results in the silencing of affected genes. The most common mark is found at symmetrical CpG dinucleotides, the methyl group being added by cytosine methyltransferases of the Dnmt1/MET1 class. Although methylation is found at other non-CpG sites—CpXpG, for example—little has been known about the enzymes that make or maintain these marks. Lindroth *et al.* (p. 2077) have now performed a genetic screen in the plant *Arabidopsis* to isolate a gene involved in methylation of CpXpG, the cytosine methyltransferase homolog *CHROMOMETHYLASE3*. Mutations in the gene result in the activation of normally inactive retrotransposon sequences and demonstrate that CpXpG methylation is also involved in gene silencing.

Just-in-Time Delivery

The transcription of the genes required in the assembly of the bacterial flagellum appears to be carefully timed for maximal efficiency. To understand the organization of genes needed to build functioning flagella without interfering directly with the genetic pathway, Kalir *et al.* (p. 2080) measured promoter activity by means of a panel of fluorescently labeled reporter plasmids. A precise temporal program of expression was observed which suggested that timing might be regulated by the binding affinity of transcription factors to promoter regions

The Longest Wake

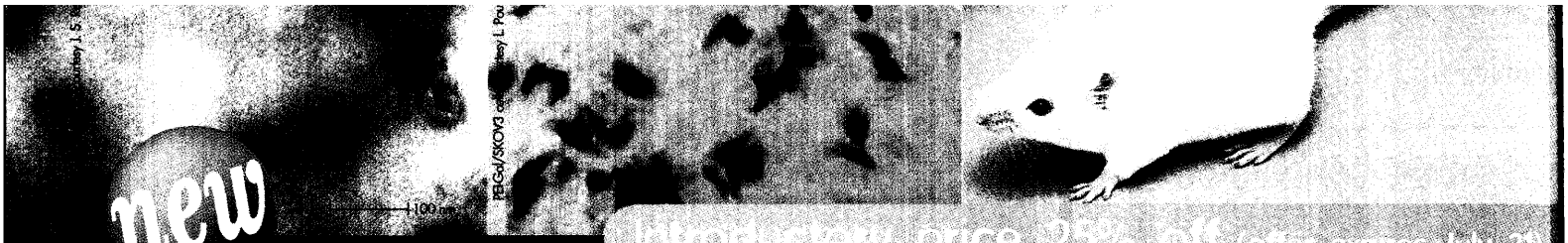


Islands disrupt prevailing winds to create ocean wakes, but these wakes generally extend no more than a few hundred kilometers. Xie *et al.* (p. 2057) document an exception to this norm: The wake downwind from the Hawaiian Islands. Satellite observations of this wake reveal that it persists for 3000 kilometers. These long structures are maintained by an interaction between the atmosphere and the ocean that creates a positive feedback involving sea surface temperature, clouds, and wind patterns. This wake is more than just a curiosity—it modifies local atmospheric and oceanic circulation and may influence atmospheric transport of aerosols and trace gases as well as the distribution of plankton and other fishery resources.

Vitamin C and Cancer

The use of high doses of antioxidants such as vitamin C to protect against cancer has been controversial. Advocates cite epidemiological studies that have revealed an association between high intake of vitamin C and reduced risk of cancer, as well as laboratory studies that demonstrate roles for vitamin C in free-radical scavenging and immune stimulation. Opponents cite the negative results of randomized clinical trials testing antioxidant therapy as well as laboratory evidence that vitamin C might also have a prooxidant effect. Lee *et al.* (p. 2083) now present in vitro data, which show that vitamin C can induce genotoxin formation (agents that damage DNA). If generated in significant amounts, these genotoxins, which are the products of vitamin C–enhanced decomposition of lipid hydroperoxides, could generate cancer-causing mutations.

CREDIT: XIE *ET AL.*



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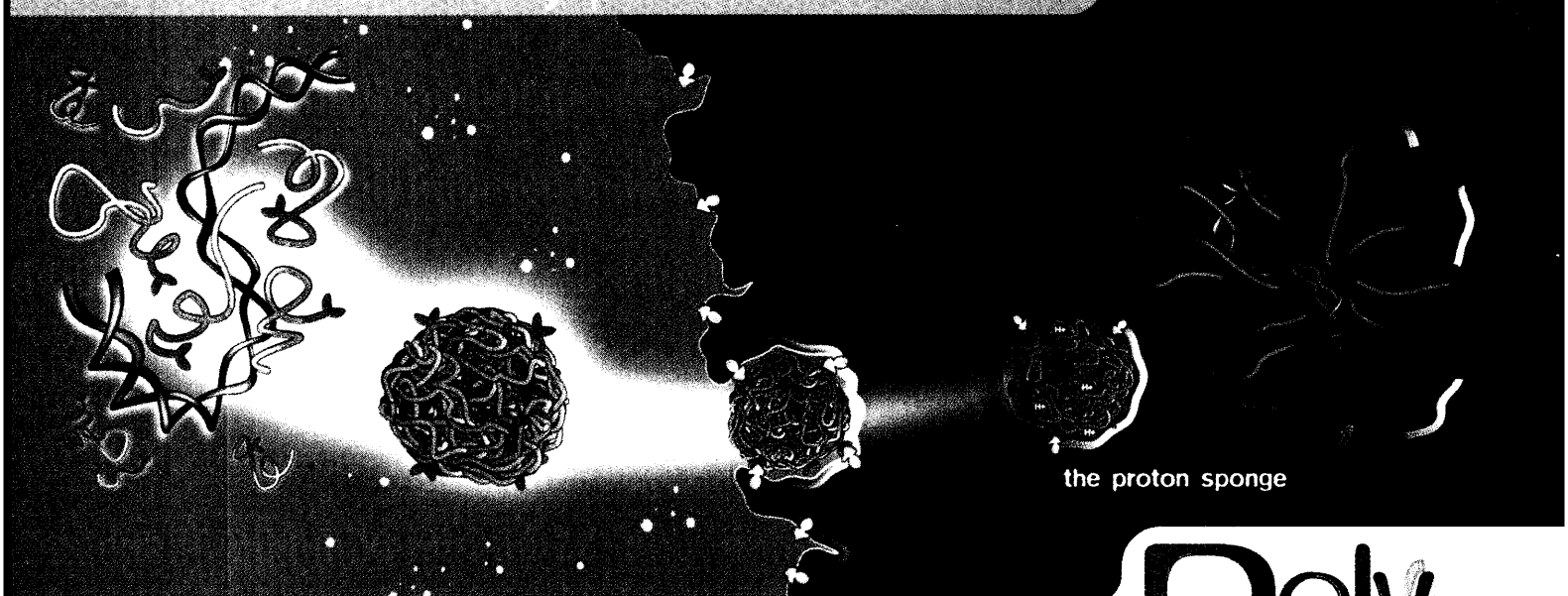

