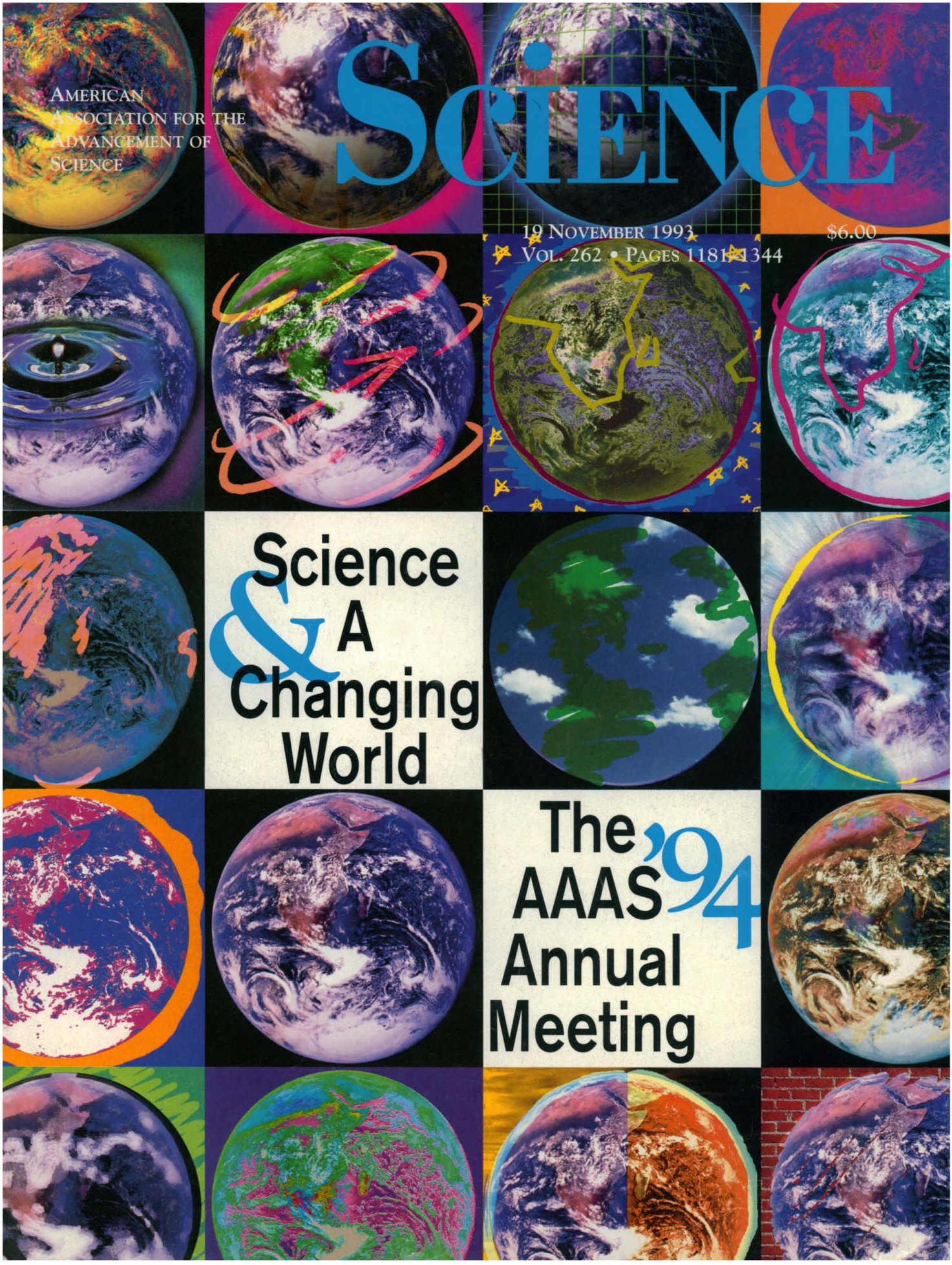
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SCIENCE
19 NOVEMBER 1993
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Science & A Changing World
The '94 AAAS Annual Meeting

Breaking the Mold

In Thermostable Enzyme Research

The Commitment to Discovery

Stratagene is committed to thermostable enzyme research. We literally go to the ends of the earth looking for novel microorganisms which may contain useful thermostable enzymes. Our goal is to make recombinant DNA methodologies more efficient and less time-consuming by exploiting these newly discovered enzymes that excel at elevated temperatures.

