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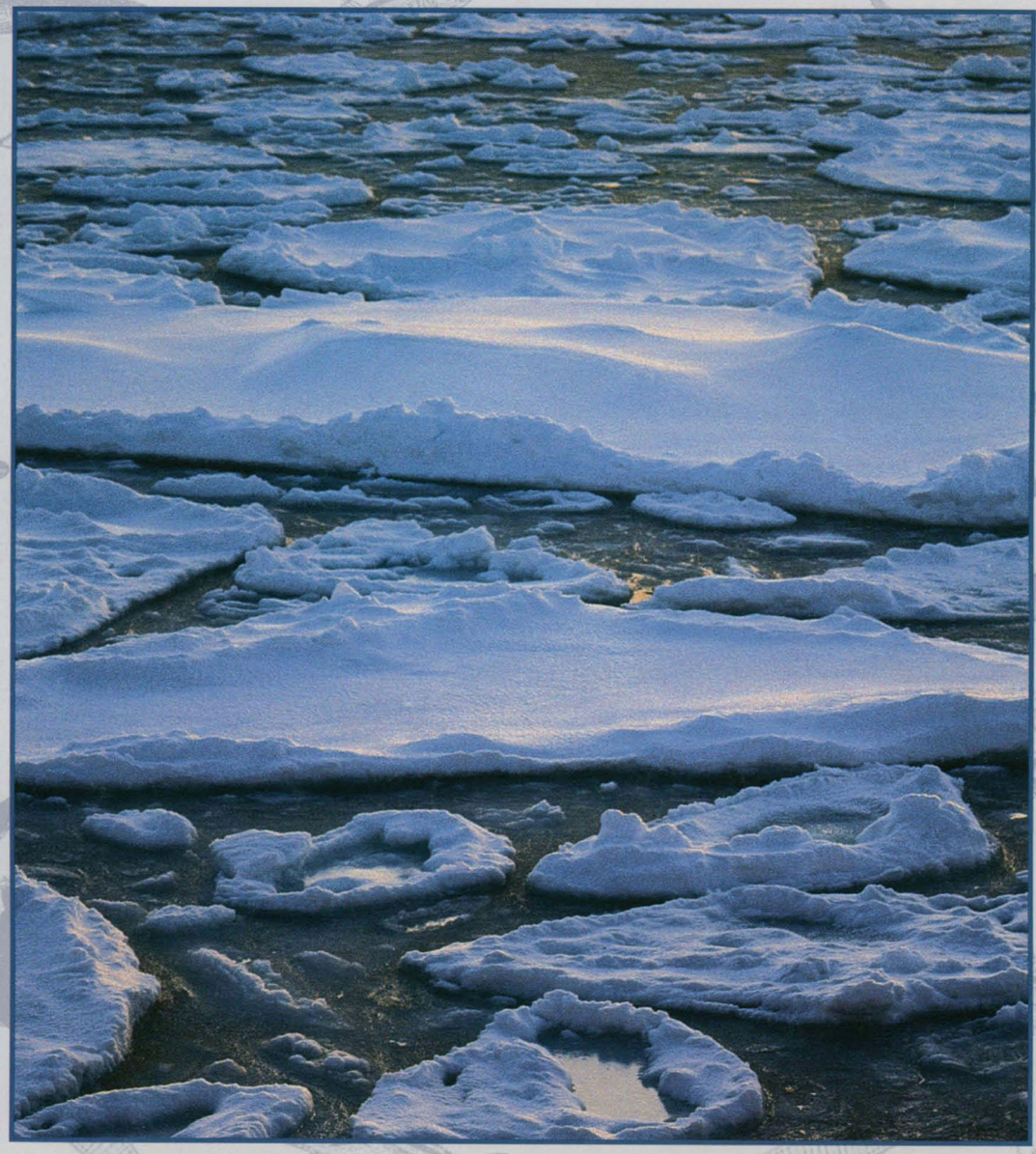
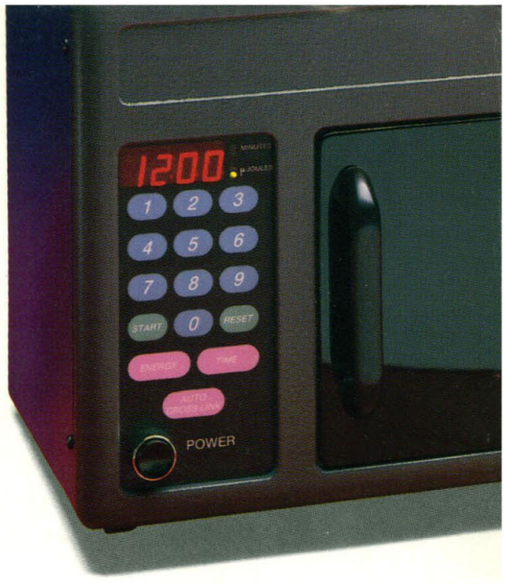
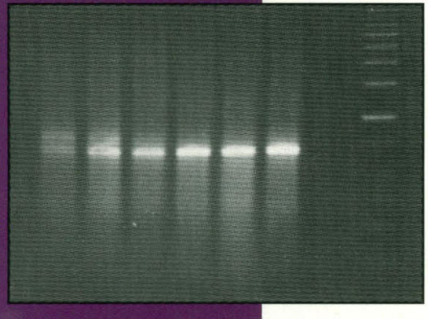


From the Innovators of UV Crosslinking Apparatus

Stratalinker® UV Crosslinker*



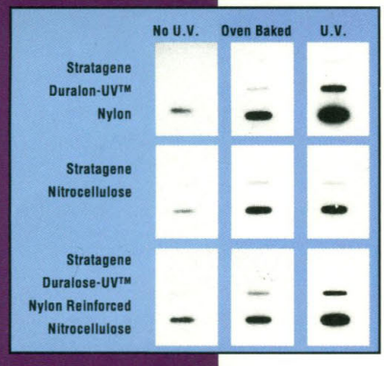
- **Reduce PCR** False Positives**
- **UV Crosslink DNA or RNA to Hybridization Membranes**
- **Fast Results**



Reduce PCR False Positives:

Contaminating DNA can be rendered inactive as a template for PCR by treatment in the Stratalinker® UV crosslinker. Reagents suspected of being contaminated can be pre-irradiated with 254 nm light (either in measured energy doses or by using the timer option) to prevent extraneous sequences from being amplified, which may result in false positives. Because of its simplicity and speed, pretreatment with the Stratalinker® UV crosslinker can be easily integrated into PCR protocols as a precautionary measure.