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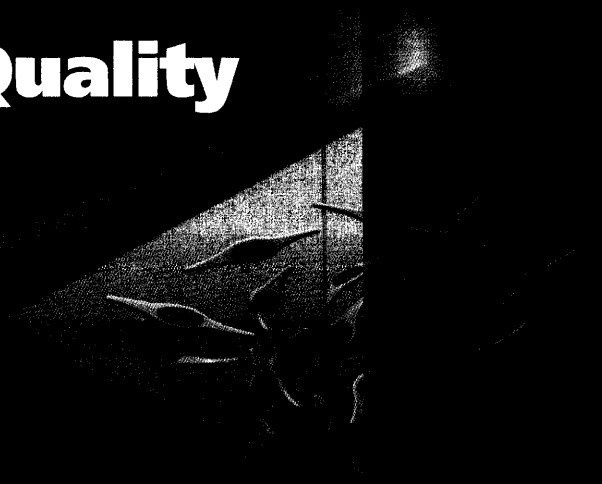
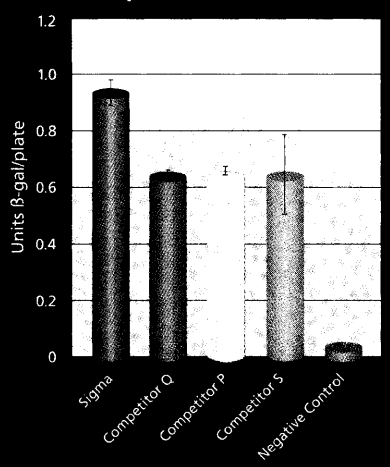
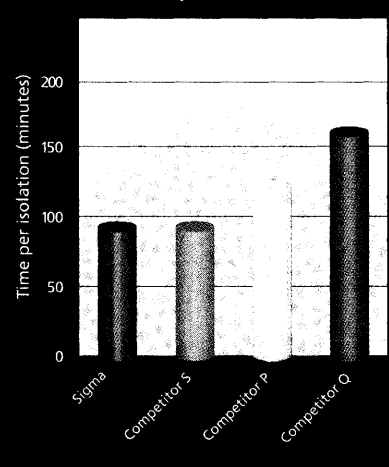


Comparison of Transfection Efficiency



The data shows the average of six replicates for each endotoxin-free plasmid isolation method. All transfections were in CHO-K1 cells. The OD measurements were taken at 420 nm and the units of ß-gal/plate were found using the Sigma ß-Galactosidase Reporter Gene Activity Detection Kit. The cells were grown to ~60-75% confluency and transfected using 3µg of plasmid DNA/15µg of Escort IV. ß-Gal activity was determined ~60-70 hour post-transfection.

Time Required Per Isolation



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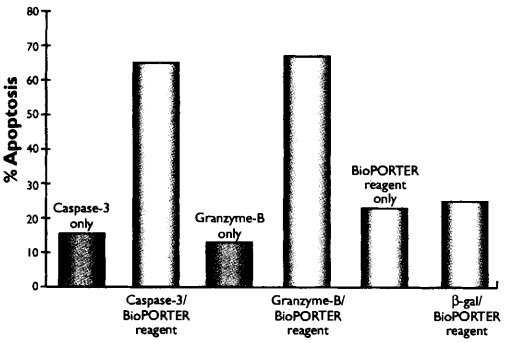

Granzyme B (450 ng), Caspase-3 (3.3 ng) or β-galactosidase (2 µg) were added to Ki-Ras 267 cells (prostate cancer) with or without BioPORTER reagent. 24 hours after protein delivery, cells were directly analyzed for apoptosis by flow cytomerty using an Annexin V-FITC assay kit.

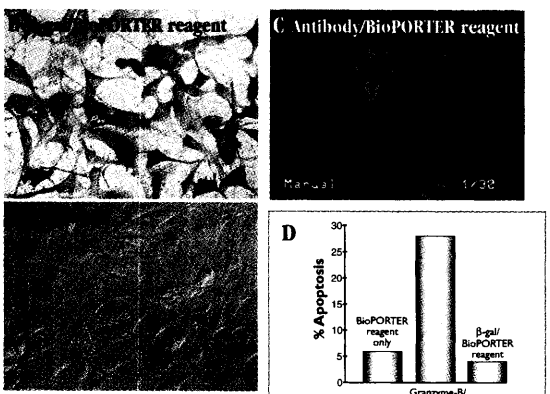

Fig. B/C FITC-Antibody or β-gal Delivery Into Mouse Fibroblasts
FITC-labeled antibody (2 µg) or β-gal (0.5 µg) were delivered with 2.5 µl of BioPORTER reagent into NIH/3T3 cells grown on coverslips in serum free conditions. Cells were examined 4 hours after protein delivery.

Fig. D Functional Granzyme-B Delivery Into Jurkat Cells
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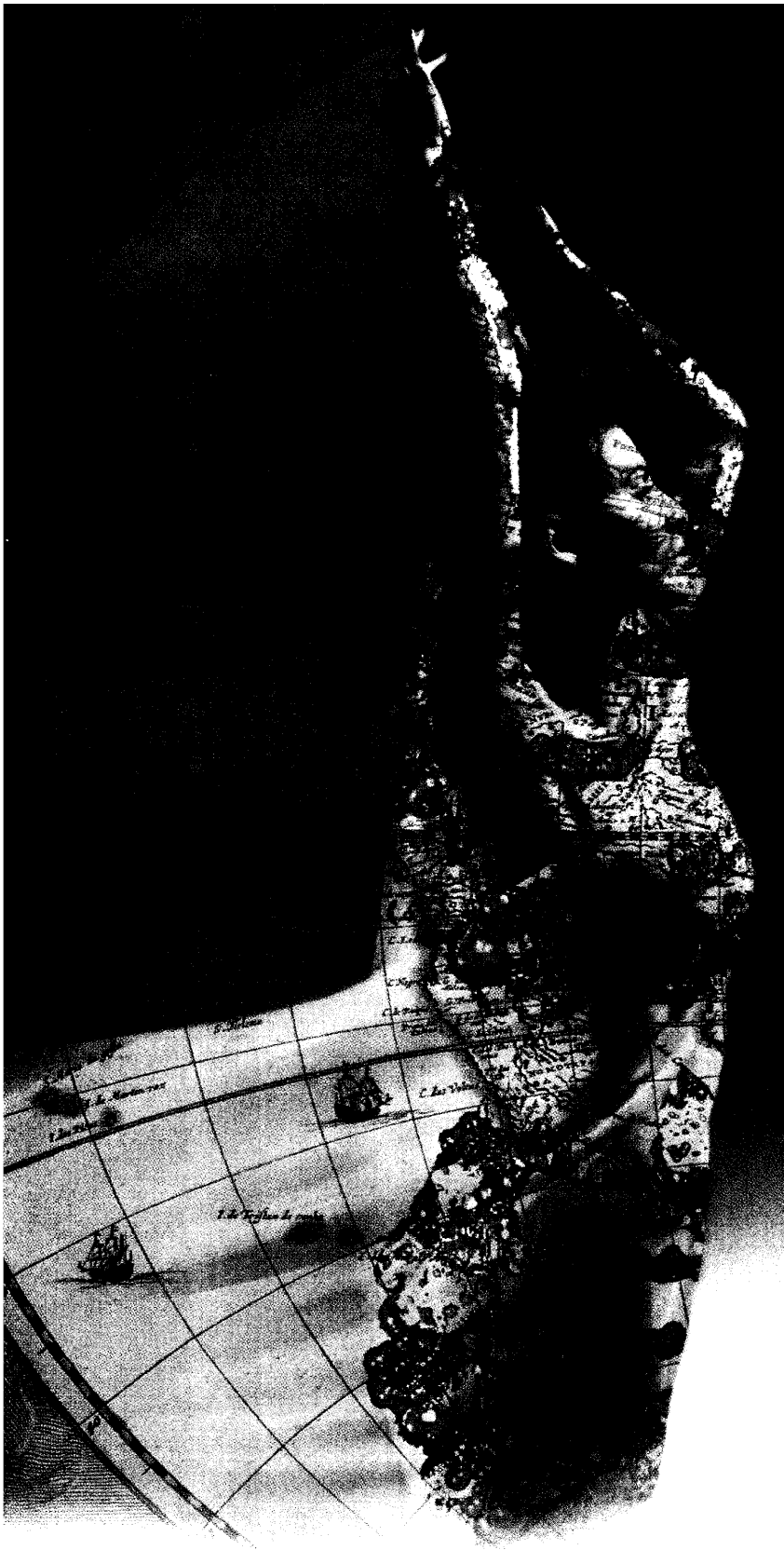