The Results of Our Search

Stratagene's search has been quite fruitful. We have broken new ground with thermostable enzymes isolated from the hyperthermophilic marine archaeon, *Pyrococcus furiosus* (*Pfu*)[1]. This extremely thermophilic microorganism grows optimally at 100°C and as may be expected, possesses a host of exceptionally thermostable enzymes.

Scientists at Stratagene have recently cloned *Pfu* DNA ligase*[2,3], which remains active following one hour incubation at 95°C and functions superbly in the ligase chain reaction (LCR)[4,5]. Cloned *Pfu* DNA polymerase* exhibits 12-fold higher fidelity than *Taq* polymerase[6,7]. The exonuclease-deficient mutant of *Pfu* DNA polymerase can be used to directly sequence PCR** products with ^{35}S-dATP[8].

This is just the beginning of Stratagene's commitment to explore thermophilic enzymes and their applications. Just the beginning of the already unmatched line of Stratagene enzymes that can take the heat.

Products

Cloned *Pfu* DNA ligase Extremely thermostable. Exhibits higher specificity with substantially less blunt-ended activity than *Tth* DNA ligase, making it ideal for use in LCR. Cat# 600191

Cloned *Tth* DNA ligase Until now, the only commercially available thermostable DNA ligase. The original LCR technique employs this enzyme. Cat# 600193

LCR Kit Includes *Pfu* DNA ligase, reaction buffer, positive and negative control oligonucleotides, control plasmid template and a detailed LCR protocol complete with experimental design and troubleshooting section. Cat# 200520

Cloned *Pfu* DNA Polymerase Extremely thermostable. Exhibits 3' to 5' exonuclease-dependent proofreading activity and the highest fidelity of any thermostable DNA polymerase. Cat#'s 600153, 600154, 600159

Native *Pfu* DNA polymerase The original high-fidelity *Pfu* polymerase isolated from the hyperthermophilic archaebacterium, *Pyrococcus furiosus*. Cat#'s 600135, 600136

Exo-minus *Pfu* DNA polymerase The genetically engineered mutant of *Pfu* polymerase possesses no detectable exonuclease activity. Ideal for cycle sequencing PCR products with ^{35}S nucleotide analogs and for other high-temperature primer extension reactions that do not require high-fidelity DNA synthesis. Cat# 600163

Cyclist™ Exo-minus *Pfu* DNA sequencing kit Contains all the reagents required for cycle sequencing with Exo-minus *Pfu*. Designed for direct sequencing of PCR products or purified plasmid templates, labeled with ^{35}S-dATP. Cat# 200326

Native *Taq* DNA polymerase Traditionally used for high-temperature primer extension reactions. Stratagene's *Taq* DNA polymerase is purified using a proprietary technique that makes the enzyme extremely thermostable. Cat#'s 600131, 600132

Cyclist™ *Taq* DNA sequencing kit Contains all the reagents required for cycle sequencing with *Taq* polymerase. Designed for direct sequencing of PCR products, plasmids from colonies or phage from plaques, using ^{32}P- or ^{33}P-dATP. Cat# 200325

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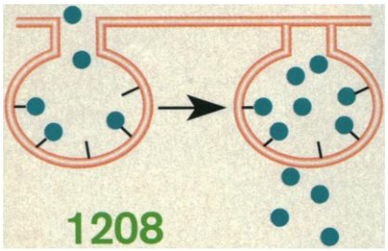
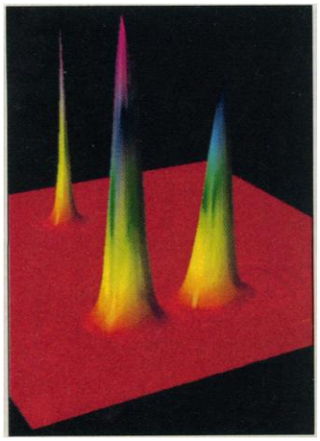


Caveolae's role in cell explored



1229

Laser-stabilized atoms

NEWS & COMMENT

RESEARCH NEWS

POLICY FORUMS

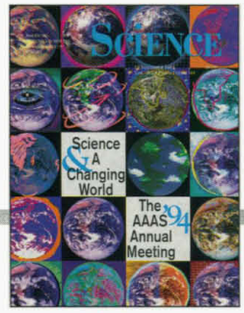
PERSPECTIVE

ARTICLE

DEPARTMENTS

COVER

Science and a Changing World is the theme for the 1994 AAAS annual meeting in San Francisco from 18 to 23 February. This is the 160th AAAS meeting, and it upholds our interdisciplinary tradition as researchers from every facet of science—including medical, environmental, evolutionary, physical, social, and technological—convene to exchange and publicize new knowledge. See page 1287 for a complete program and registration information. [Earth image: National Aeronautics and Space Administration. Additional illustration: Tracy Keaton Drew, Washington, DC]



