American Association for the Advancement of Science

Science

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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

REFERENCES

1. Fiala, G. and Stetter, K. (1986) *Arch. Microbiol.* 145:56-61.
2. Marsh, E., *et al.* (1992) *Stratagies* 5:73-76.
3. Mathur, E., *et al.* (1992) *The San Diego Conference on Nucleic Acids* Abstract #10.
4. Barany, F. (1991) *PCR Methods and Applications* 1:5-16.
5. Barany, F. (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193.
6. Lundberg, K., *et al.* (1991) *Gene* 108: 1-6.
7. Mathur, E., *et al.* (1991) *Nucleic Acids Res.* 19:6952.
8. Hedden, V., *et al. Stratagies* (1992) 5:79-80

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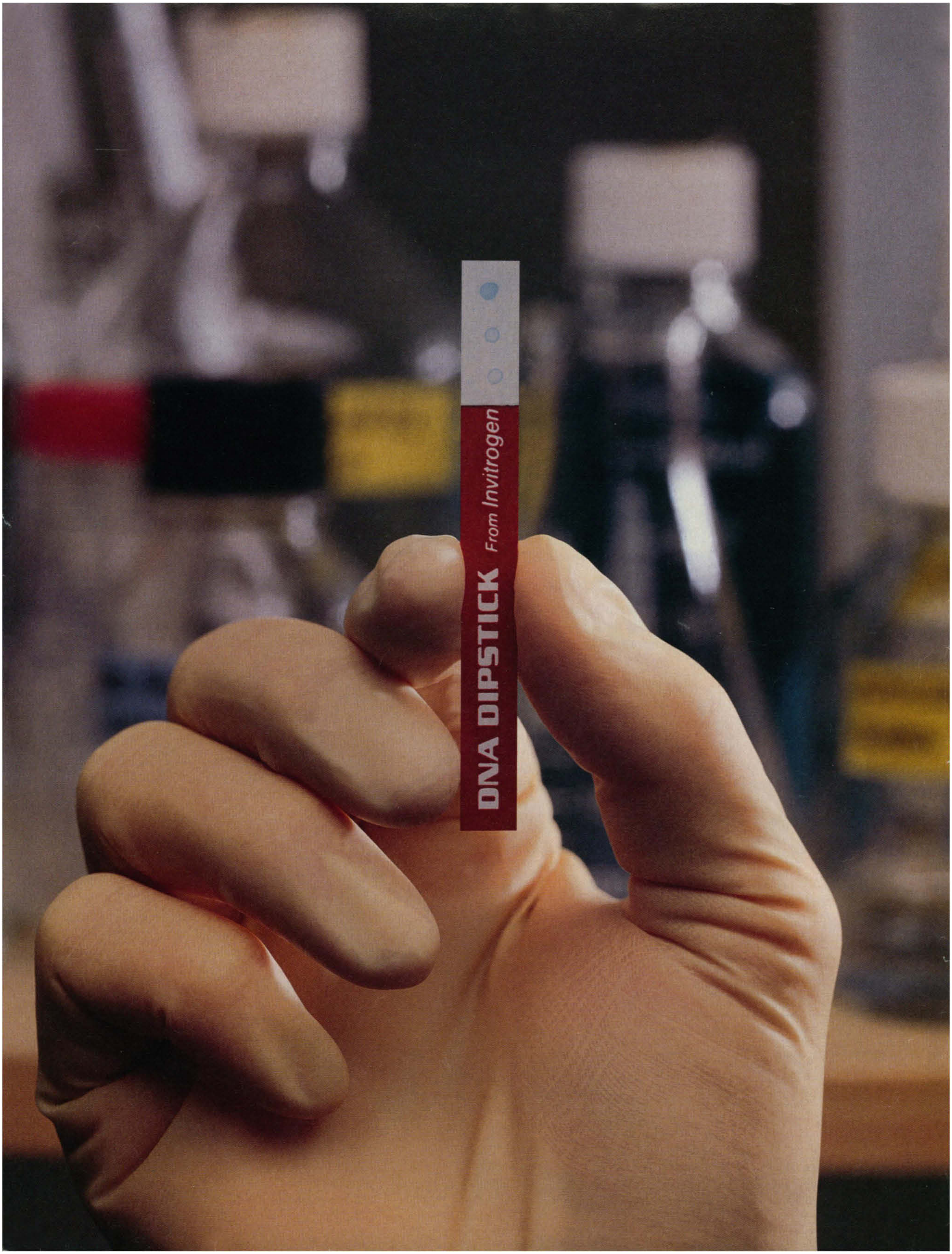
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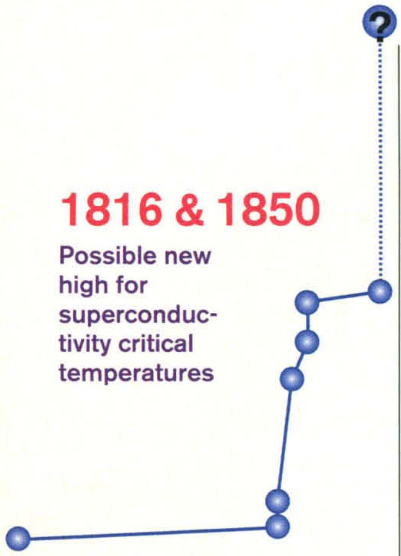
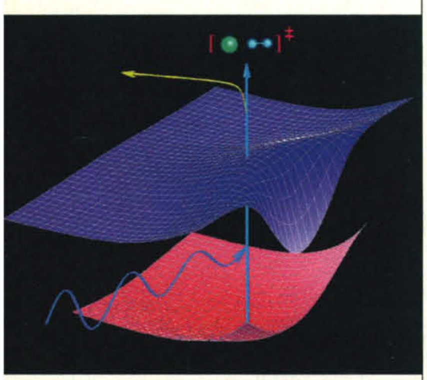