Six identical tubes, each containing 100 ng of contaminating DNA (4.8 kb linear fragment) in reaction buffer were irradiated at successively higher energy levels in the Stratalinker UV crosslinker. Following treatment, 100 ng of control DNA (4.1 kb linear fragment) was added to each tube, along with recommended amounts of primers, nucleotides, and polymerase. PCR was performed according to the GeneAmp† kit (Perkin-Elmer Cetus Instruments) instructions. Amplification of the contaminating DNA yields a 1.8 kb fragment, whereas the control DNA with the same primers yields a 1.1 kb fragment. Lanes 1-6: 0, 25, 50, 100, 200, and 400 mJ/cm2 UV energy, respectively. Lane 7: Stratagene's Kb size ladder (500 ng).



UV Crosslink DNA or RNA to Membranes:

The degree of ultraviolet crosslinking can dramatically affect the binding and sensitivity of nucleic acid hybridization (see accompanying chart). The versatile Stratalinker® UV crosslinker can be used for the following applications:

- Binding RNA or DNA to nitrocellulose, nylon or hybrid membranes for Northern, Southern, dot, or slot blot analysis (1, 2).
- Linking DNA to filters for bacterial or phage library screening.
- DNA nicking in agarose gels prior to blotting (3).
- Dimer formation to perform partial digests for rapid gene mapping (4).
- Confirming recA+ versus recA- genotypes in *E.coli* strains through UV sensitivity testing (5).

1. Khandjian, E.W. *Biotechnology* 5 February 1987.
2. Church, G.M., Gilbert, W. *P.N.A.S.* 81: 1991-1995, 1984.
3. Vollrath, D. and Davis, R.W. Nucl.Acids Res. 15:7865-7876, 1987.
4. Whittaker, P.A., Southern E.M. Gene 41: 129-134, 1986.
5. Maniatis, T., et al Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory.

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* patents pending

**The polymerase chain reaction (PCR) process is covered by patents issued to Cetus Corporation.
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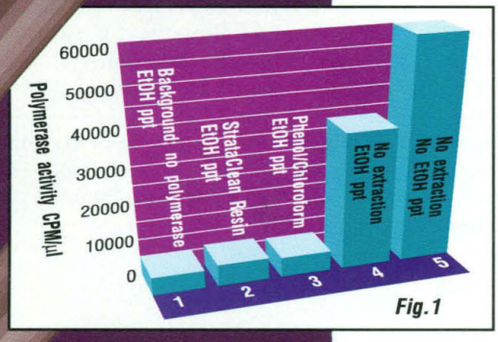

Fig. 1

Remove Polymerase Activity after PCR with StrataClean Resin

StrataClean™ resin has proven to be a safe, fast, and efficient method for the removal of polymerase activity from PCR reaction mixtures. Using StrataClean resin, it is also possible to eliminate an ethanol precipitation step before cloning PCR amplified DNA.

Figure 1. Comparison of StrataClean resin and phenol/chloroform extraction methods for removal of Polymerase activity following amplification reactions. Four identical 100µl amplification reactions were treated as follows: no extraction; two extractions with StrataClean resin; two extractions with phenol/chloroform; and a single precipitation with ethanol. The samples were then assayed for polymerase activity at 72°C using a modified activated calf thymus/gap filling assay, essentially as described by Maki; *et. al.* (2). Background activity was measured by assaying an amplification reaction without any added polymerase.

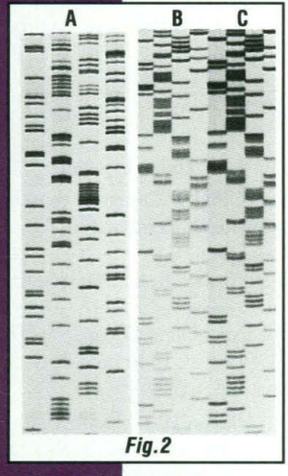

Fig. 2

Sequence Quality Plasmid Templates from Mini-preps Using StrataClean Resin

StrataClean resin has also been shown to produce sequencing quality plasmid templates when used in either a rapid boiling mini-prep or as a substitute for phenol in a standard alkaline lysis mini-prep. The key to the rapid mini-prep is that no precipitation step is required. StrataClean resin is used to remove the unwanted cellular protein quickly and efficiently.

Figure 2. Sequence derived from DNA purified using StrataClean resin with both the standard rapid boiling mini-prep procedure and the alkaline lysis mini-prep procedure. Panel A: StrataClean resin rapid boiling mini-prep; Panel B: phenol/chloroform alkaline lysis mini-prep; Panel C: StrataClean resin alkaline lysis mini-prep.

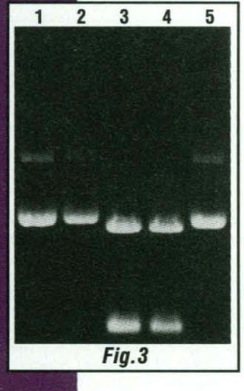

Fig. 3

Extract Restriction Endonucleases and many DNA Modifying Enzymes from Nucleic Acids

Quantitative removal of restriction enzymes from DNA can be accomplished in a matter of minutes with StrataClean resin and eliminates the hazards associated with liquid phenol extractions. The StrataClean resin extraction relies on the use of patented hydroxylated silica particles which exhibit characteristics similar to phenol[1,2]. It is supplied as a 25% slurry which is non-toxic, non-flammable and odor-free. StrataClean resin has a very high affinity for proteins and a very low affinity for nucleic acids at neutral pH (>1900:1 respectively).

1. U.S. Patent Serial No. 4,923,978 2. Strategies Vol. 3 Number 4

Figure 3. Ethidium stained agarose gel. Lane 1: control uncut plasmid DNA, Lane 2: the same DNA after standard StrataClean resin extraction, Lane 3: plasmid DNA digested with 4 units *Pvu* II, Lane 4: plasmid DNA after standard StrataClean resin extraction then digested with *Pvu* II, Lane 5: 24 units *Pvu* II extracted with StrataClean resin from 20 microliters of 1X Universal buffer, plasmid DNA then added and incubated at 37°C for 18 hours.

* The polymerase chain reaction (PCR) process is covered by patents issued to Cetus Corporation.