RESEARCH ARTICLE

REPORTS

1277

Behavior of *Salmonella flgM* mutants on motility plates

AAAS Board of Directors

◩ Indicates accompanying feature

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edited by PHIL SZUROMI

Vox pox

Smallpox virus, which requires a human host, has been virtually eradicated. Many people have no immunity against this lethal virus, so should the remaining lab stocks be destroyed as scheduled at the end of this year? Or should they be preserved for future research and in case some virus still survives in the wild? In a pair of Policy Forums, Mahy *et al.* (p. 1223) and Joklik *et al.* (p. 1225) debate this issue.

Helping form an meaningful attachment

Gram-negative bacteria become attached to eukaryotic cells through fibers called pili during the early stages of infection. The assembly of these protein fibers is mediated by chaperone proteins, such as PapD in *Escherichia coli*, which transports pilus subunit proteins such as PapG, an adhesion, to the outer membrane for assembly. Kuehn *et al.* (p. 1234) have analyzed the molecular basis of this interaction. Peptides derived from the carboxyl terminal of pilus subunit proteins bind to PapD and inhibit its chaperone activity. An x-ray structure of a carboxyl-terminal peptide from PapG bound to PapD reveals that binding occurs in an immunoglobulin-like cleft through the interaction of positively charged peptide residues with the arginine-8 and lysine-112 residues of PapD. Mutation of these highly conserved residues in PapD inactivates its chaperone activity in vivo.

Electron ejection

Atoms can be ionized by the photoelectric effect; as the light intensity increases, the probability that an electron is stripped off rises as well. Computer simulations have recently shown that this trend may reverse at very high light intensity, as discussed by Eberly and Kulander (p. 1229). Laser-induced stabilization occurs when the atomic electron's wave function is distorted by the strong electromagnetic field of the light, which in turn alters the ionization rate and decreases the probability of ejection. The authors review the computational results and examine the experimental possibilities.

Silicon spectra

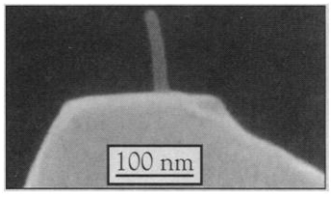
Bulk silicon cannot emit light by luminescence, but an etched, porous silicon surface can. Does the light come from a chemical species at the silicon surface, or is it enhanced photon emission from small quantum-confined nanoparticles? Wilson *et al.* (p. 1242) addressed this question by carefully precipitating size-selected nanocrystals. Their time-resolved photoluminescence and absorption spectroscopy measurements offer direct evidence that, like bulk silicon, the nanocrystals behave as indirect band gap emitters, and that quantum confinement is the source of the luminescence.

Single molecule dynamics

Vibrations of a single molecule have been directly observed by Watanabe *et al.* (p. 1244). They used a newly developed femtosecond field emission camera to follow the motion of a single copper phthalocyanine molecule adsorbed onto a tungsten tip. The adsorbed molecule modifies the electron emission properties of the tip as the electrons pass from the metal into the vacuum through the molecule. A modified current is observed that is modulated by the vibrations of the molecule relative to the metal surface.

Nanowire magnets

Iron filaments less than 10 nanometers in width and hundreds of nanometers in length have been fabricated on silicon substrates. Kent *et al.* (p. 1249) used the scanning tunneling microscope to dissociate gas-phase iron carbonyl. Depending on the bias conditions and pressure,



either relatively pure body-centered-cubic metallic iron or a disordered carbonaceous phase can be formed. Such structures should prove useful in exploring the technological limits of miniaturized magnetic structures.

Tracking HIV variability

Identifying variant strains of the human immunodeficiency virus (HIV), whether in an infected individual or as the virus moves through a population, is normally a time-consuming, large-scale sequencing exercise. Delwart *et al.* (p. 1257) have developed an assay for variation based on the decrease in gel mobility of DNA heteroduplexes with sequence divergence that arises from gaps and mismatches. The authors use this method to construct a phylogenetic tree relating HIV strains found in different parts of the world.

