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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 ³⁵S-dATP⁸.

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 ³⁵S 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 ³⁵S-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

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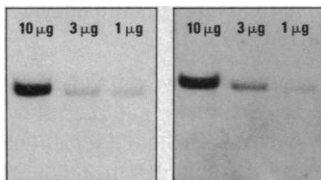
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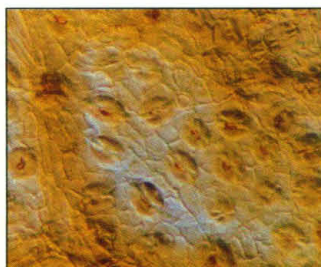
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Mean sea surface elevation in the North Atlantic, showing the influence of ocean floor topography and the flow of cold and warm currents on surface levels. This image was constructed from data from the radar altimeter of the European Space Agency's ERS-1 oceanographic

satellite, a good example of European scientific collaboration. See page 1742. Beginning on page 1733, this special issue features News reports, Perspectives, and a Policy Forum on science in Europe. [Image: Carel Wakkers]



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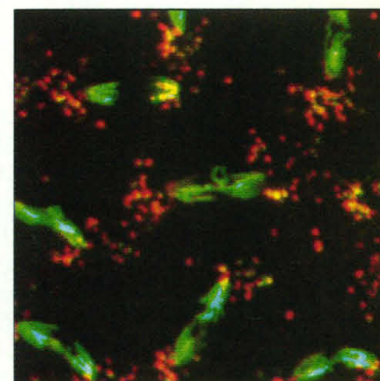
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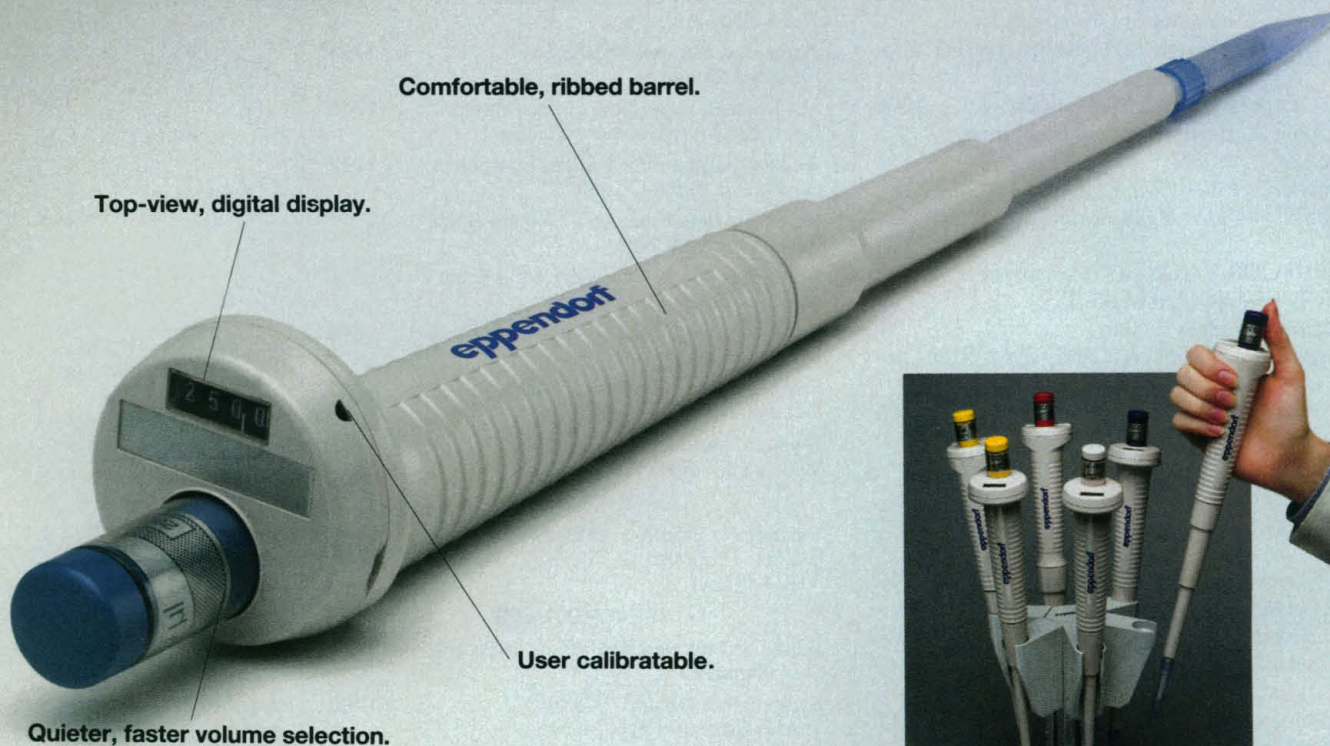
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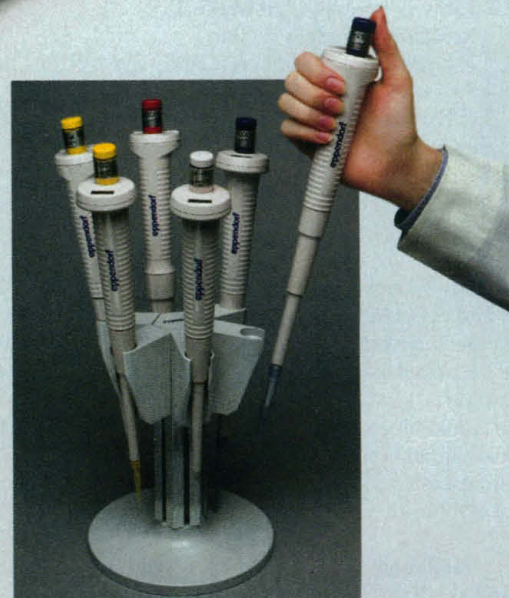
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Frictional account