1816 & 1850

Possible new high for superconductivity critical temperatures



1828 & 1852

Making a quick transition

NEWS & COMMENT

RESEARCH NEWS

PERSPECTIVES

ARTICLE

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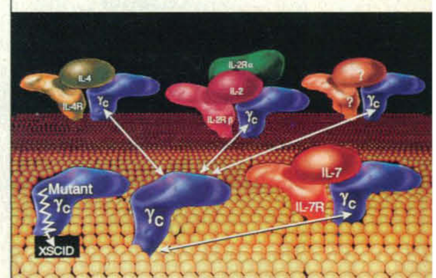
COVER

Model of the protein lysin superimposed on the shells of a red abalone. To fertilize the egg, the abalone spermatozoon must disrupt the protective vitelline envelope. Lysin binds to the filamentous glycoproteins that form the envelope and creates a hole by means of a nonenzymatic process. The lysin-glycoprotein association also contributes to the species recognition between sperm and egg. See page 1864. [Cover design: Peggy Myer. Digital photography: Bob Turner. Molecular model: Mike Pique]

REPORTS



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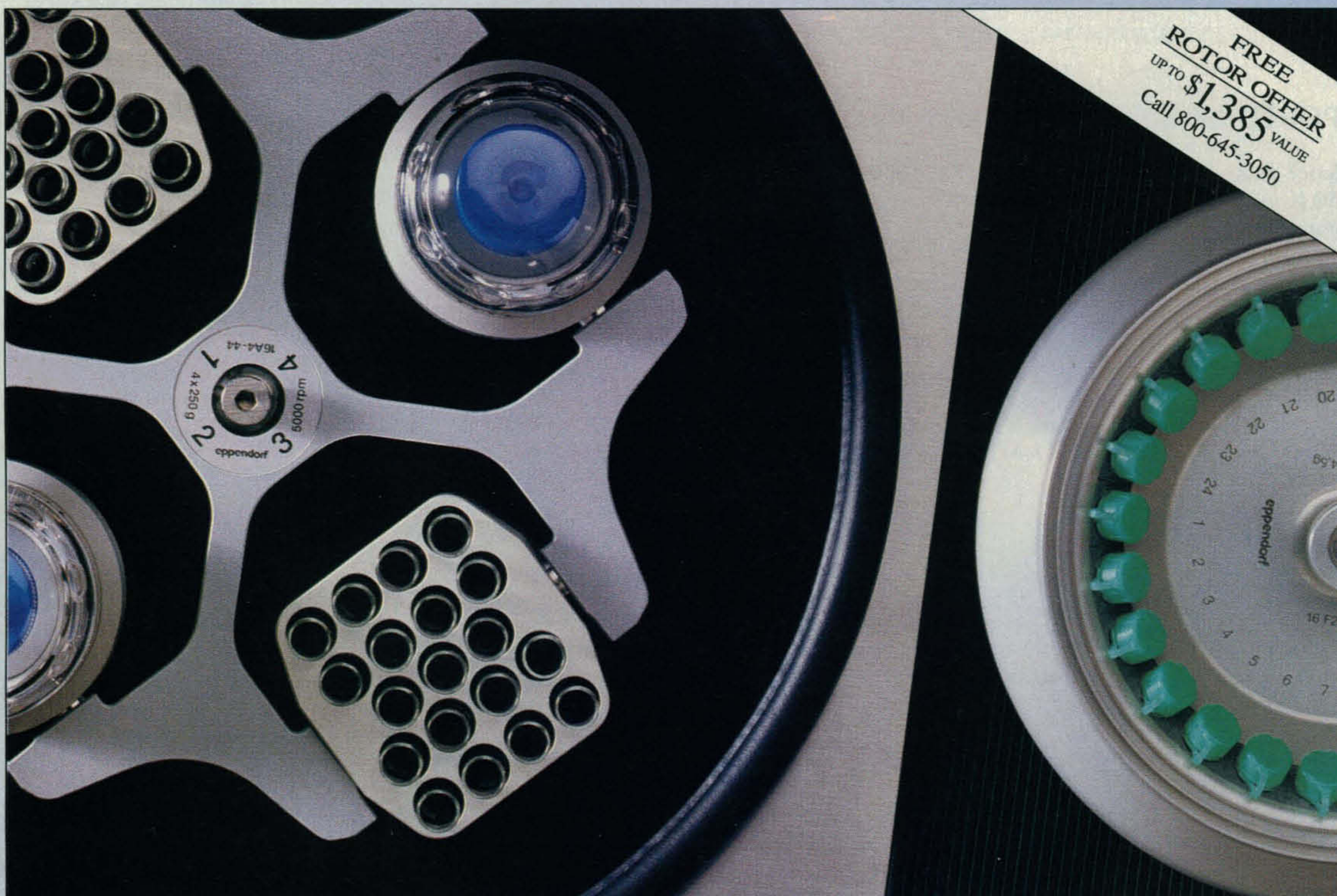

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edited by PHIL SZUROMI

Origins and silence

In yeast, extra copies of the mating-type genes are silenced, or maintained in an inactive state. Silencing requires flanking sequence elements (silencers). One silencer, HMR-E, contains an ARS consensus sequence, a sequence found at yeast replication origins. A link has now been made between transcription silencing and DNA replication (see the Perspective by Newlon, p. 1830). Foss *et al.* (p. 1838) found a mutation in a gene *ORC2* that disrupts both transcriptional silencing and the cell cycle. The sequence of this protein matches that of a protein subunit (ORC2) of the origin recognition complex (ORC) characterized by Bell *et al.* (p. 1844). Li and Herskowitz (p. 1870) screened for proteins that bind to the yeast ARS sequence and found another ORC subunit, ORC6, which was also characterized by Bell *et al.*

Pinning down the transition state

Although numerous studies have been made of the elementary reaction between F and H_2, quantitative agreement between theory and experiment has been elusive. Manolopoulos *et al.* (p. 1852; see the Perspective by Schatz, p. 1828) used photoelectron spectroscopy to characterize the FH_2^- intermediate, which undergoes electron photodetachment near the transition state, for the *para* and *normal* populations of the hydrogen rotational state. Exact quantum reactive scattering simulations of these spectra on a highly accurate ab initio potential energy surface yielded excellent agreement. Such agreement could not be obtained with semiempirical potential energy surfaces that have normally been used.

Pushing superconductivity to 250 kelvin?