Bcl-2 and cell death

Expression of the proto-oncogene *bcl-2* can inhibit apoptosis and necrotic neural cell death, apparently by inhibiting the net generation of oxidative species in the cell. The neural cell line GT1-7 is highly sensitive to toxicity by buthionine sulfoximine (BSO), which depletes cells of reduced glutathione (GSH) that helps protect cells from oxidative injury. Expression of Bcl-2 in these cells lessens BSO toxicity. Although cells expressing Bcl-2 have higher GSH concentrations, Kane *et al.* (p. 1274) show that Bcl-2 still has a protective effect even when GSH is depleted by exposure to diethyl maleate. However, Bcl-2 expression decreases the concentration of hydrogen peroxide and hydroxyl radicals and can rescue yeast mutants lacking superoxide dismutase, an enzyme that protects cells against oxidative damage.

From start to finish

Flagella construction in *Salmonella typhimurium* requires the coordinated regulation of three gene hierarchies; each class of genes is necessary for the expression of the subsequent class. Hughes *et al.* (p. 1277; see the Perspective by Losick and Shapiro, p. 1227) have studied how the cell can detect the synthesis of the initial components of the flagella (hook–basal body complex) and then turn on the late genes to finish the flagella. The intracellular levels of FlgM are regulated in concert with the construction of the flagella. The expression of the late gene class is prevented by a negative regulator FlgM. FlgM is expelled from the cell through an opening in the flagellar hook–basal body complex to allow expression of the late genes.

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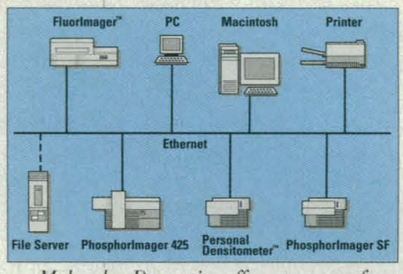

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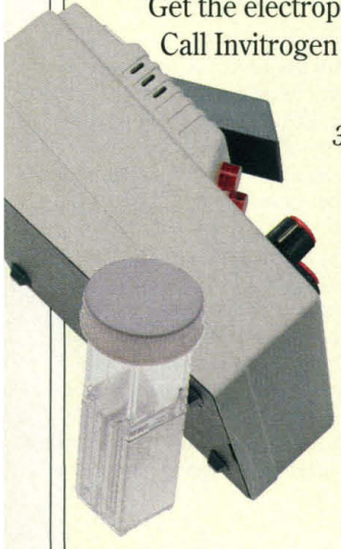

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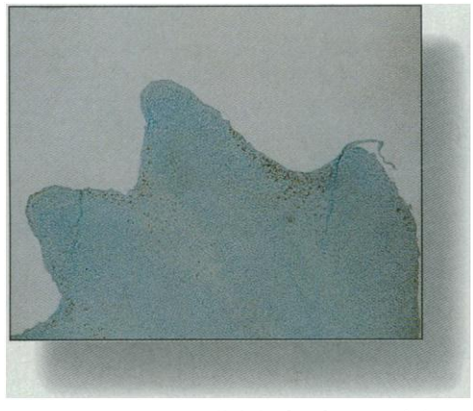
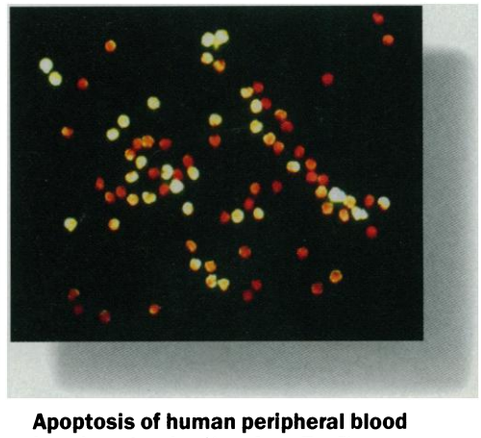
Apoptosis in physiological cell turnover of epithelium in human duodenum[1], ApopTag™ Peroxidase Kit and methyl green (X400)



Developmental cell deletion in remodeling of mouse forelimb bud (14-day embryo), ApopTag™ Peroxidase Kit and methyl green (X200)[2]



Apoptosis of human peripheral blood lymphocytes *in vitro*, ApopTag™ Fluorescein Kit (yellow) and propidium iodide (red) (X400)[3]

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[3]X Li, W James, F Traganos & Z Darzynkiewicz, (1993) manuscript submitted.