In most of the theoretical models for the movement of DNA through a gel during electrophoresis, the viscous friction between the moving polymer chain and the solvent provides the drag that results in separation. Burlatsky and Deutch (p. 1782) show that solid frictional effects contributed by the gel itself could greatly reduce mobility as the chain becomes entangled in the gel over several points. The effect is analogous to pulling on a rope looped through several pegs; if there are too many pegs, the rope pulls itself taut and will not move.

■

Cooperative catalyst

Hydroformylation generates more than 5 billion kilograms a year of alcohols and aldehydes from olefinic precursors. This process is catalyzed by a monometallic rhodium catalyst. Broussard *et al.* (p. 1784) report improved product selectivity and reactivity with a bimetallic rhodium catalyst. Studies with model compounds suggest that an intramolecular hydride transfer is facilitated in the *racemic* form of their catalyst.

■

Turning over an old leaf

The record of past atmospheric CO₂ levels and thus the relation between CO₂ levels and climate become obscure at times before about 150,000 years ago, the extent of the ice core records. Stomatal cells in leaves, which regulate gas exchange, may hold the clue. Van Der Burgh *et al.* (p. 1788) developed a stomatal index and show that for durmast oak (*Quercus petraea*) the index correlates with CO₂ levels over the last

Linking solar rotation and the solar cycle

Solar rotation rates can be inferred from frequency splittings observed in the acoustic (p-mode) oscillations of the sun. An analysis of Doppler images by Woodard and Libbrecht (p. 1778) show that the sun's subsurface rotation rate increased between 1986, a sunspot minimum, and during 1988 to 1990, near the solar maximum. The increase was highly latitude-dependent and was greatest near 60°. These results may give important clues that may connect solar rotation to the magnetic dynamo that is thought to drive sunspot formation.

120 years. Analysis of fossil leaves of this oak suggests that CO₂ concentrations fluctuated between 280 and 370 parts per million by volume during the past 10 million years in conjunction with inferred changes in climate.