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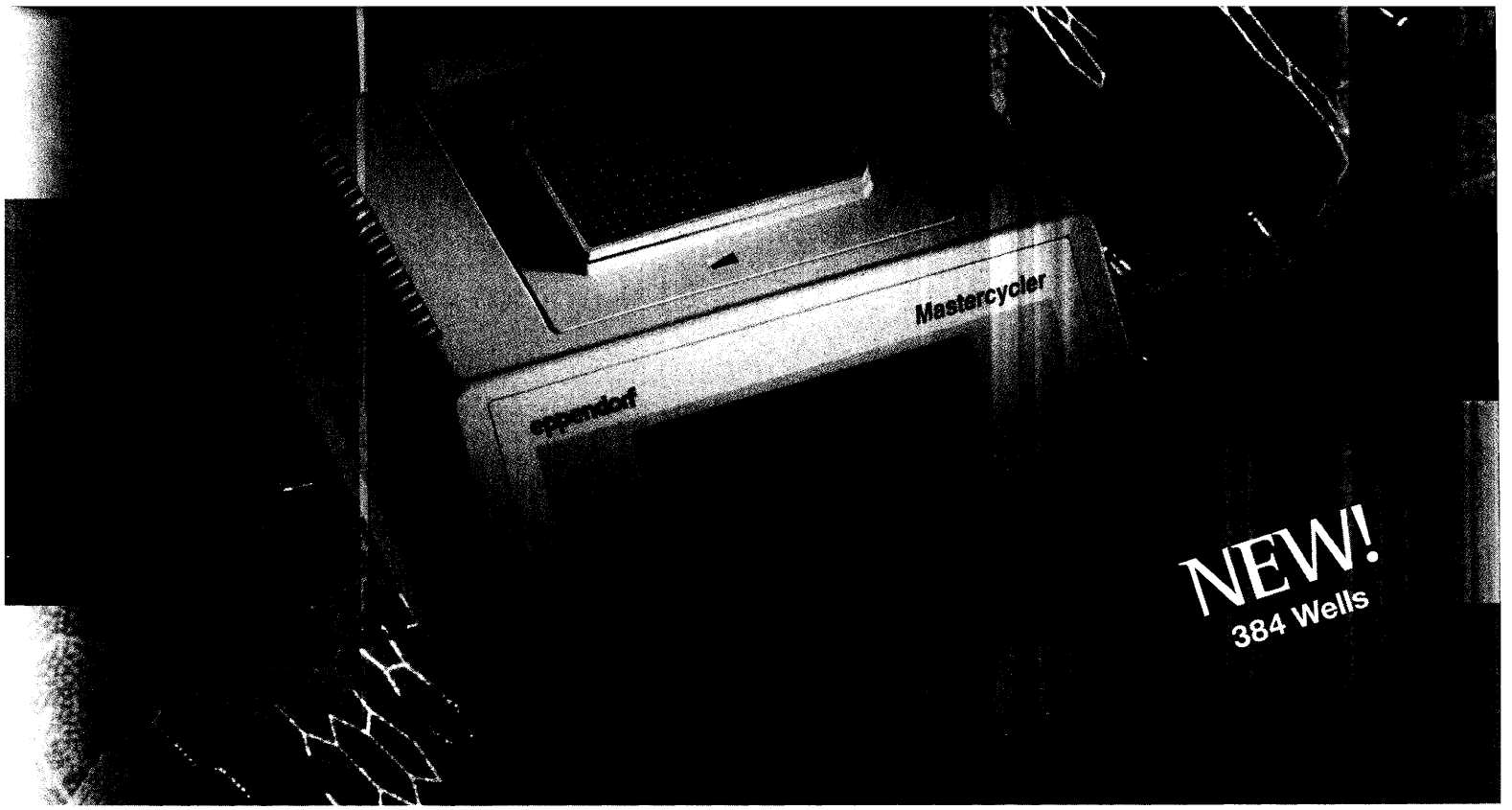

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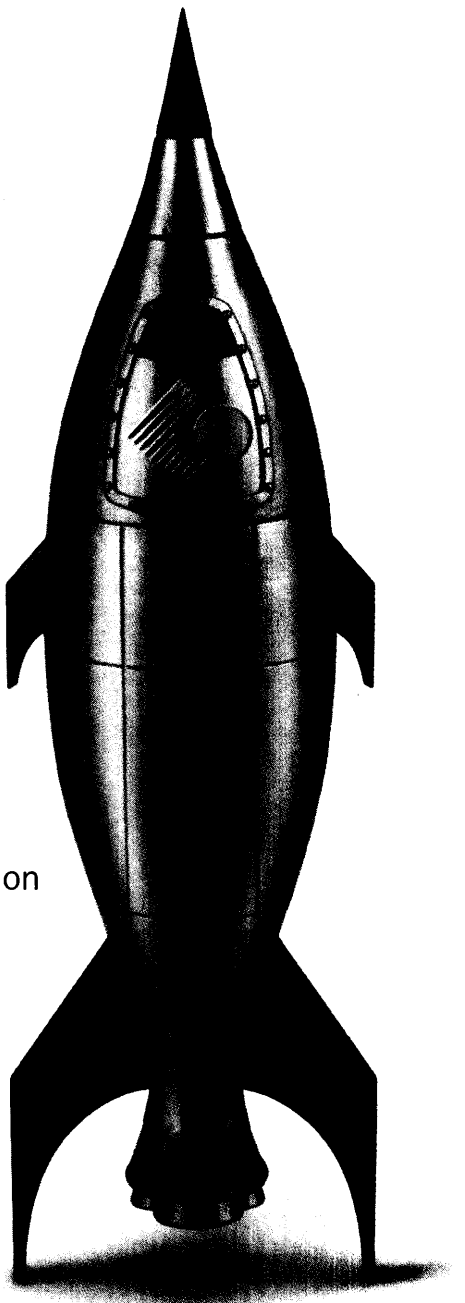

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The Principal Investigators will be offered awards of up to €1.3m (US$1.2m) per annum, to a maximum of €6.5m (US$6m) over five years. These awards will cover the salaries and other costs of the teams (possibly up to 12 people) over a three to five year period.

Research Areas

The objective of the Foundation is to create a critical mass of excellence in basic research within Biotechnology and Information and Communications Technologies (ICT). This will underpin the enterprise environment in Ireland, particularly for industries such as Biopharmaceuticals, Healthcare, Agri-Food, Information and Communications.

The areas eligible for funding in this Call are:

Biotechnology

- **Signal Transduction in Cellular Contexts;**
- **The Immune Process, including Molecular Medicine;**
- **Functional Genomics**

and the relevant underlying platform technologies (such as DNA chip array technology, proteomics, bioinformatics and structural biology). The Foundation is also prepared to consider outstanding proposals in other areas of fundamental importance to the advancement of Biotechnology.