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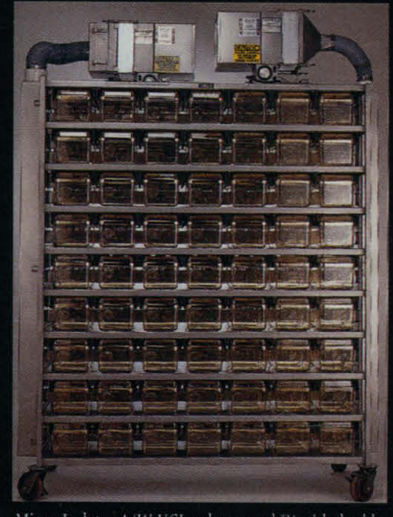

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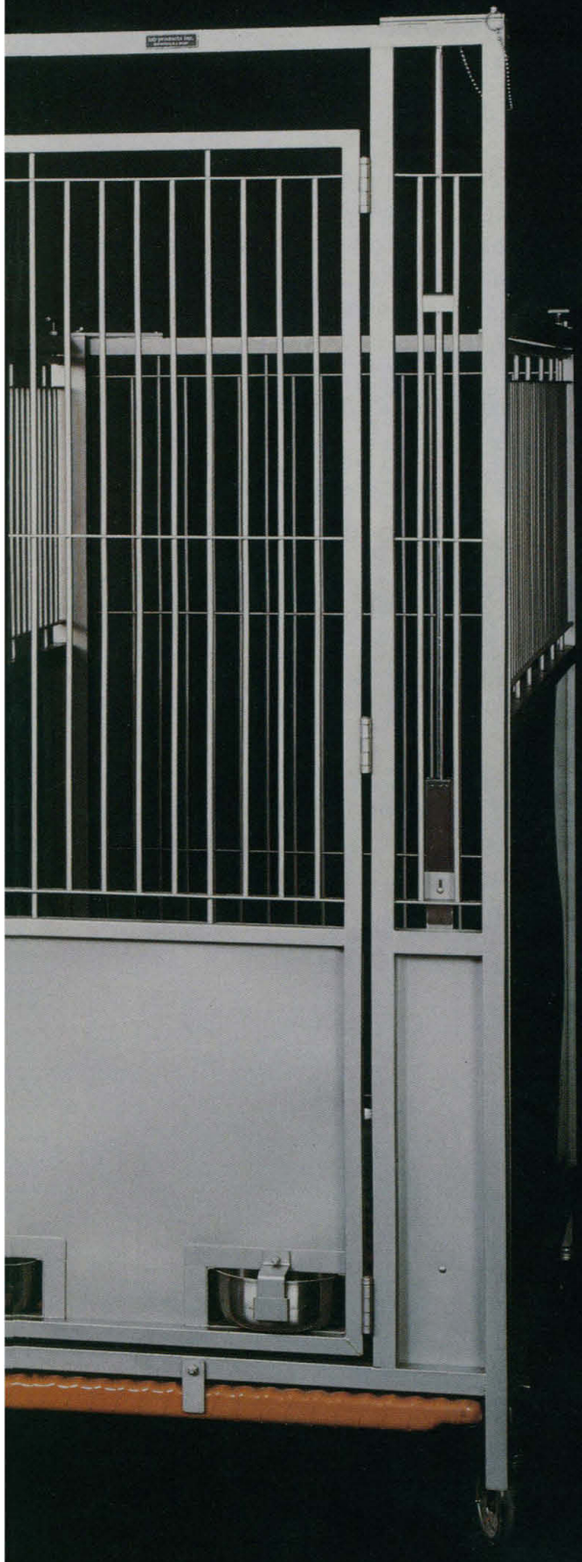
to modify, to maintain and to move.)



Lab Products, Inc. Mobile Kennels incorporate the advantages and benefits of our fixed Formed Beam Kennels.™ The design allows for easy assembly of modular frame units that snap together without the use of fasteners or tools. The kennel frame supports a variety of panels for easy expansion and animal socialization when required. The Mobile Kennels can be linked together as integrated

units without tools by a simple locking device, and they are quickly disassembled for very flat compact storage.