■

Dimer dependence

The cytokines interleukin-6 (IL-6), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF) bind to receptors that share a signal transducing component, gp130. Davis *et al.* (p. 1805) show that the CNTF receptor consists of the CNTF binding protein, LIFR β (the LIF binding protein), and gp130. Signaling from the CNTF and LIF receptors depends on heterodimerization of LIFR β with gp130, whereas activation of the IL-6 receptor occurs through homodimerization of gp130 in association with the IL-6 binding subunit. Dimerization leads to tyrosine phosphorylation of the receptors. Murakami *et al.* (p. 1808) find that tyrosine kinase activity is associated only with dimers of gp130 formed in the presence of IL-6. A gp130 mutant that forms dimers but is not tyrosine phosphorylated does not transduce a signal. Dimerization of receptor subunits may be a common mechanism for transmembrane signaling.

Two on one

Lymphocytes could in principle express up to four different types of antigen receptors, but under physiological conditions B cells and $\alpha\beta$ T cells express only one functional type of antigen receptor. Davodeau *et al.* (p. 1800) found that this rule of allelic exclusion is not followed by human $\gamma\delta$ T cells. They identified a population of cells that expressed two distinct functional γ chains in their T cell receptors. Such bispecific T cells, if they encounter ligands for both of their receptors, could possibly initiate autoimmune responses.

■

Reforming killers

T cell responses to pathogens generally fall into two types—CD8⁺ cells, or killer T cells, mediate the cytolytic response, and CD4⁺ cells, or helper T cells, generate cytokines in the presence of antigen and interleukin-4 (IL-4). Erard *et al.* (p. 1802) show that mature mouse CD8⁺ cells that are activated in the presence of IL-4 develop further into a noncytolytic population of CD4⁺CD8⁺ cells. These cells switched to a helper phenotype, producing large amounts of IL-4, IL-5, and IL-10 and activating resting B cells. Infectious agents may evade killer cell responses through overproduction of IL-4.

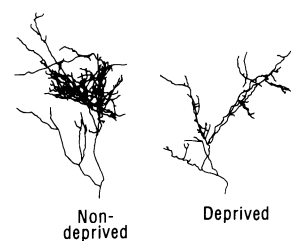
Transferring incompatibility

Infection of insects with the rickettsial symbiont *Wolbachia* leads to cytoplasmic incompatibility. In *Drosophila simulans*, infected males mated to uninfected females produce few if any progeny, yet infected females produce normal progeny from both infected and uninfected males. Boyle *et al.* (p. 1796) show that host factors help determine the degree of infectivity. They transfected *Wolbachia* from infected *D. simulans* egg cytoplasm by microinjection into uninfected eggs as well as into eggs from *D. melanogaster*. Low levels of infection were seen in the *D. melanogaster* strains, but lines could be bred that expressed high densities of *Wolbachia* and high rates of egg mortality, as did *D. simulans* populations that were reinfected from *D. melanogaster*.

■

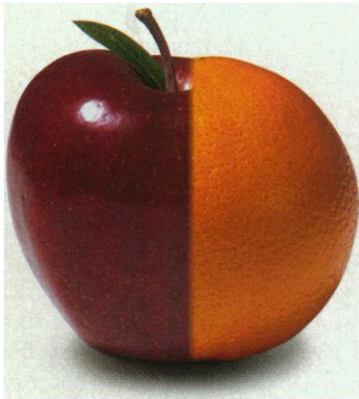
Quick change

Neurons in the primary visual cortex of animals with binocular vision can be strongly affected by imbalances in visual input during early postnatal life. Long-term visual deprivation of one eye can greatly decrease the cortical domains that respond to the deprived eye. Antonini and Stryker (p. 1819) show that even short periods of visual dep-



privation (6 or 7 days) resulted in striking anatomical changes caused by elimination of axonal branches.