Information & Communications Technologies (ICT)

The Foundation is particularly interested in basic research which is fundamental to the advancement of ICT in:

- **Software Reliability in Complex Software-Intensive Systems;**
- **Optical Transmission and Switching Technologies (including related Nanoscience).**

The Foundation is also prepared to consider outstanding proposals in other areas of fundamental importance to the advancement of ICT.

Applications

This Call for Proposals is a 'rolling call' with no set closing date; applications may be submitted at any time and will be reviewed periodically.

Please refer to **www.sfi.ie** for more complete details of this Call for Proposals.

Further assistance can be obtained by contacting our infodesk at

Email: **info@sfi.ie**
Telephone: + **353 1 607 3200**
Fax: + **353 1 607 3201**

Proposals, which can only be accepted on the application form provided, should be submitted to the Foundation at the following address:

Science Foundation Ireland (SFI),
Call for Proposals - SFI Principal Investigators,
Forfás,
Wilton Park House,
Wilton Place,
Dublin 2,
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eIF-2α	1	PLCγ-1	1
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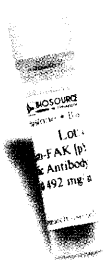

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UNITED KINGDOM

* 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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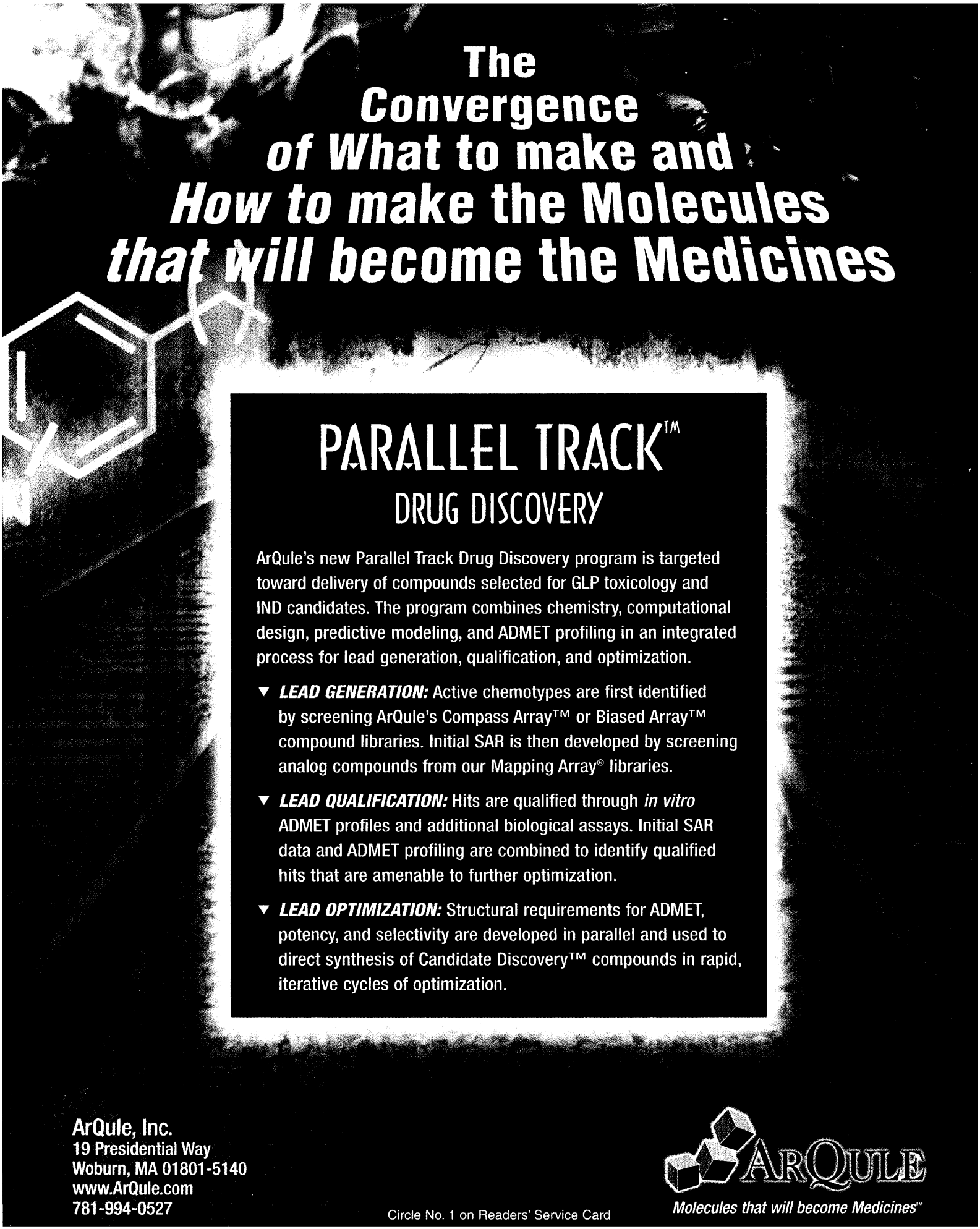
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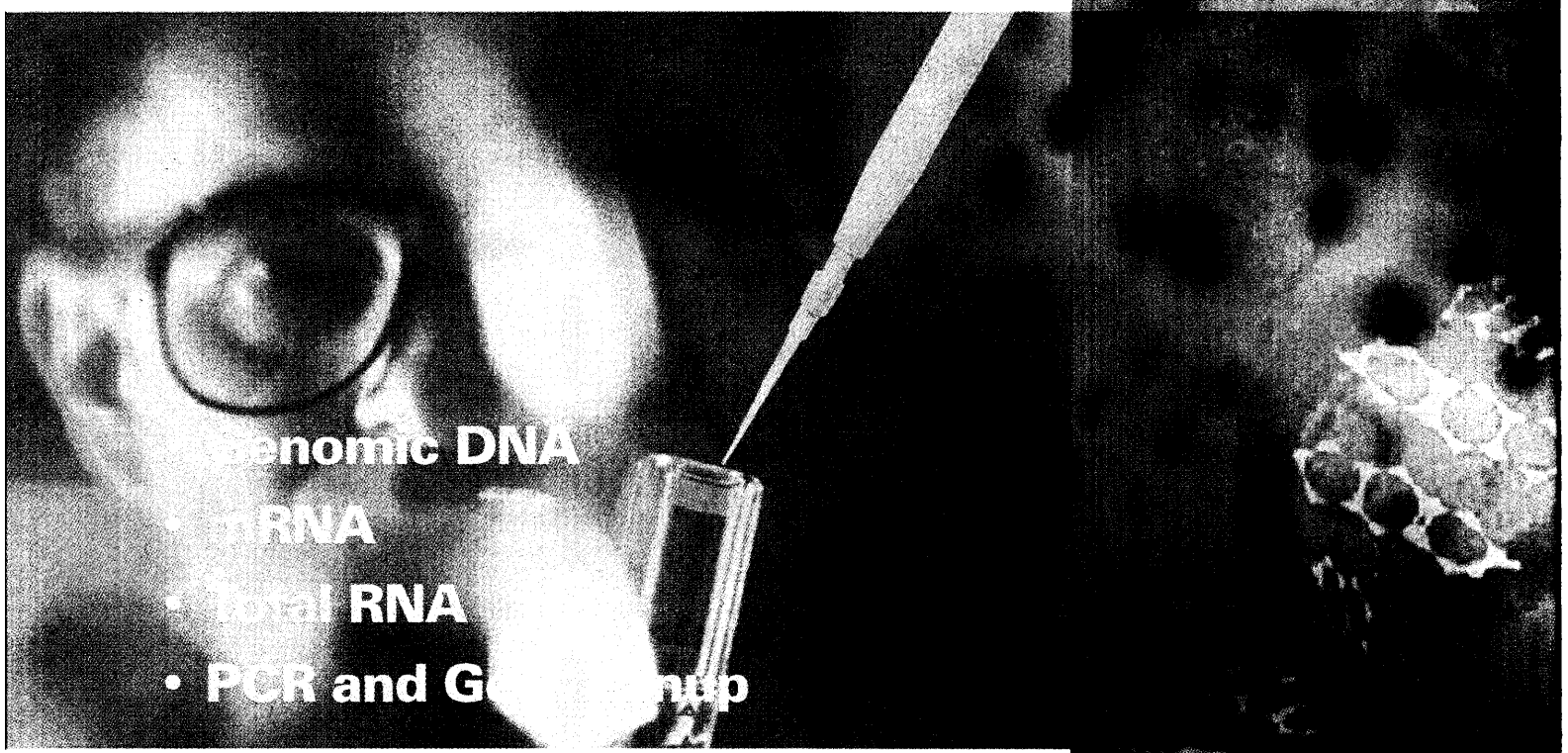