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COVER Pack ice in the marginal ice zone (MIZ) of the Southern Ocean. Springtime melting of the ice results in strong vertical stratification of the water column, which restricts phytoplankton blooms to the near-surface waters of the MIZ. This stability within the MIZ potentially restricts the growing phytoplankton to depths at which midultraviolet (UVB) irradiance has increased significantly in areas beneath the Antarctic ozone hole. See page 952. Detrimental effects of this increased UVB flux on phytoplankton productivity might propagate up the food chain. [Photo by R. C. Smith]

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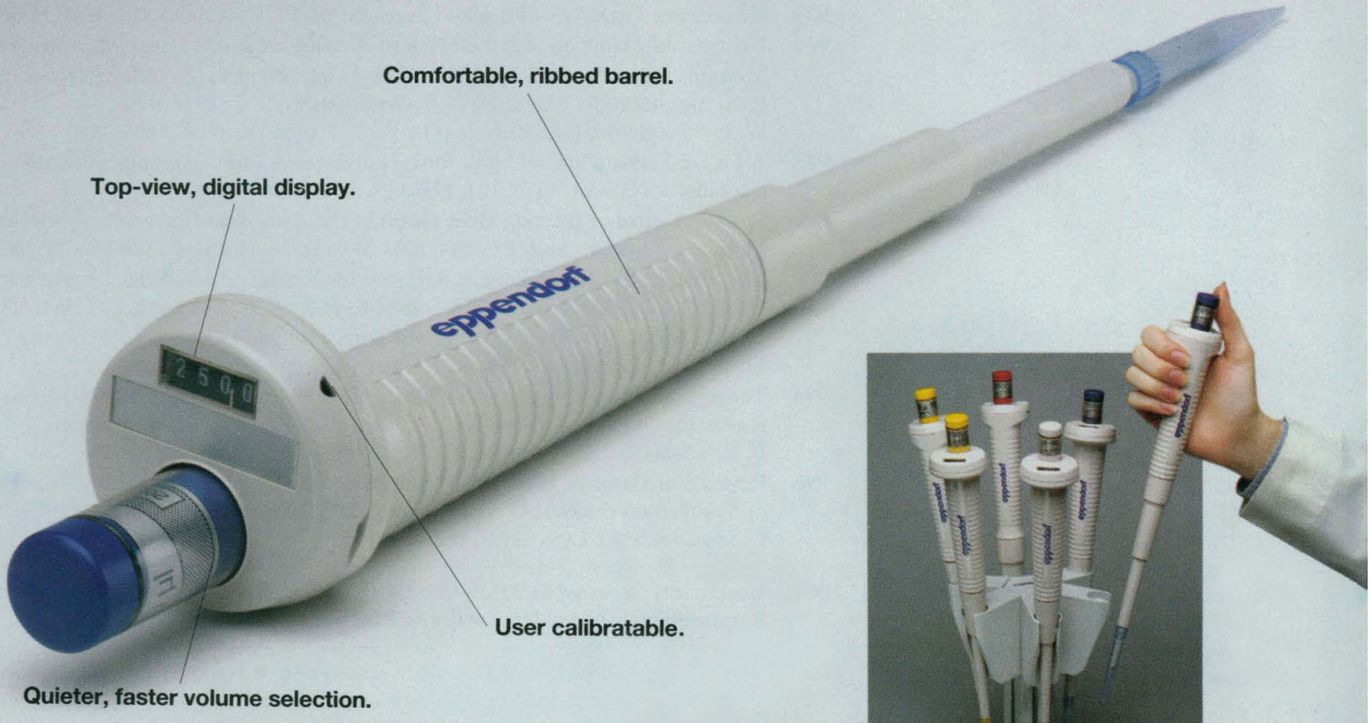

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This Week in

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Class and health

Are the higher rates of mental illness generally seen in the lower social classes caused by stress due to lower class status (social causation), or do the mentally ill fall in social class or fail to rise (social selection)? Dohrenwend *et al.* (p. 946) compared disorder rates between two ethnic groups at two social class levels. He used a large cohort of young adults in Israel, comparing those of European descent with others of North African descent who experience prejudice. Social selection was implicated in schizophrenia and social causation was implicated in depression, antisocial behaviors, and substance abuse.

Fitting in antigen

Induced fit in antibody-antigen recognition was seen by Rini *et al.* (p. 959), who compared the x-ray structures of an unliganded antibody, Fab 17/9, and its complex with a peptide antigen. Fab 17/9 is a specific antibody to a peptide immunogen corresponding to 36 residues from influenza virus hemagglutinin (HA1); the peptide antigen corresponds to a nine-residue sequence in HA1. Antigen binding induces a major rearrangement in the hypervariable loop H3 of the antibody to create a binding pocket for a β turn in the peptide and to accommodate a Tyr residue. Flexibility in the antibody combining site allows the peptide to be in a conformation similar to that in HA1.

Polymer displacement

A strongly adsorbing polymer should in principle displace a more weakly adsorbed polymer from a surface, but the more weakly bound polymer can avoid displacement by being sterically pinned to the surface by the network of loops formed by its stronger neighbor. Johnson and Granick (p. 966) point out that the non-Arrhenius time evolution of their experimental results shows similarity to phenomena in bulk glasses.

Organic aerogels

Silica aerogels, with their open structure, make good thermal insulators; Lu *et al.* (p. 971) made organic aerogels with improved thermal properties. These resorcinol-formaldehyde aerogels have considerable mechanical flexibility, in contrast to the more rigid silica aerogels. The material may provide an alternative to chlorofluorocarbon foams for thermal insulation.

Predicting diversity

Another central problem in ecology is predicting community diversity. Hochberg and Hawkins (p. 973) present a model in which refuges are essential in explaining global patterns of richness of parasitoid species. Parasitoids, insects which parasitize and can kill other insects, thrive in diverse communities of ten or more species per host. In numerical simulations of many generations of these predator-prey communities, factors such as initial densities, carrying capacity, and search efficiency for predators were varied. If a factor was not included for refuges from parasitoid attack, the model failed to reproduce known diversity patterns.

Seeing diversity

Amazonian rain forests are a species-rich area, but greater mammalian diversity appears to occur in the drylands, according to Mares (p. 976). Data on the distribution of 883 species of South American mammals in six major habitats showed that the drylands, the largest habitat, are home not only to the greatest number of mammalian species but are also more diverse in supporting the greatest number of endemic taxa. If conservationists ignore this mammalian and possibly other species richness, novel taxa may not be preserved. Pimm and Gittleman (p. 940) discusses the complex issues involved in determining where the diversity is and predicting what may remain.

One-cell chemistry, or less

For years, neuroscientists have been using microelectrodes to monitor electrical activity in brains and neural tissue, often in single neurons. But behind this electrical behavior is a lot of quick and complex chemistry, also occurring on the single-cell and subcellular level. With a growing battery of analytical tools and methods (p. 925), chemists are providing neuroscientists the means for such fine-grained chemical eavesdropping.