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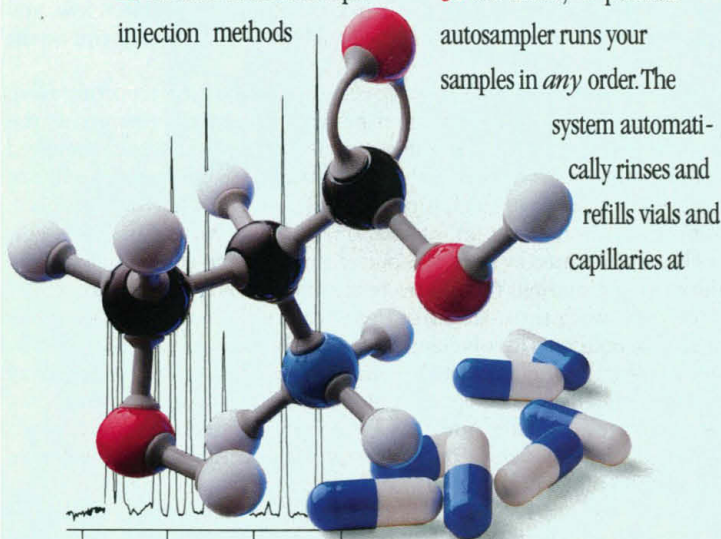
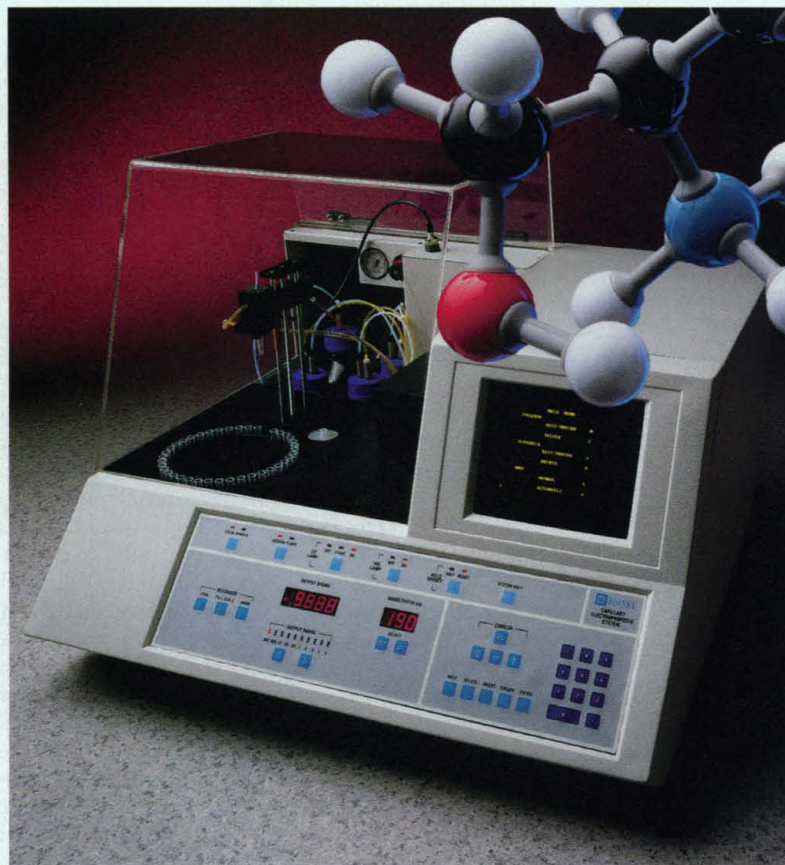
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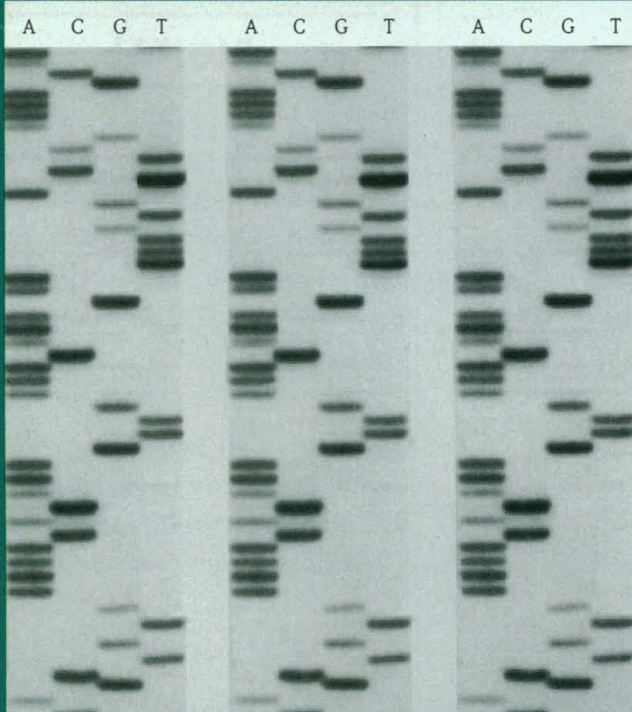