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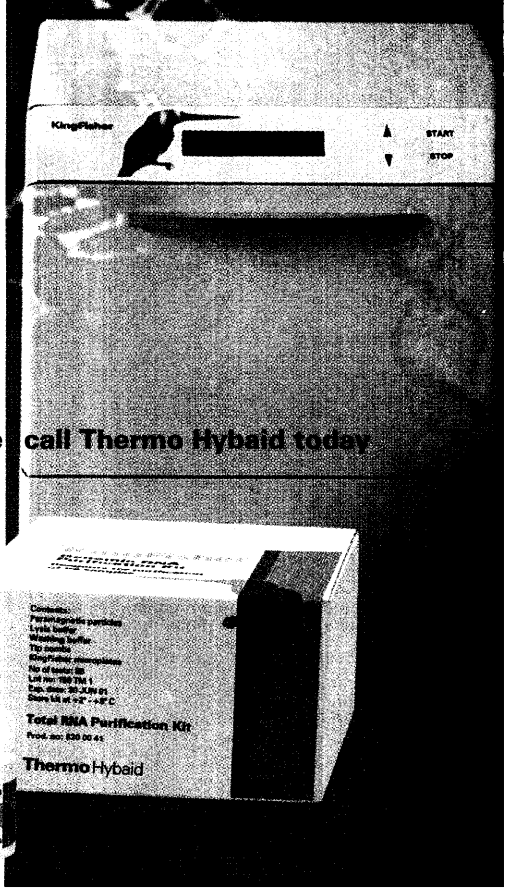

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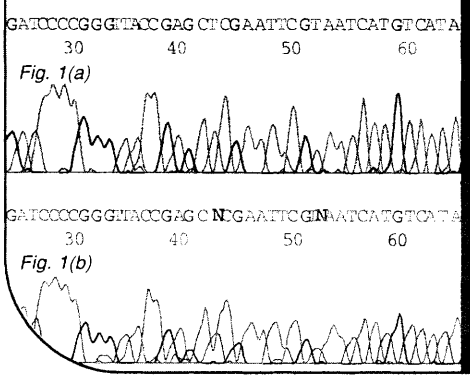
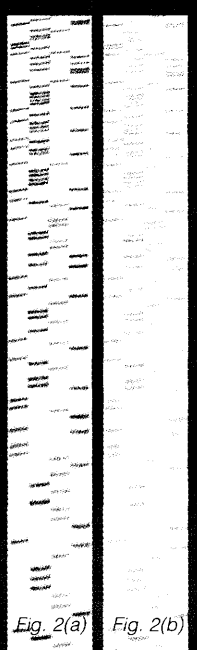
Fig. 2. Autoradiograms of a 20.7 kb Lambda PCR fragment sequenced with MBL202 Fwd primer using USB's Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit. PCR clean-up performed with: (a) ExoSAP-IT; (b) a column designed for PCR clean-up.