One characteristic of the various families of high-temperature cuprate superconductors is that the transition temperature is roughly a function of the number of CuO_2 layers. However, it has proven difficult to synthesize pure phases for materials with more than three layers. Laguës *et al.* (p. 1850; see news story by Pool, p. 1816) report the epitaxial growth of a thin film of an artificial cuprate compound of the BiSrCaCuO family with building blocks of eight adjacent cuprate layers. This material was grown by sequentially imposed layer epitaxy on a single crystal of $SrTiO_3$ at a substrate temperature of 500°C. The resistivity of this material drops five orders of magnitude between 280 and 250 K, and it exhibits a diamagnetic variation of susceptibility and magnetization below 290 K. These and other results suggest the possibility of a superconducting transition at 250 K.

Riding high

Nitrous oxide (N_2O), a greenhouse gas, is currently increasing in abundance in the atmosphere. Like CO_2, its global budget seems to be out of balance between known sources and sinks. To estimate fluxes from sources better, Kim and Craig (p. 1855) measured nitrogen and oxygen isotopic ratios of N_2O in several soils and in stratospheric gas samples. The data imply that there is a large back flux of N_2O from the stratosphere, but this flux appears to be so large that it must in turn be balanced by a large input of N_2O from the oceans.

Separation anxiety

The abundances of siderophile (metal-loving) elements such as nickel and iridium in the Earth's mantle seem too high to account for equilibrium fractionation of the iron-rich core at temperatures up to 1600°C, the limit of earlier measurements. A recent controversial proposal is that this imbalance can be explained by the separation of the core at higher pressures and temperatures where the distribution coefficients for siderophile elements between iron melt and silicate mantle are closer to unity. Walker *et al.* (p. 1858) report measurements of distribution coefficients for some of the problematic elements at temperatures up to 3000 K. Coefficients for germanium, gold, sulfur, and nickel do decrease significantly with temperature, but discrepancies among elements are still evident. The data for sulfur also suggest that it or carbon, rather than oxygen, may be the light element in the core.

Share and share alike

Mutations in the g chain of the interleukin-2 receptor (IL-2Rg) can lead to X-linked severe combined immunodeficiency (XSCID) in humans, yet mice that do not produce IL-2 still develop a population of mature mature T cells. The IL-2Rg was found to be a component required for high-affinity binding by the IL-4 receptor (Kondo *et al.*, p. 1874, and Russell *et al.*, p. 1880) as well as the IL-7 receptor (Noguchi *et al.*, p. 1877). The cytokines IL-4 and IL-7 both exhibit T cell growth factor activity; disabling activation in several receptor systems may account for the virtual absence of T cells in XSCID patients (see news story by Nowak, p. 1818).

Oxygen signal

When infected by a pathogen, plants may produce both local and systemic defensive responses. This phenomenon, termed systemic acquired resistance, is mediated in part by salicylic acid (SA). Chen *et al.* (p. 1883) have cloned an SA-binding protein that has catalase activity and is inhibited by SA. Inhibition increases the amount of active oxygen species and induces the expression of defense-related genes. Active oxygen species as well as SA seem to be critical elements in the signaling pathway from infection to systemic resistance.

In one package

Many cellular functions are controlled by guanosine triphosphate (GTP)–binding proteins. Hydrolysis of GTP by these proteins causes their inactivation. The small GTP-binding proteins like Ras are regulated by GTPase activating proteins or GAPs. However, the heterotrimeric GTP-binding proteins (G proteins) that couple receptors on the cell surface to intracellular signaling pathways have intrinsically high GTPase activity. Markby *et al.* (p. 1895) present evidence that G proteins contain a separate domain that functions as a GAP. The GTP-binding and GAP-like portions of a G protein α subunit were expressed separately and shown to retain their essential functions. The results suggest a mechanism by which GTPase activity may be enhanced.