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◀ Autoradiogram of chemiluminescent sequencing reaction using the CircumVent™ Phototope™ Kit. Sequencing reaction of a double-stranded DNA template using Vent[™]_R (exo⁻) DNA polymerase and thermal cycle sequencing procedure, followed by chemiluminescent detection. Lane order A, C, G, T.

Sears, et al. (1992)
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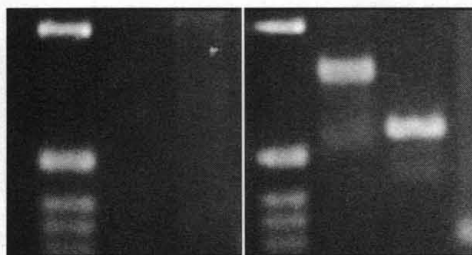
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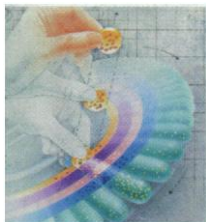
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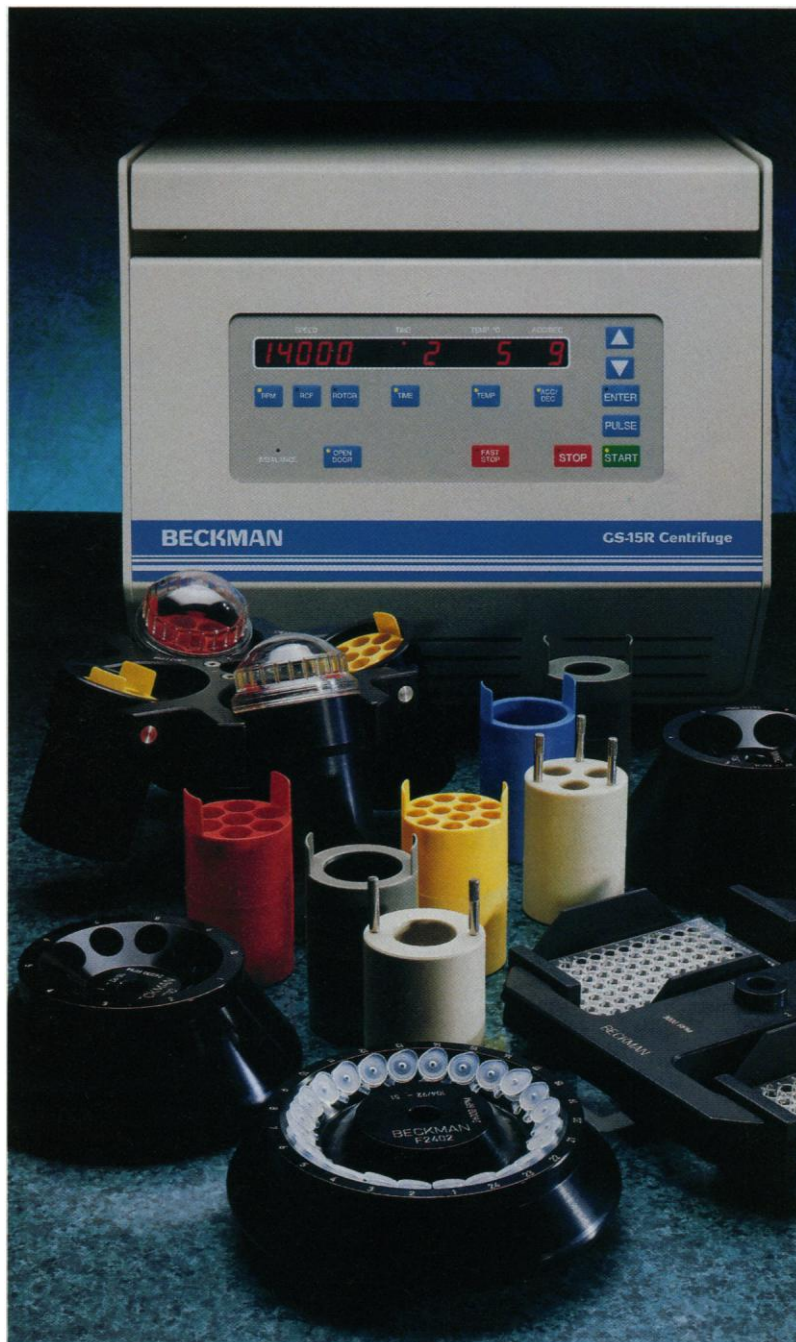
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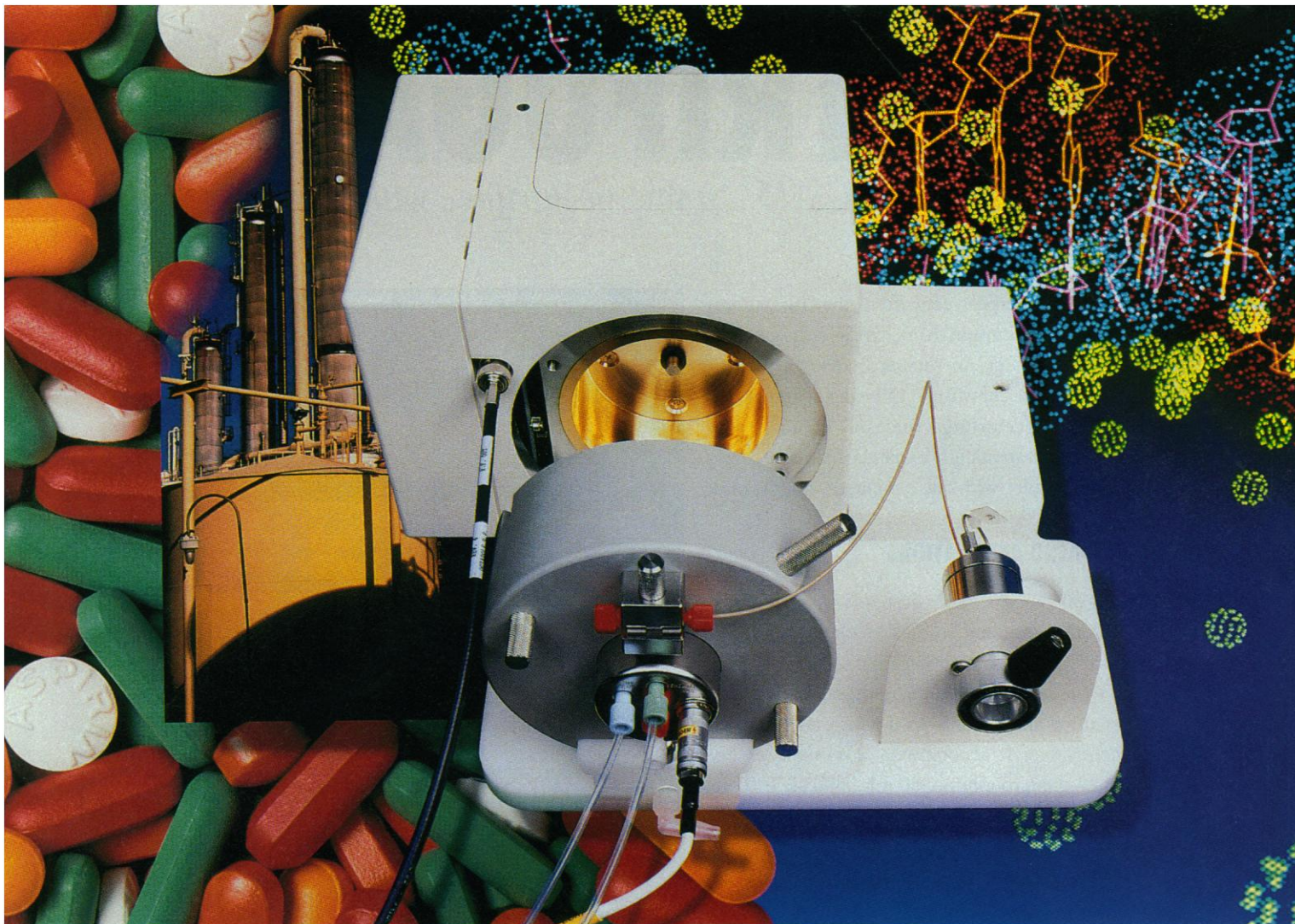