FEATURES OF THE FORMED BEAM CONSTRUCTION;

- supports a variety of flooring styles and patterns
- integrates stationary and removable panels
- designed to deflect urine and feces with absolute minimum of collection areas; and easy to clean surfaces.

- provides smooth floor transitions between kennels and removable side panels which reverse to open bar dividers for visibility
- permits opening of adjacent kennels by slide-panel doors
- highest quality materials and construction
- optional features include resting board, metabolism pan, exercise ramp and sliding panels.

With the use of our advanced 3-dimensional CAD modeling system Lab Products, Inc. will be glad to help you during the key design phase of your kennel room layouts and the selection of the appropriate kennel. Call or Fax for sales and service information.

lab products inc
a bioMedic company

255 West Spring Valley Avenue, P.O. Box 808, Maywood, New Jersey 07607
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U.S. Patent Number 5,048,460

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science pour l'art

CALL FOR SUBMISSIONS

The LVMH MOËT HENNESSY • LOUIS VUITTON Group announces the 1994 Science for Art Prize

"UNPREDICTABLE BEHAVIOUR OF MATTER"

This year's Prize would like to stress unpredictable aspects in the behaviour of matter and its manifestations on all imagined or experimental levels. Thus, works in the following scientific fields are particularly relevant ***mathematics, physics, chemistry, physical-chemistry*** *and* ***biology****, along with all* ***simulations techniques, analytical tools*** *or* ***processes*** *related with these phenomena.*
Priority will be given to those dossiers that deal with ***both*** *aspects, theoretical and experimental or applied.*

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A Scientific Award *for the scientific study offering the largest field of investigation of fundamental or applied research.*

An Innovation Award *for the most promising applied research with a technological or commercial value.*

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1993 Samuel EDWARDS, Cambridge Cavendish Laboratory, United Kingdom

Winners of the INNOVATION AWARD

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1992 Nicolas FRANCESCHINI, CNRS Marseille, France
1993 Wolfgang HELFRICH, Freie Universität Berlin, Germany

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The deadline for submitting an application is ***January 29, 1994***
Further details and guidelines for application may be obtained from :

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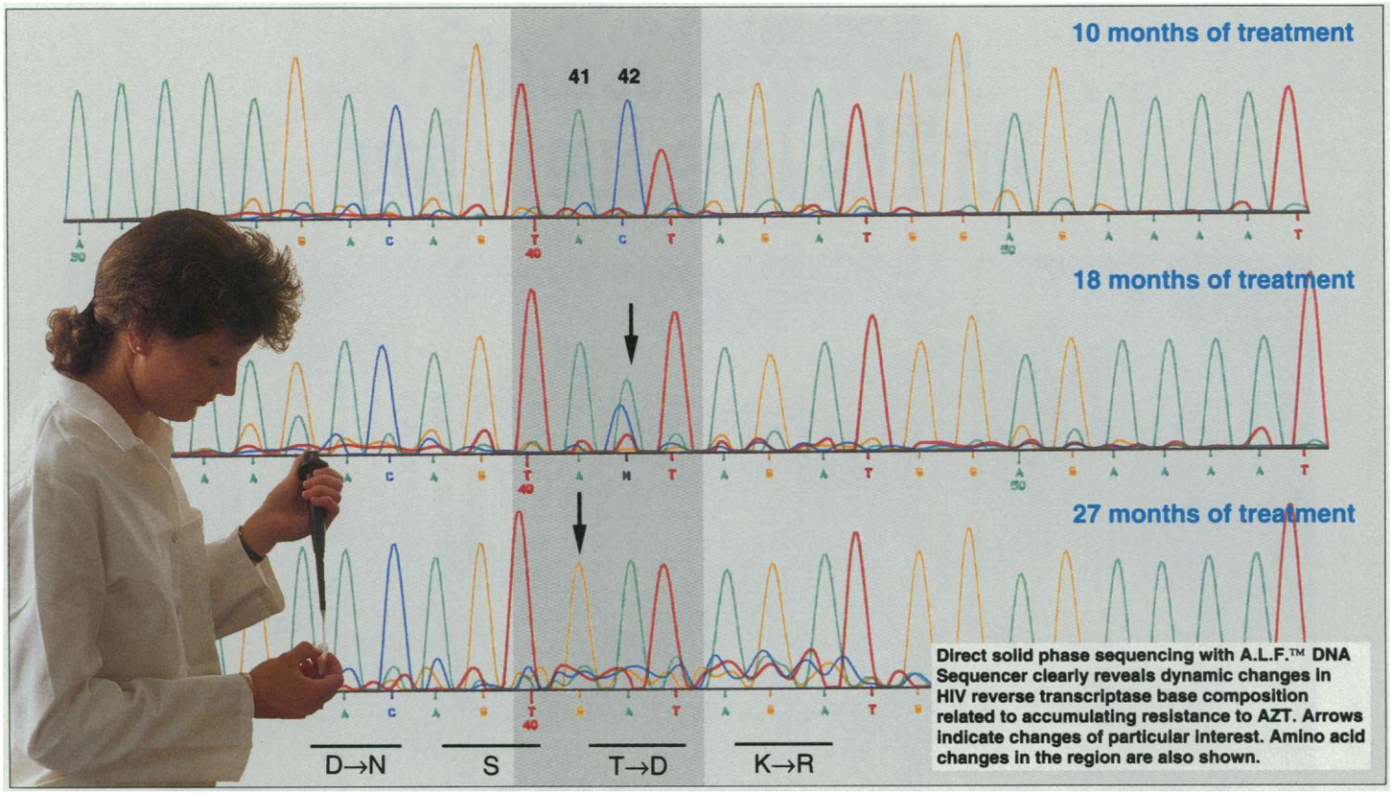