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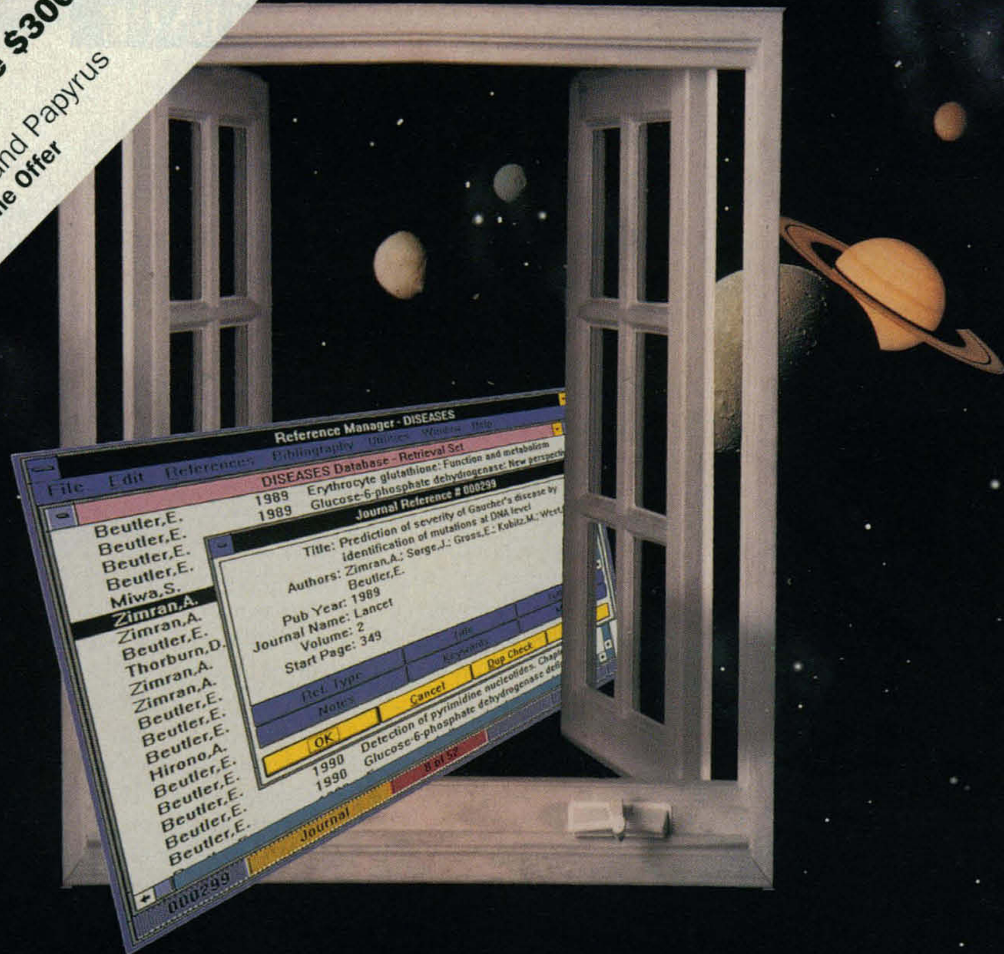