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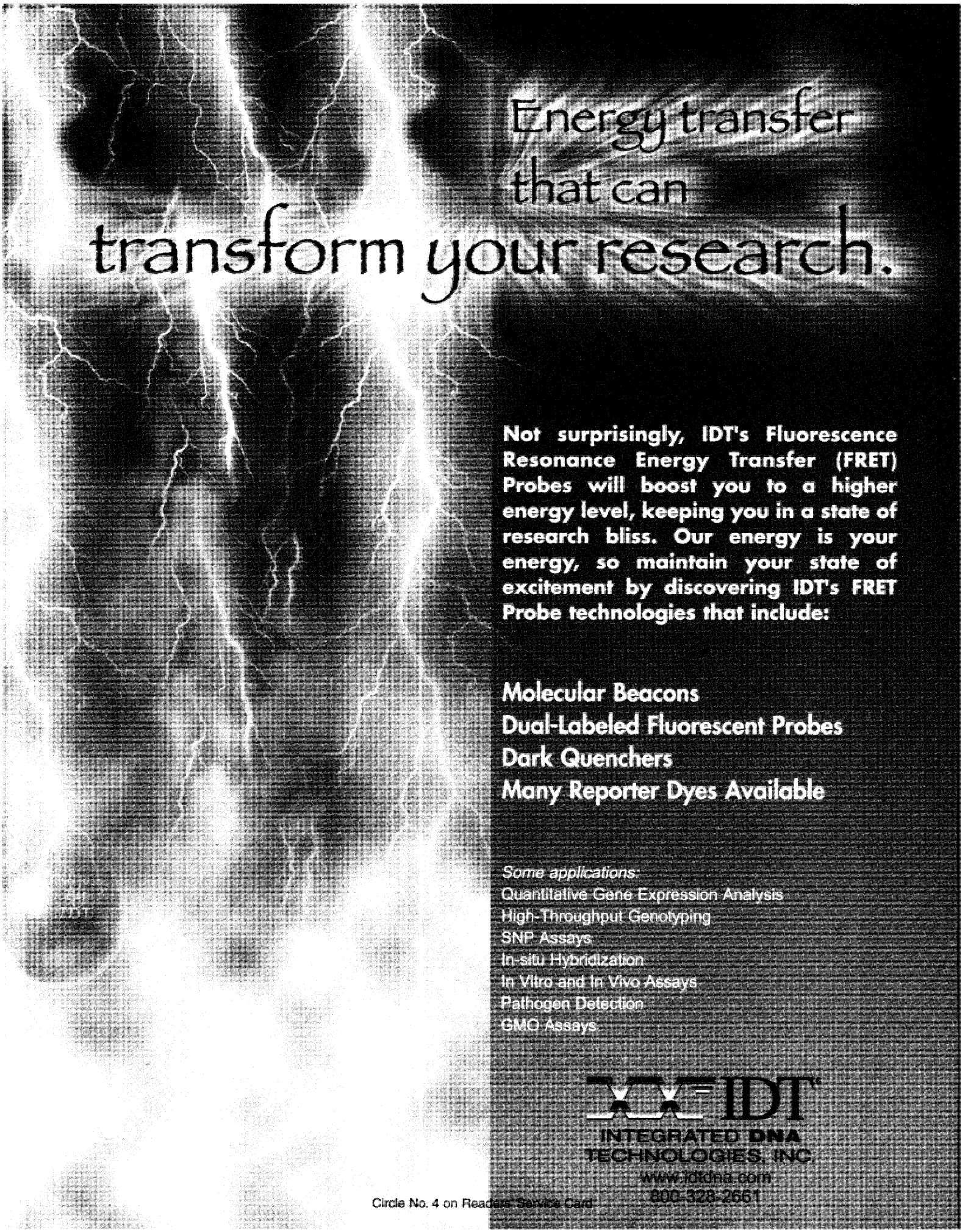
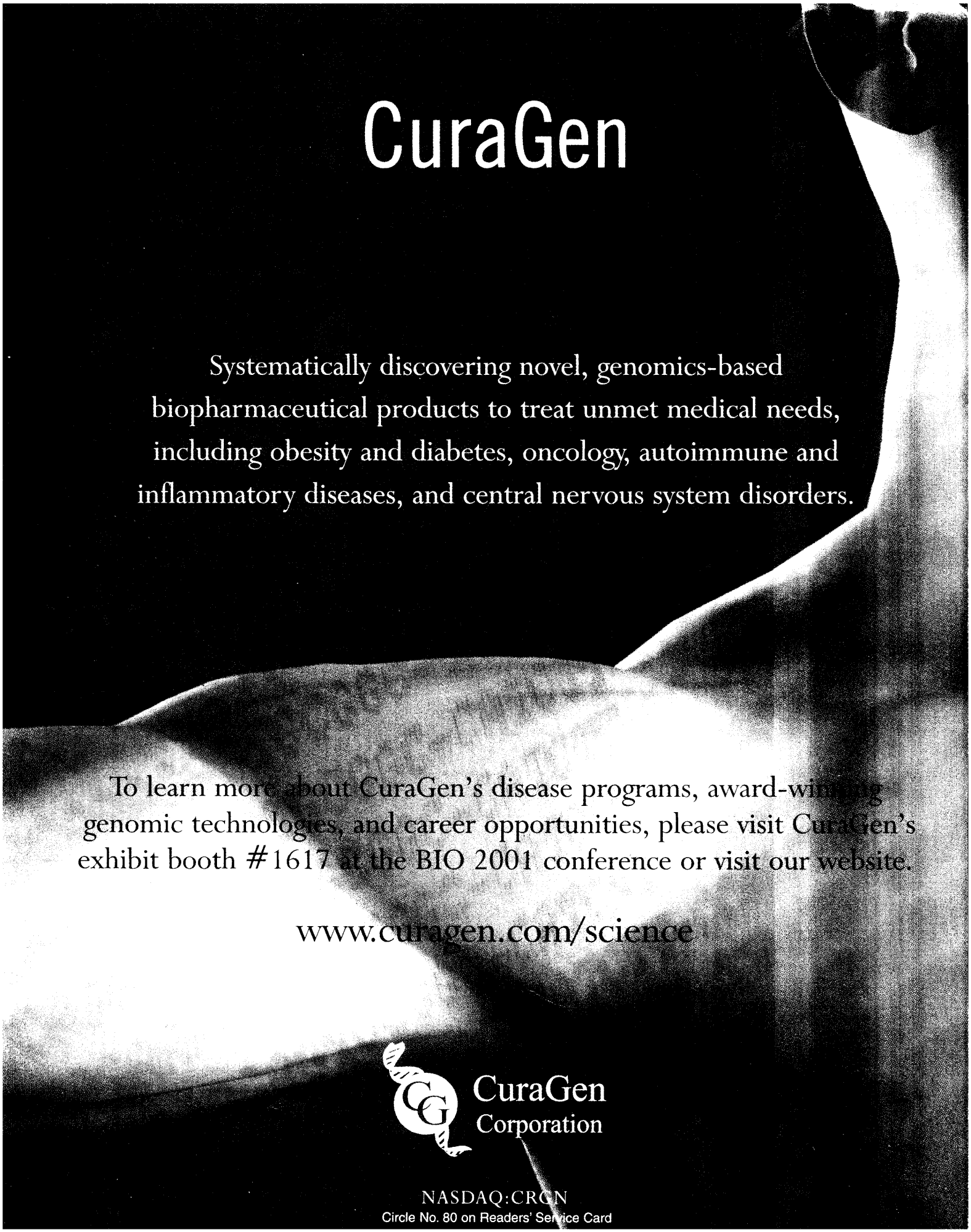
† The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F. Hoffmann-La Roche Ltd. ‡Patent pending on product. The method of use is covered by the following patents: 5,756,285 and 5,741,676.

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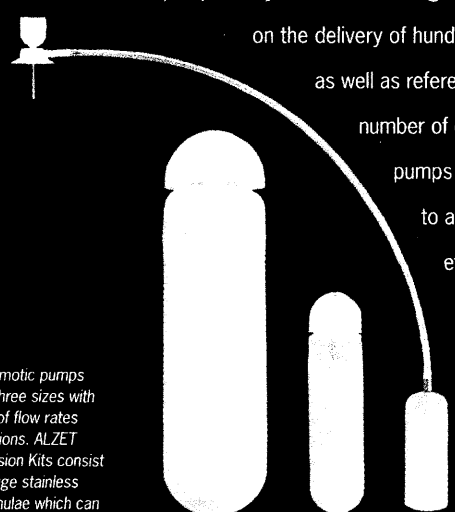

Fig. 2

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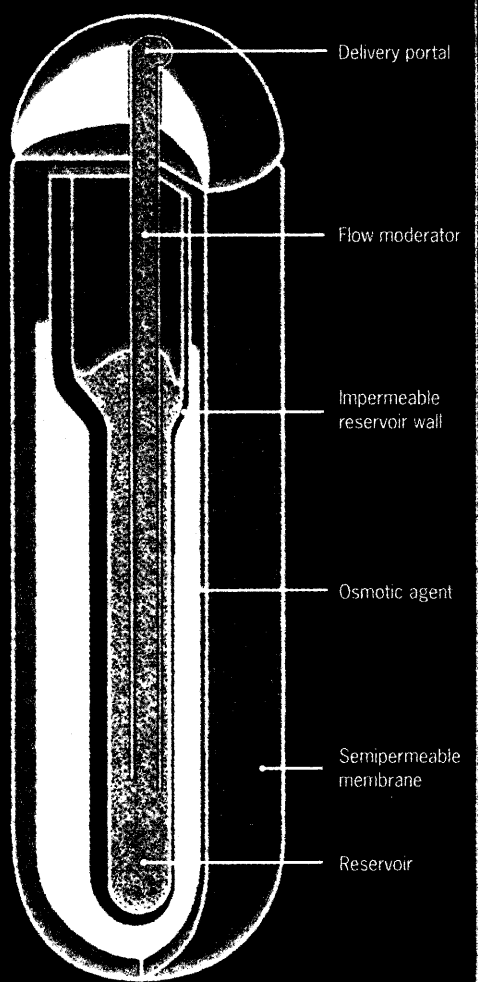

Fig. 1

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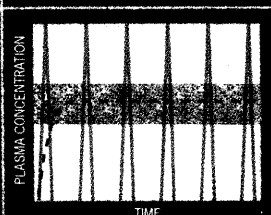

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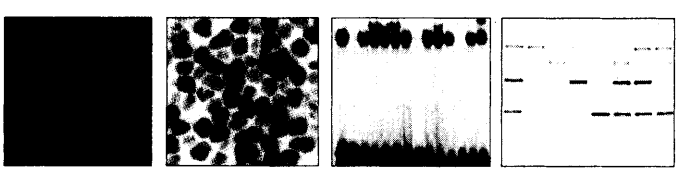