Choosing sides

Developmental signals can establish pattern formation in oocytes and early embryos. Mowry and Melton (p. 991) studied localization in *Xenopus* oocytes of VG1 RNA, which is found exclusively in the vegetal hemisphere of mature oocytes and is an early indicator of axis formation. A 340-nucleotide signal in the 3' untranslated region is sufficient to direct RNA localization. In early *Drosophila* development, the gap gene *knirps*, required for posterior patterning, appears to operate through a different mechanism than the anterior pattern-formation genes. Pankratz *et al.* (p. 986) show that *knirps* has the potential to be expressed throughout the embryo; its borders of expression are set by negative regulation. In the anterior, *bicoid* sets the borders of *hunchback* expression by directly activating it.

RNA mimicry

Threonyl–transfer RNA synthetase (ThrRS) in *Escherichia coli* can negatively regulate its own translation by binding to its mRNA leader sequence, which has structural analogies to $tRNA^{Thr}$. Graffe *et al.* (p. 994) used tRNA identity rules to create a leader sequence in ThrRS mRNA that would be recognized by methionyl-tRNA synthetase (MetRS). This identity change caused translation of ThrRS to be regulated by MetRS.

EDITED BY PHILLIP D. SZUROMI

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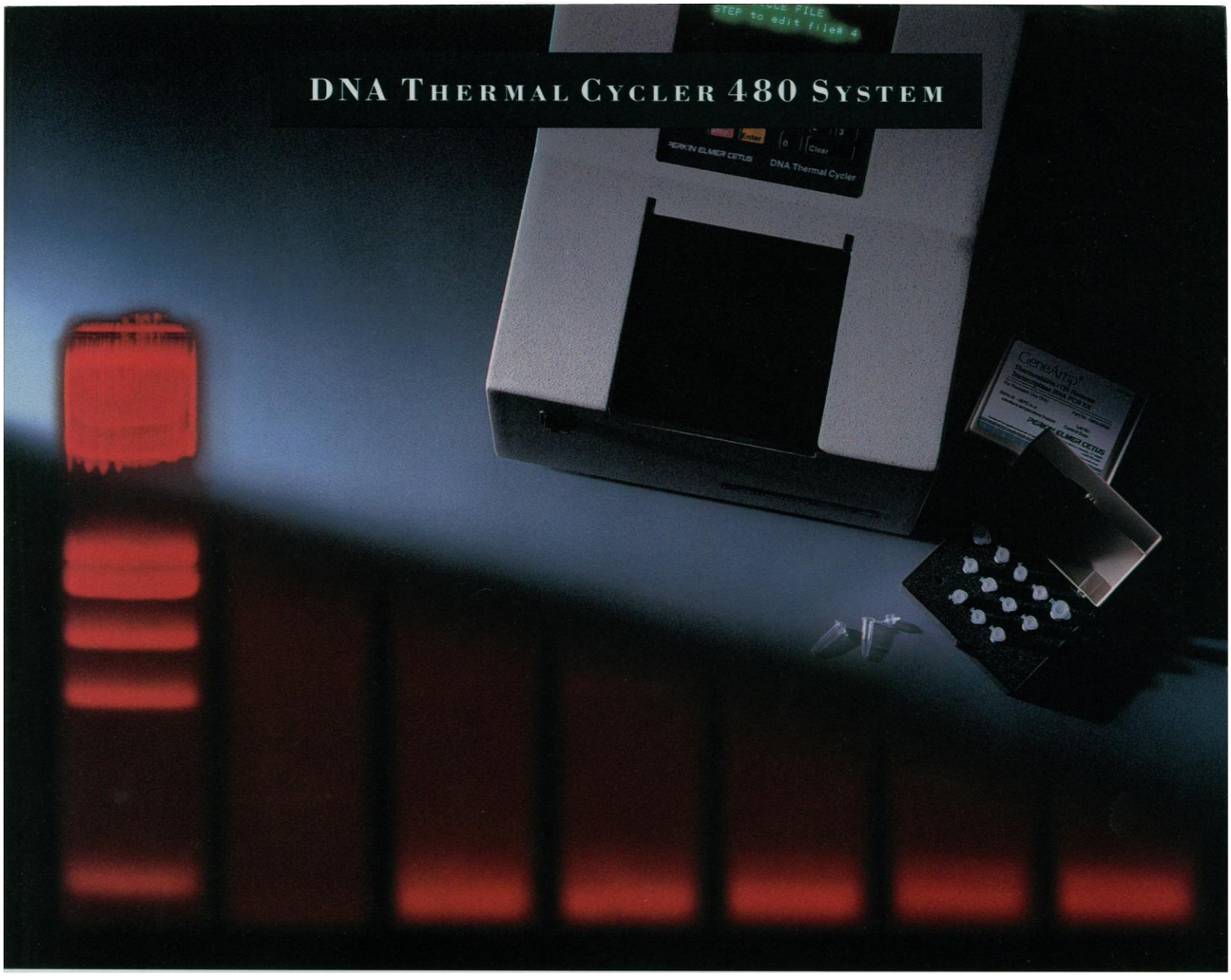

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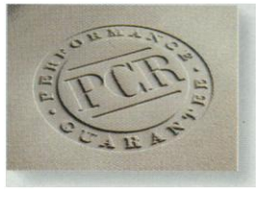

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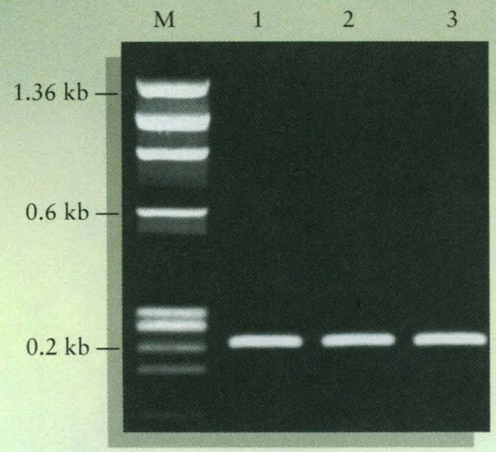
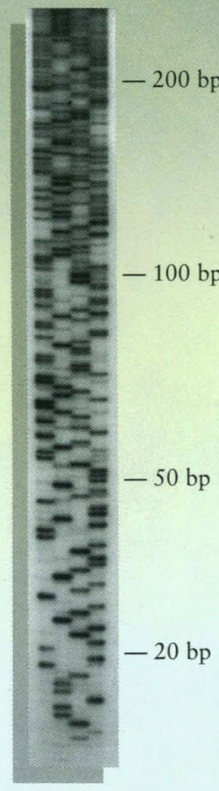


PCR† testing of stability of DNA Polymerization Mix. A target sequence in human genomic DNA was amplified using DNA Polymerization Mix which had been stored under various conditions. Lane 1, storage at -20°C for three months; lane 2, after 50 freeze/thaw cycles (-20°C to 37°C); and lane 3, stored at 37°C for three months. The marker (M) is φX-174 RF DNA-*Hae* III (27-4044-01).