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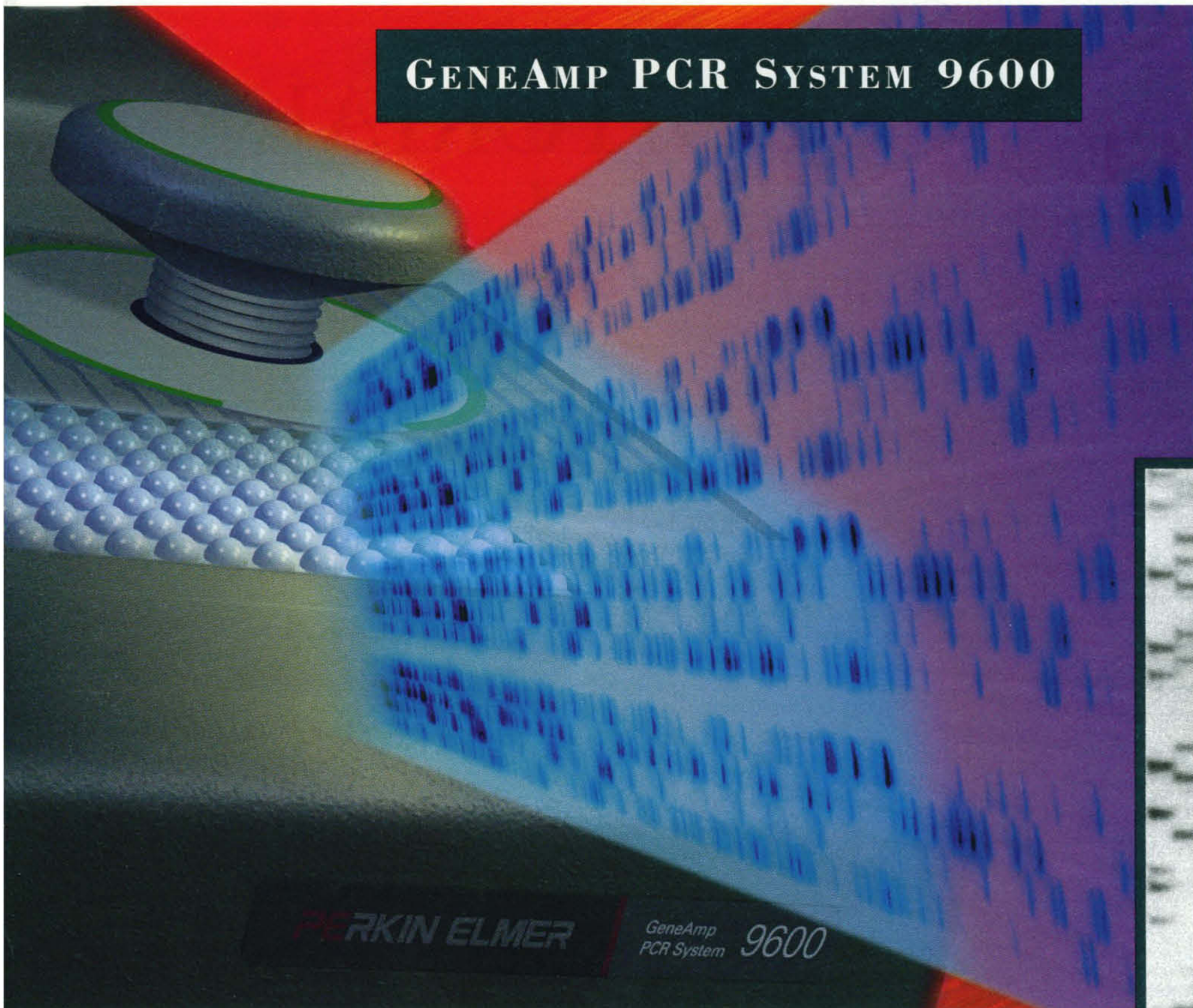

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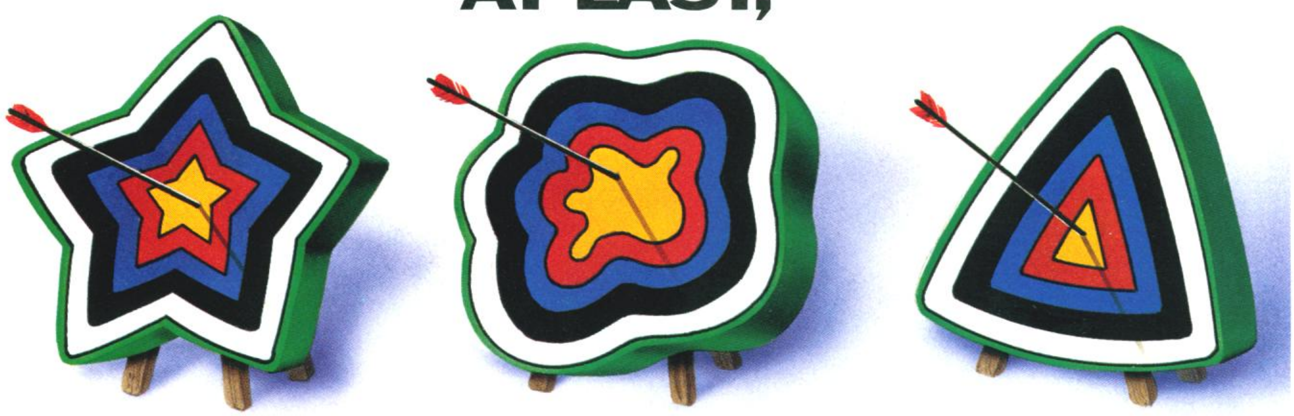