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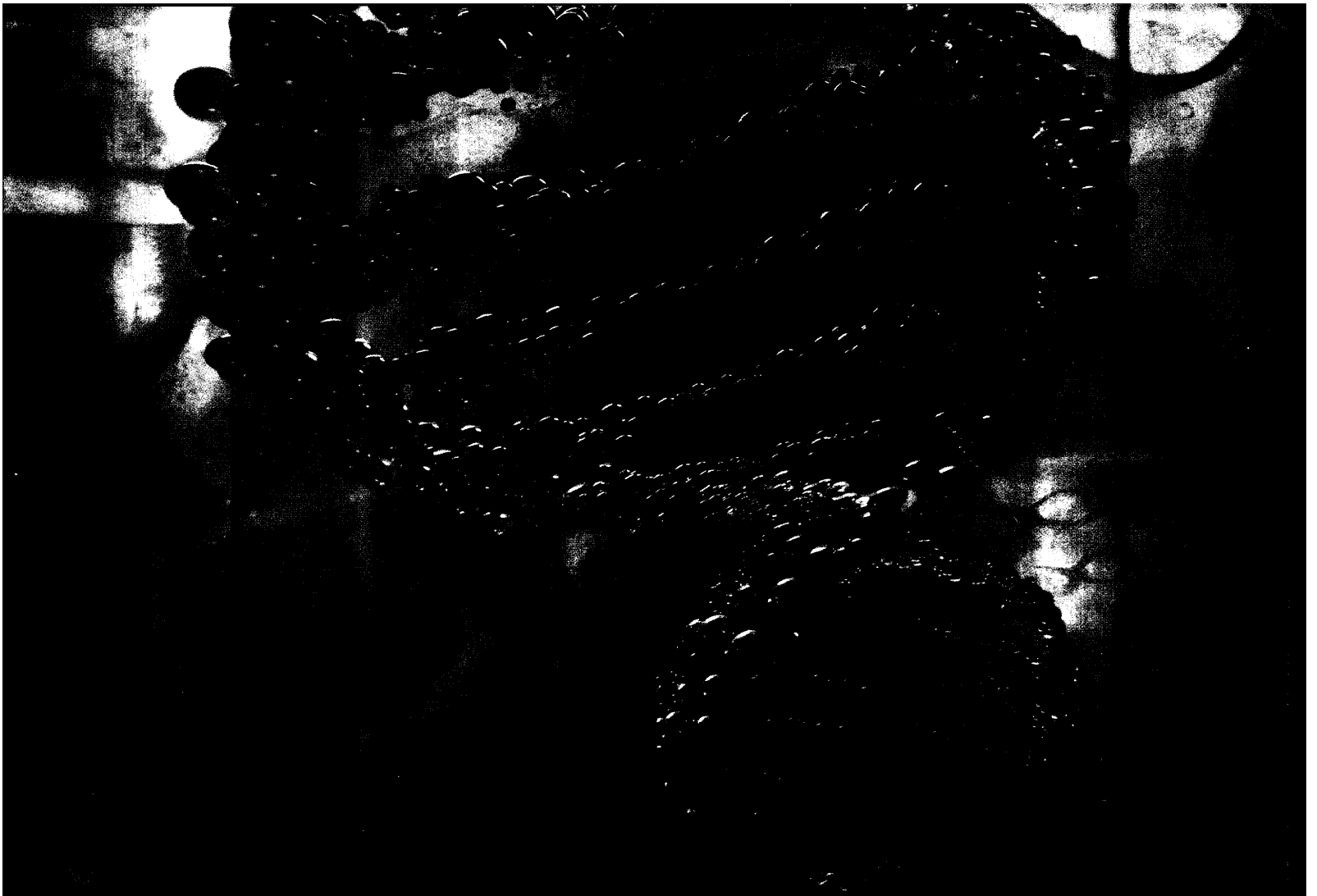

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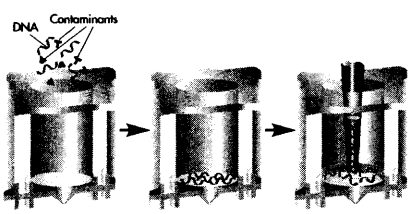

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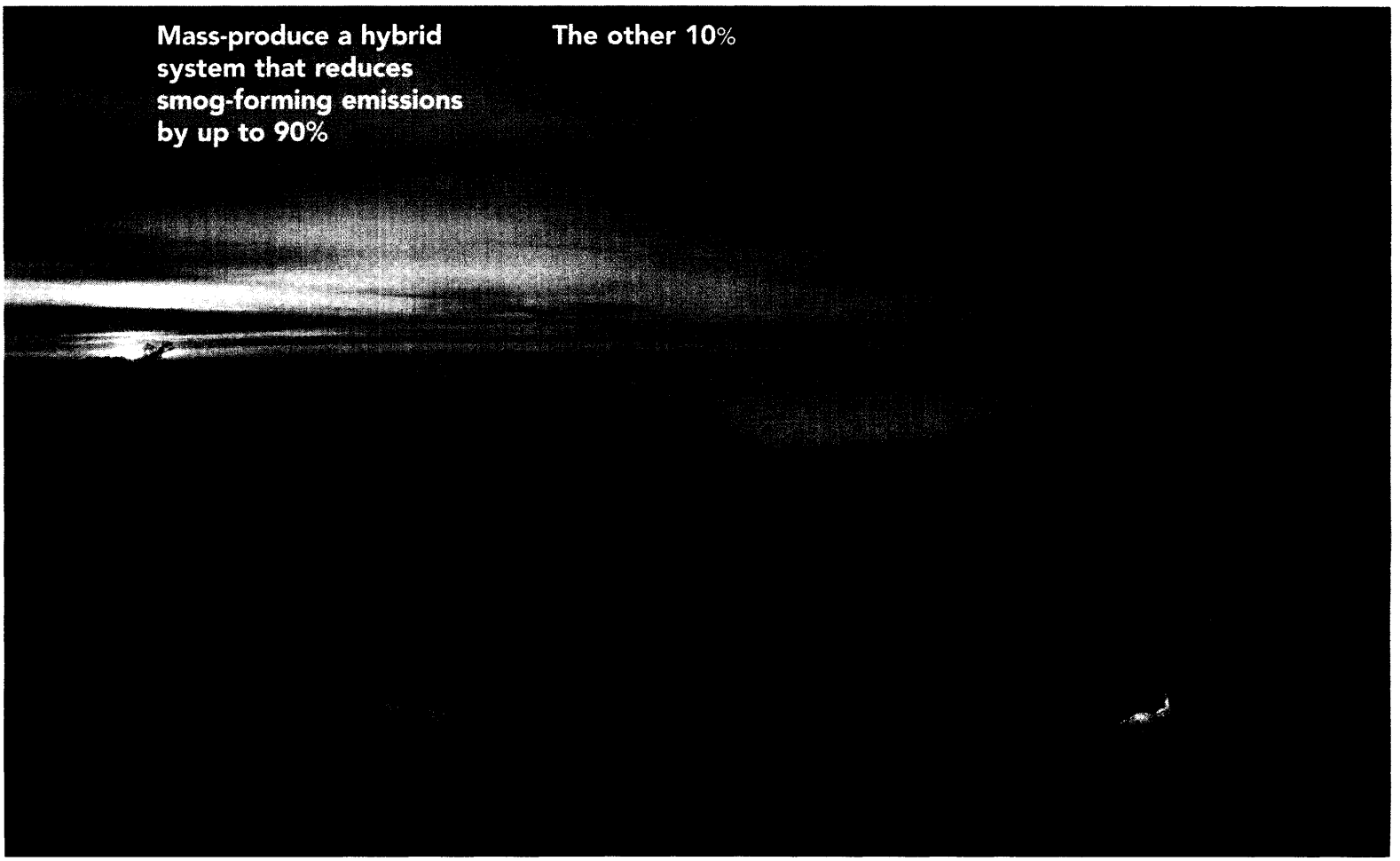