Sequencing results obtained with column-purified PCR† products. A Miniprep Spun Column (27-5101-01, -02) was used to remove primers and unincorporated nucleotides from the amplified DNA prior to sequencing. The figures in the margin denote distance from the 5'-end of the sequencing primer.

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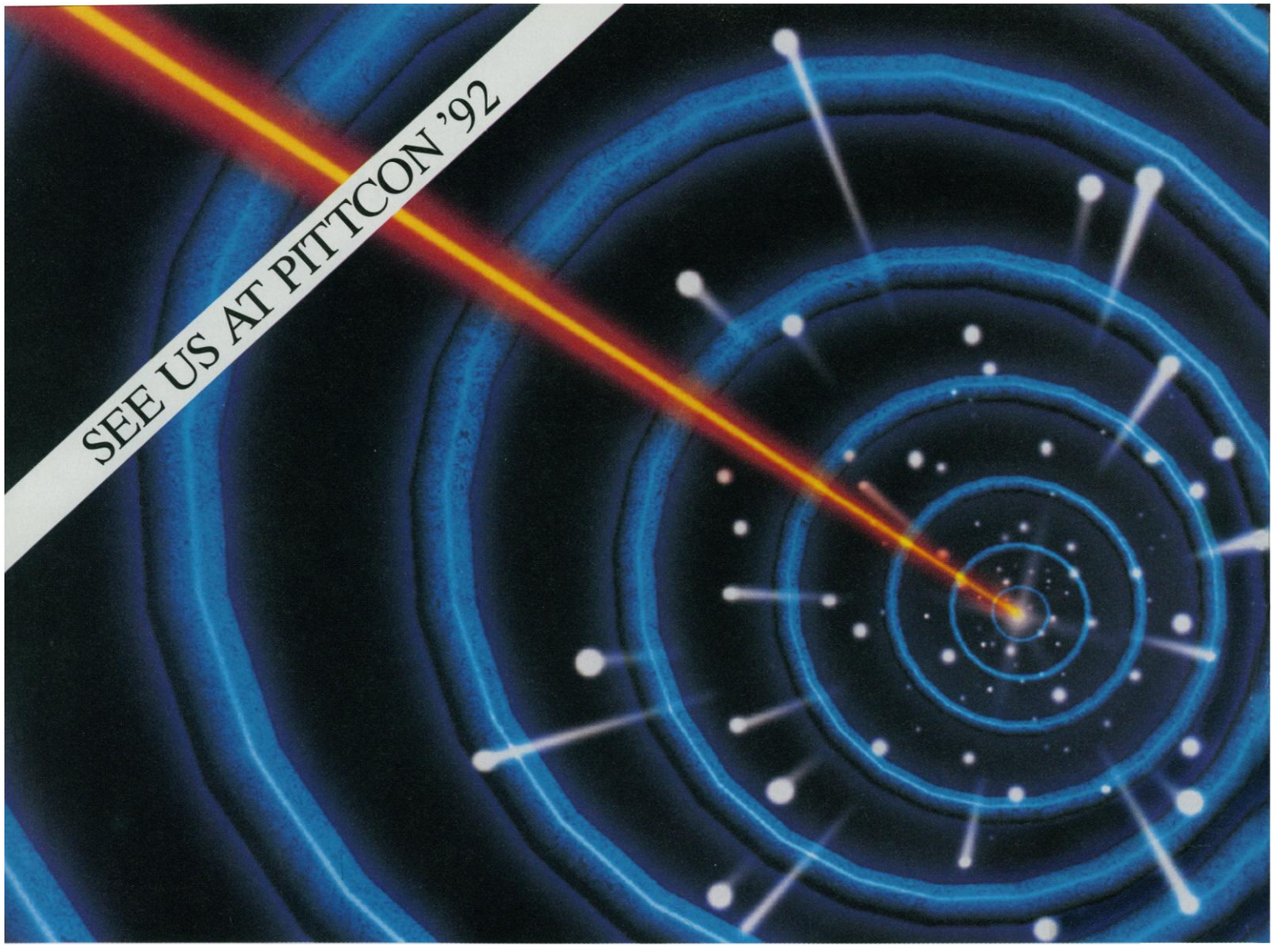

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**patent pending.* *mRNA model courtesy of* BIOSYM

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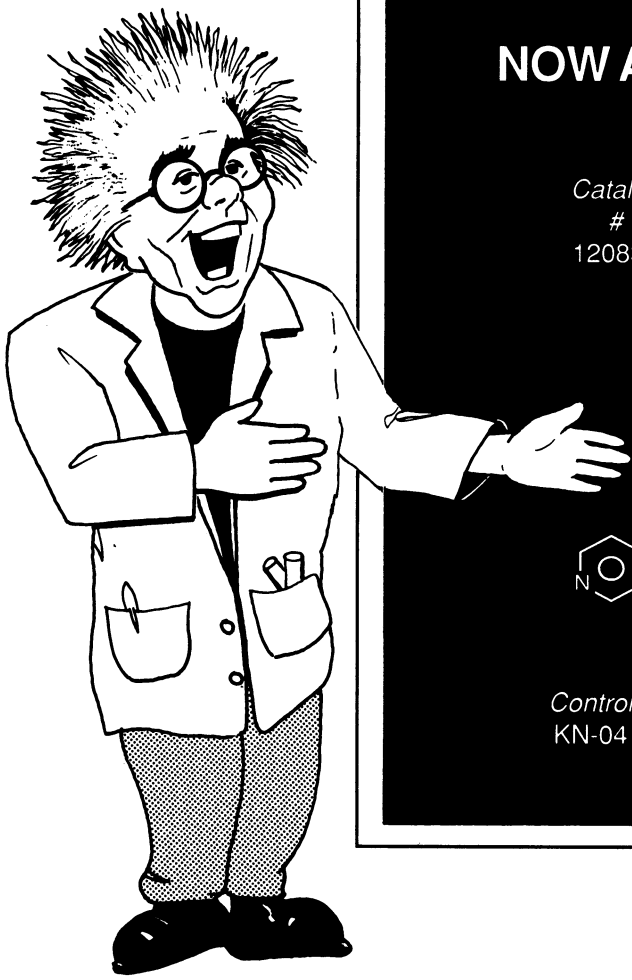