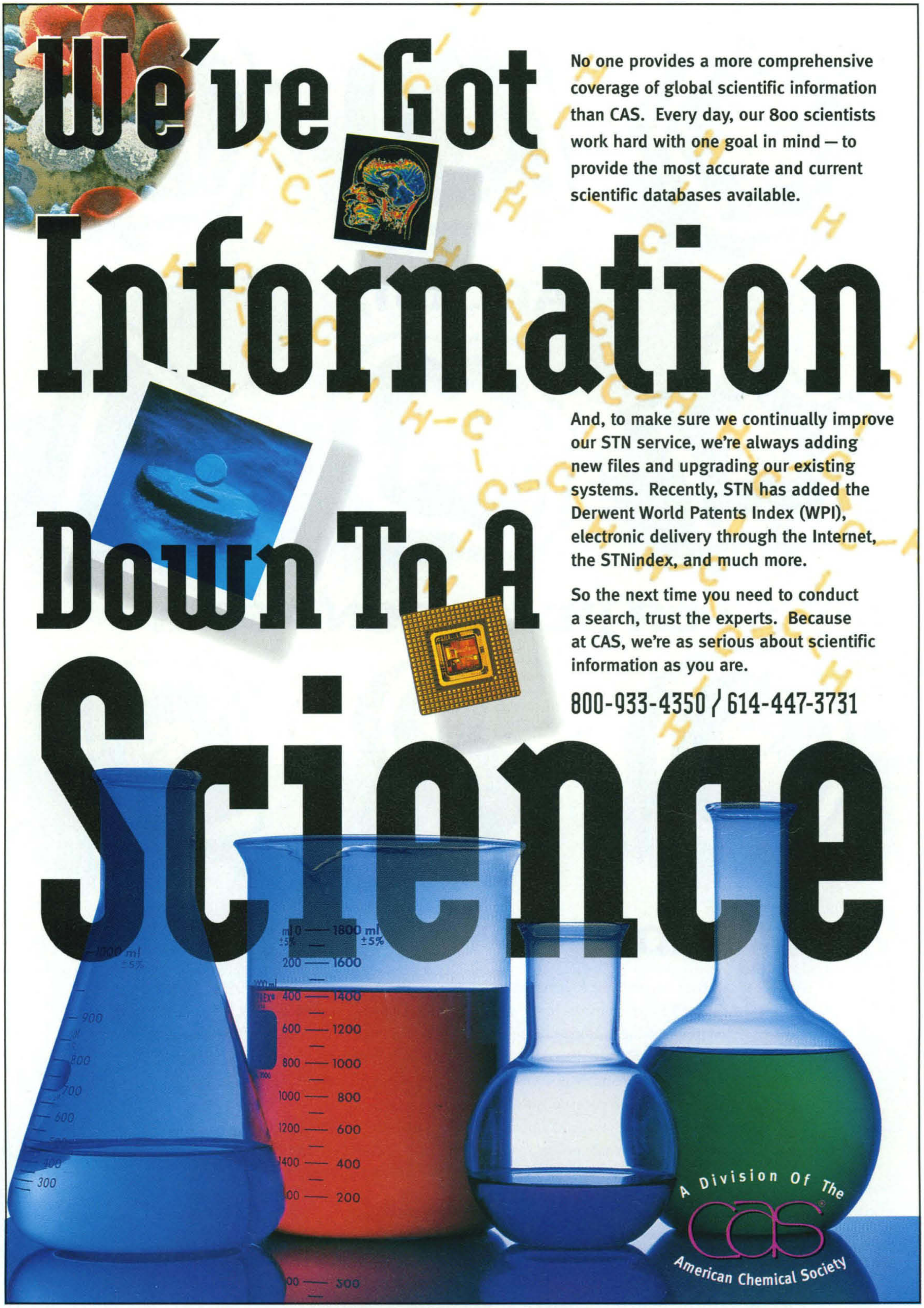
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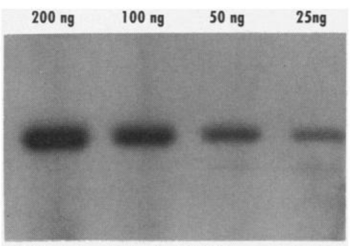