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SpotArray™ Product Family

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- Confocal design for optimum signal-to-noise ratios

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Image Analysis

QuantArray®

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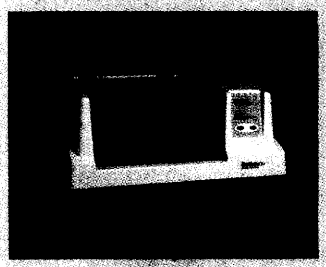
- Robust spot finding
- Spot quality measurements for array QC

Focus on Array Production



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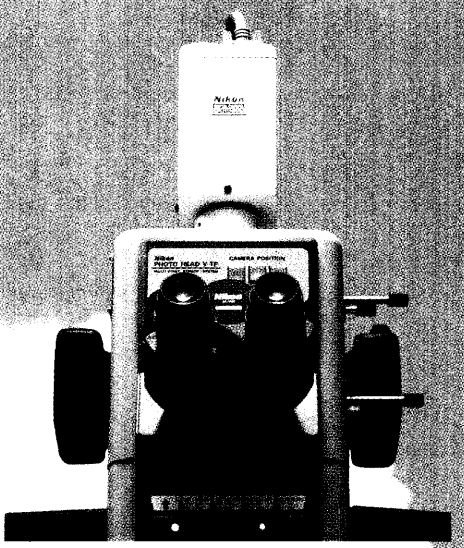

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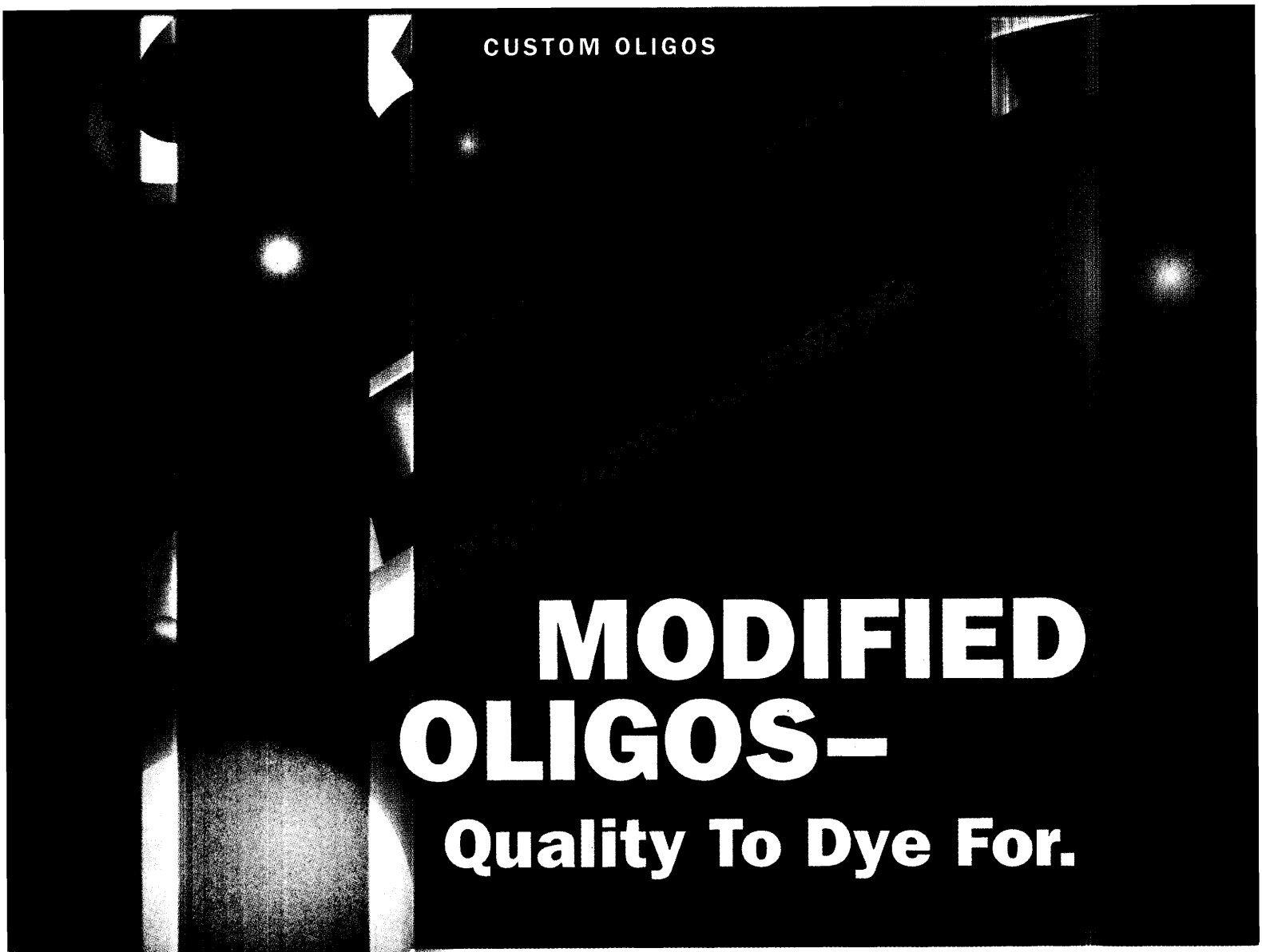

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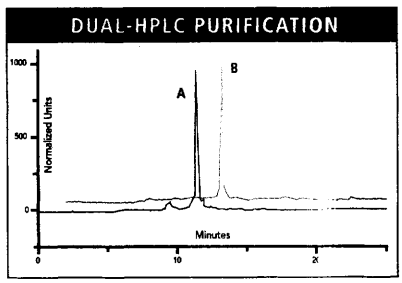

Trace A represents a crude Molecular Beacon (5'-end TET and 3'-end Dabcyl). Trace B is the same oligo after dual-HPLC purification.

Oligos are quality-checked by PAGE and statistical MALDI-TOF mass spectrometry. Dual-labeled probes and Molecular Beacons are purified by a two-step HPLC process to ensure high quality.

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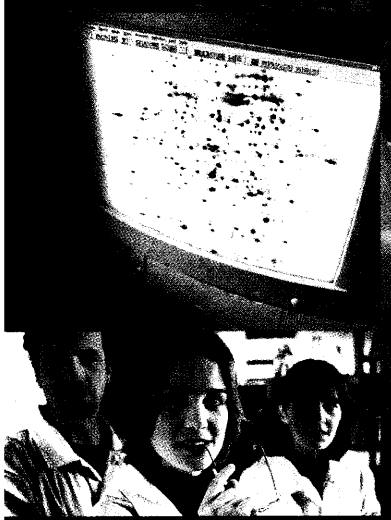

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