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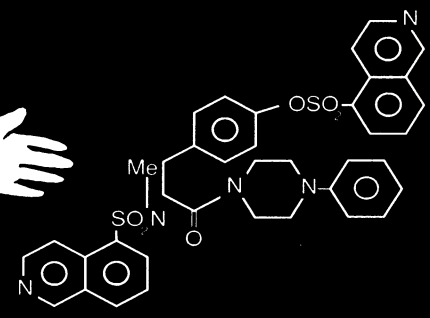

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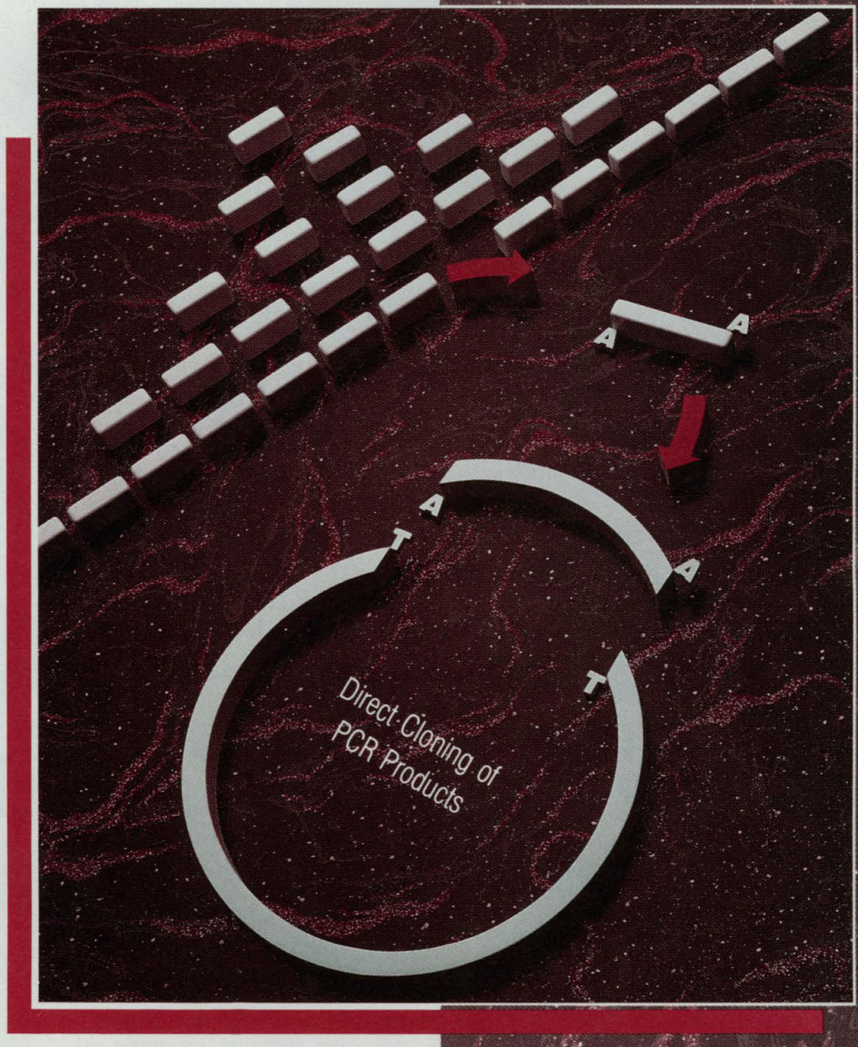

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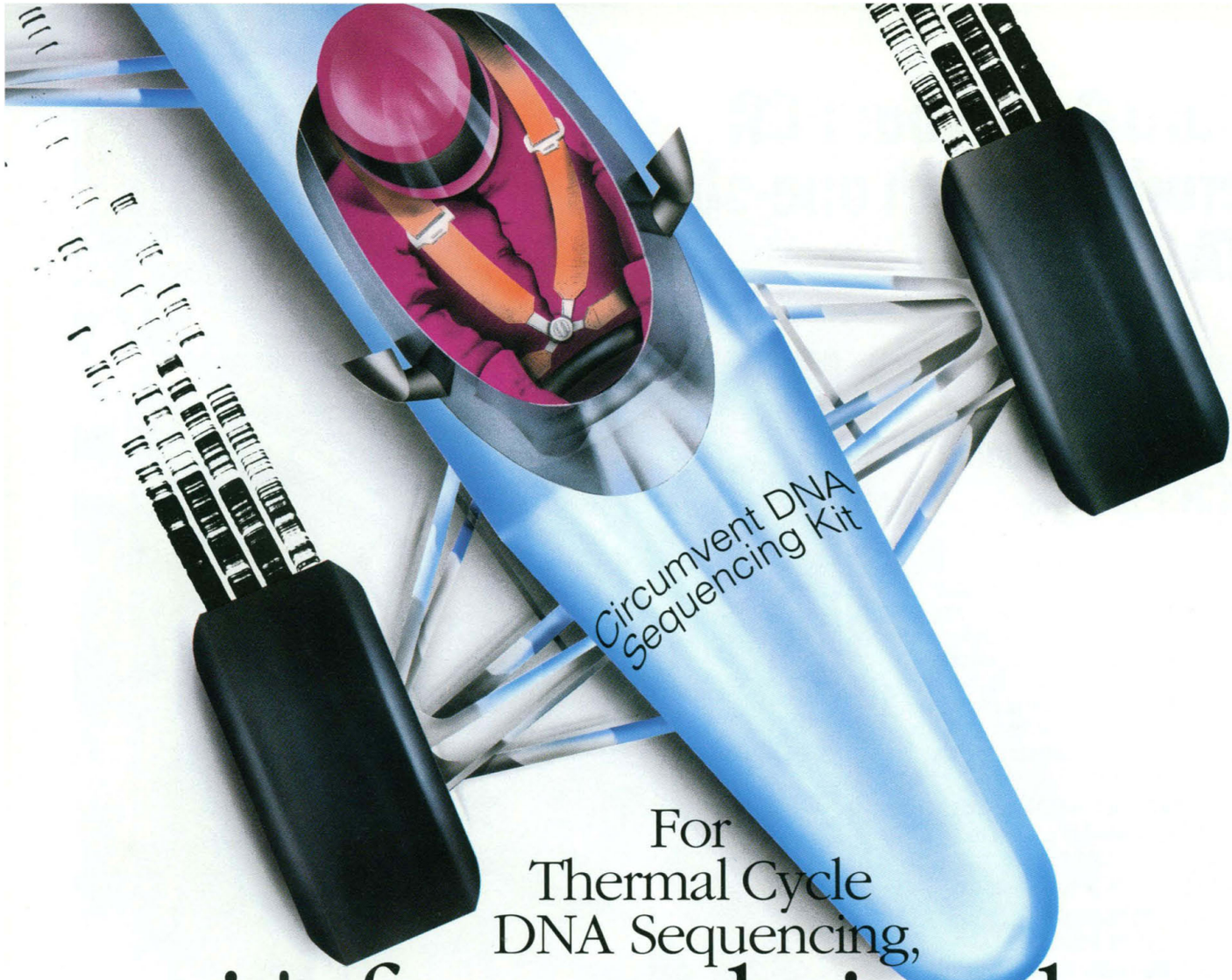