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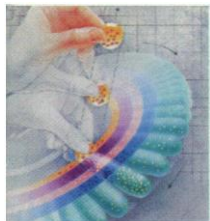
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When we published our survey of "Science in Europe" just over a year ago (24 April 1992, p. 457), Europe was well along the road to a continent without frontiers. Since then European integration has hit some bumps. In this year's special issue, we take a look at what the year's events mean for science. The surprising message: Prospects for collaboration in basic research may actually have improved (p. 1734). On the applied research front, Europe is taking a hard look at industrial projects launched in the 1980s; the results could hold lessons for the United States, now launching similar public-private research efforts (p. 1736).

This year's special issue, like last year's, is not intended to be a comprehensive look at every aspect of European science—an impossible task. Last year, we asked for readers' suggestions of topics they would like to see covered in future issues, and the lineup reflects your responses. In our coverage of scientific disciplines, we asked our reporters to take a look at European chemistry (p. 1738) and at a world-leading effort in oceanographic research (p. 1742). And we come back to an institution we reported on last year: the European Molecular Biology Laboratory, which is under new management (p. 1740). We also asked leading researchers from six countries to discuss recent developments in their own disciplines. Their Perspectives cover two areas of earth science—volcanology (p. 1759) and Greenland ice cores (p. 1766)—evolutionary biology (p. 1760), chemistry (p. 1762), plant biology (p. 1764), x-ray astronomy (p. 1769), immunology (p. 1771), and an intriguing question in human population biology: Where did the Europeans come from (p. 1767)?

Many of you wanted to know more about science in eastern Europe. This year, we have a news report and a Policy Forum on German reunification and its impact on science (p. 1744 and p. 1753), and we examine how developments in Poland (p. 1747) and the newly formed Czech Republic (p. 1748) have affected two leading research institutions. Finally, we take a look at the French government's efforts to encourage the development of centers of scientific excellence outside Paris (p. 1749), and the Swedish government's attempts to shake up basic research (p. 1751).

—Colin Norman, Katrina Kelner, and David Voss

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- Morbidity and Mortality Weekly Report 12 February 1993, **Centers for Disease Control and Prevention,** 1993 Mar 20 (Vol. 42, No. 5) Doc. No. 41
- Morbidity and Mortality Weekly Report 05 February 1993, **Centers for Disease Control and Prevention,** 1993 Mar 17 (Vol. 42, No. 4) Doc. No. 39
- Morbidity and Mortality Weekly Report 29 January 1993, **Centers for Disease Control and Prevention,** 1993 Mar 16 (Vol. 42, No. 3) Doc. No. 38
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CLINICAL ALERT

- Important therapeutic information on treatment of HIV infection in HIV-infected patients who are intolerant of or have failed zidovudine therapy: NIH clinical alert, February 1, 1993, **National Institutes of Health,** 1993 Mar 5 Doc. No. 35

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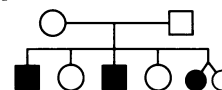
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