Direct solid phase sequencing with A.L.F.™ DNA Sequencer clearly reveals dynamic changes in HIV reverse transcriptase base composition related to accumulating resistance to AZT. Arrows indicate changes of particular interest. Amino acid changes in the region are also shown.

A.L.F.™ DNA Sequencer helps AZT fight HIV

Accurate detection of heterozygote point mutations has enormous potential as a clinical research tool. Nothing illustrates this better than the recent spectacular analysis of emerging AZT resistant HIV species by direct, solid phase genomic sequencing with A.L.F.™ DNA Sequencer.

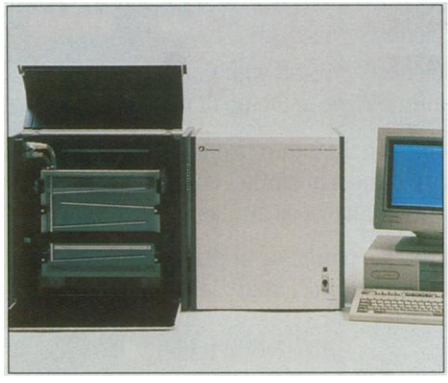
The non-edited data output above shows DNA sequences of HIV RT from a patient undergoing AZT treatment[1]. This clean sequence with little background signal emphasizes the suitability of A.L.F. for direct genomic sequencing of clinical samples. Detailed analysis revealed dynamic changes in base composition.

Take a close look at the changes A.L.F. detected at position 42. The C residue after 10 months treatment became a 50% A/C mixture at 18 months and a clear A nucleotide at 27 months. With a secondary shift from A to G at position 41, Thr69 changed to Asp, a substitution not previously reported.

Only the Automated Laser Fluorescent detection system of A.L.F. combines all the advantages for detecting point mutations like these.

Fixed laser detection of sample bases is essential to reduce background noise. With its unique fixed laser (A.L.F has no moving parts apart from the door), background noise is lower than other sequencers. Hence its base calling is more accurate.

And because A.L.F. uses just one single fluorescent label, you don't have to worry about spectral overlaps and mobility shifts, which again makes base calling more accurate.



A.L.F. DNA Sequencer accurately detects heterozygote point mutations. DNA sequencing with A.L.F. has many applications in clinical research.

Furthermore, the well-proven Sanger technique, already cited more than 20,000 times, leaves nothing to chance with the reaction chemistry.

A.L.F. thus provides the accuracy needed to yield the "consensus" sequence of viral genomes in samples from HIV-1 infected patients treated with AZT.

So with A.L.F. generating precision data like this, clinical researchers can rapidly determine the molecular basis for drug resistance and more effectively plan treatment with alternative drugs or combinations of drugs. And, of course, direct DNA sequencing with A.L.F. has plenty of other clinical applications in areas such as infectious diseases, cancer, genetic disorders and forensics.

Ask for more details and a reprint of the reference.

1. Dynamic changes in HIV-1 quasispecies from azidothymidine (AZT) treated patients. *FASEB Journal 6 (1992)*,Wahlberg, J., Albert, J., Lundeberg, J., Cox, S., Wahren, B., Uhlén, M.

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