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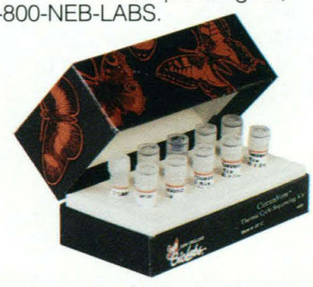

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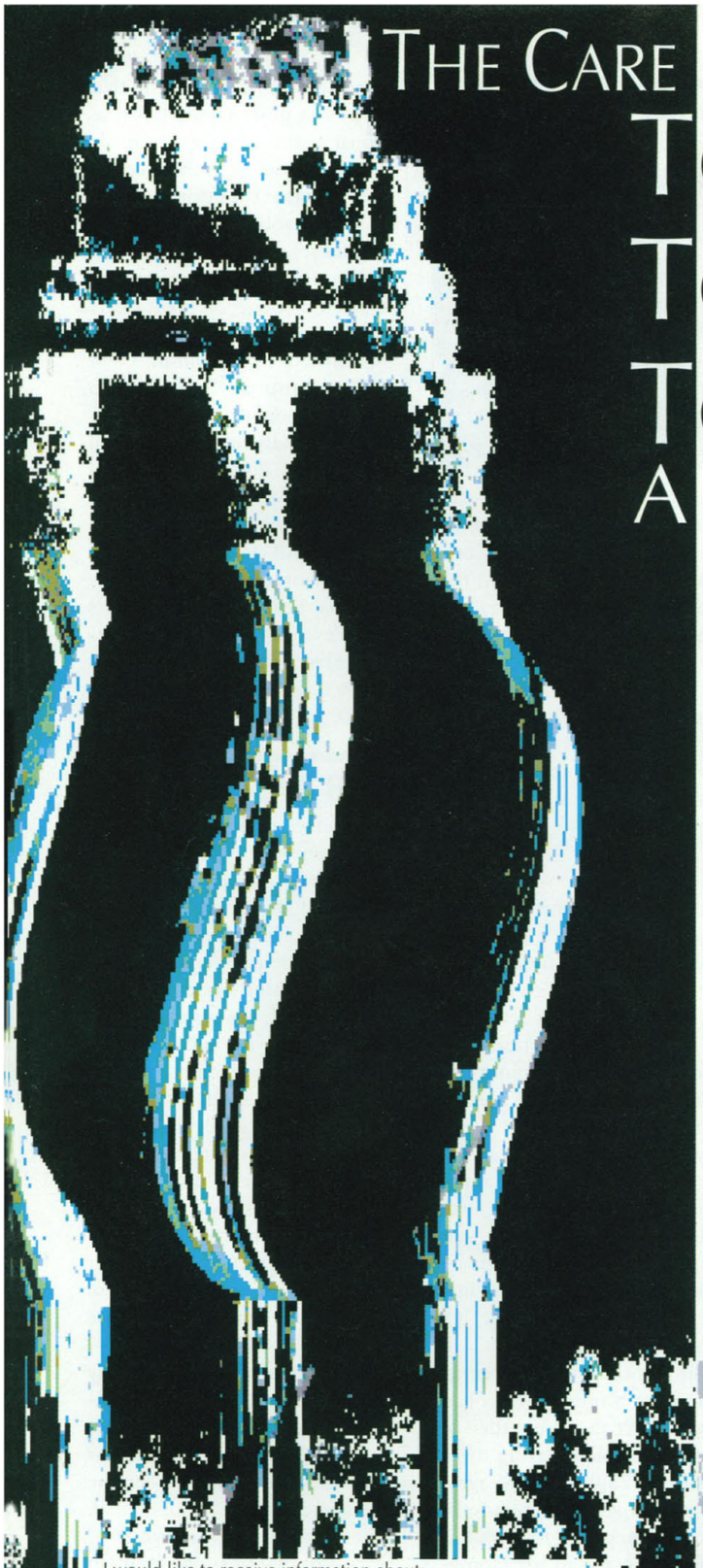

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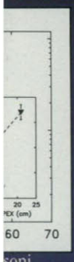

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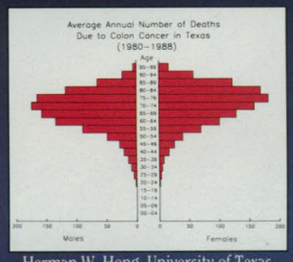

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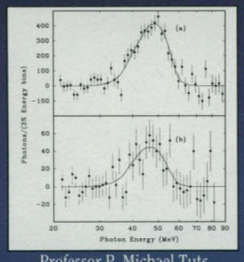

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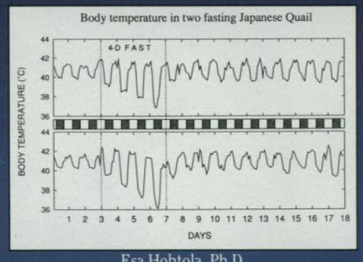

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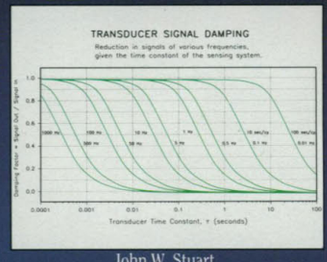

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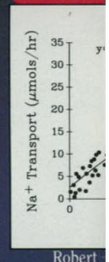

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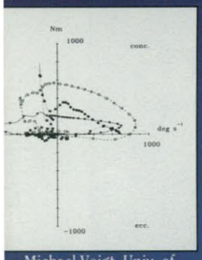