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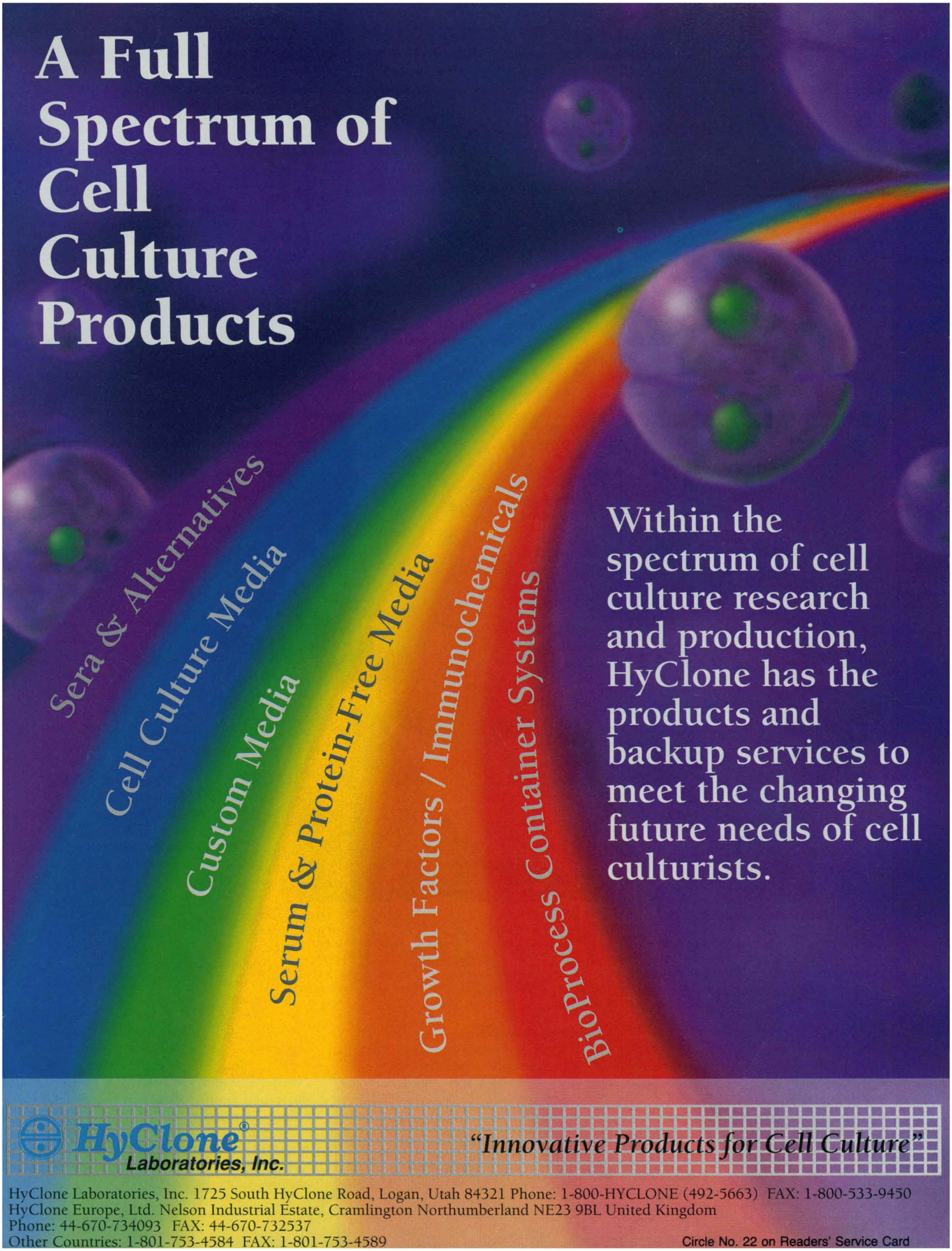
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