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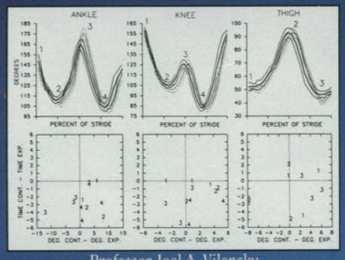

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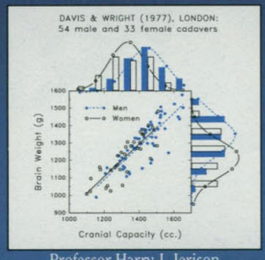

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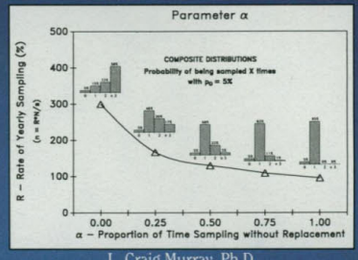

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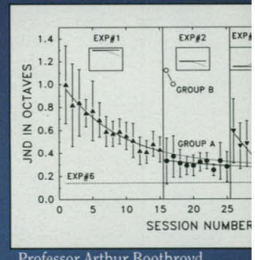

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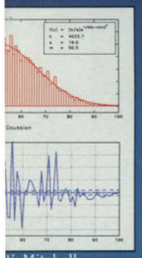

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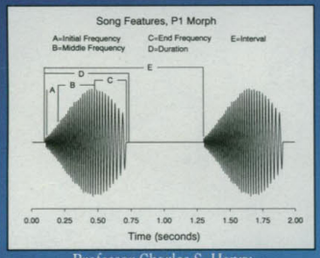

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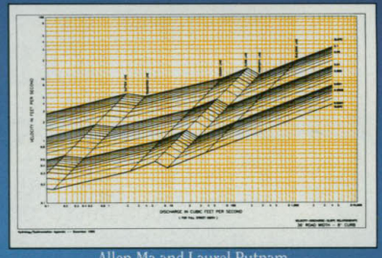

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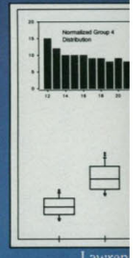

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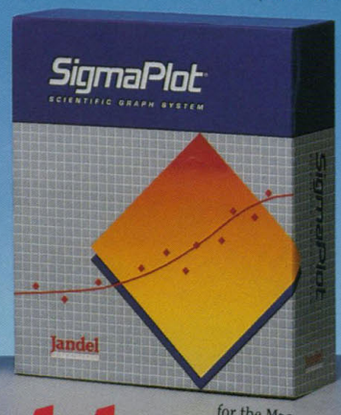

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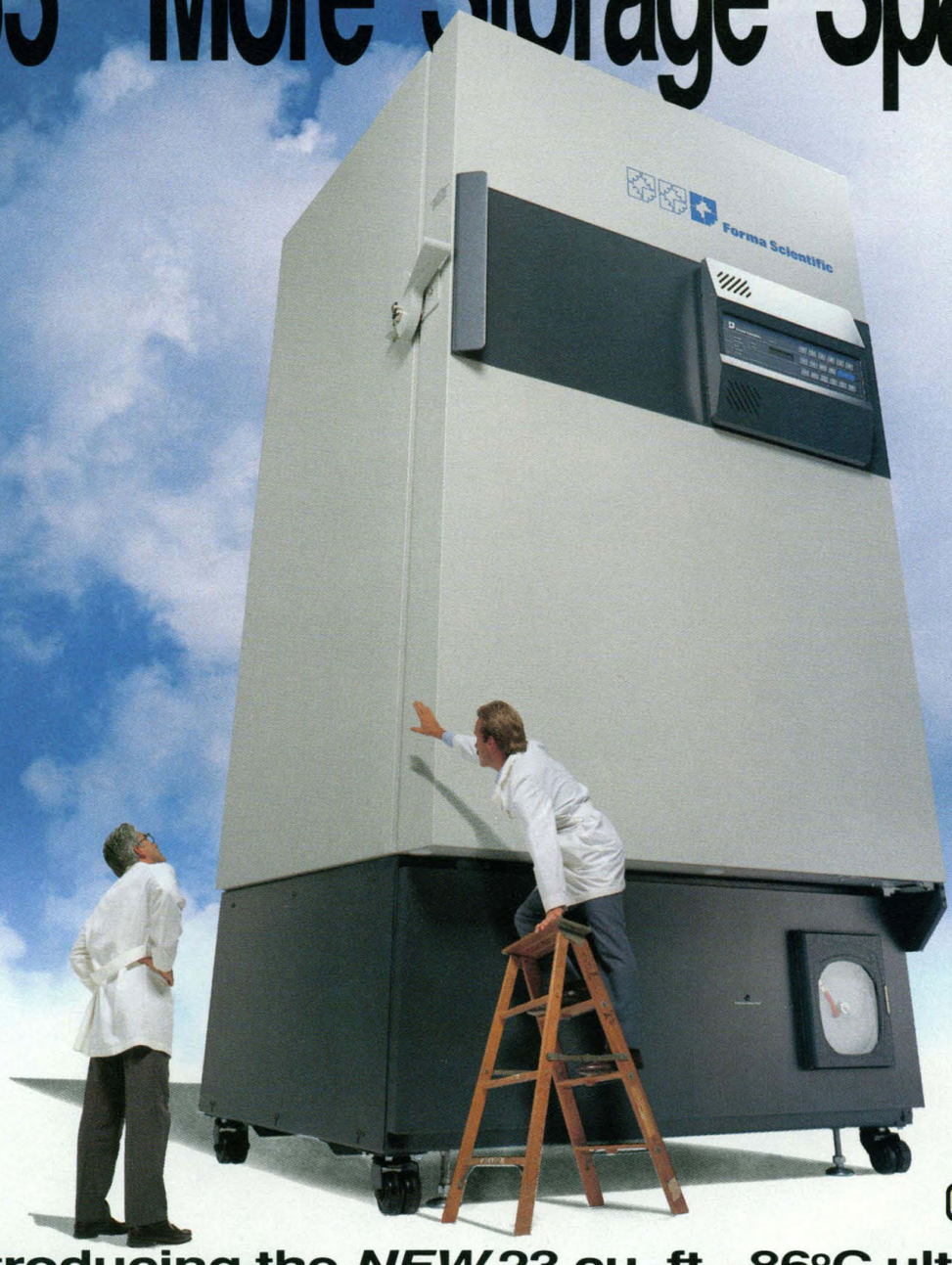

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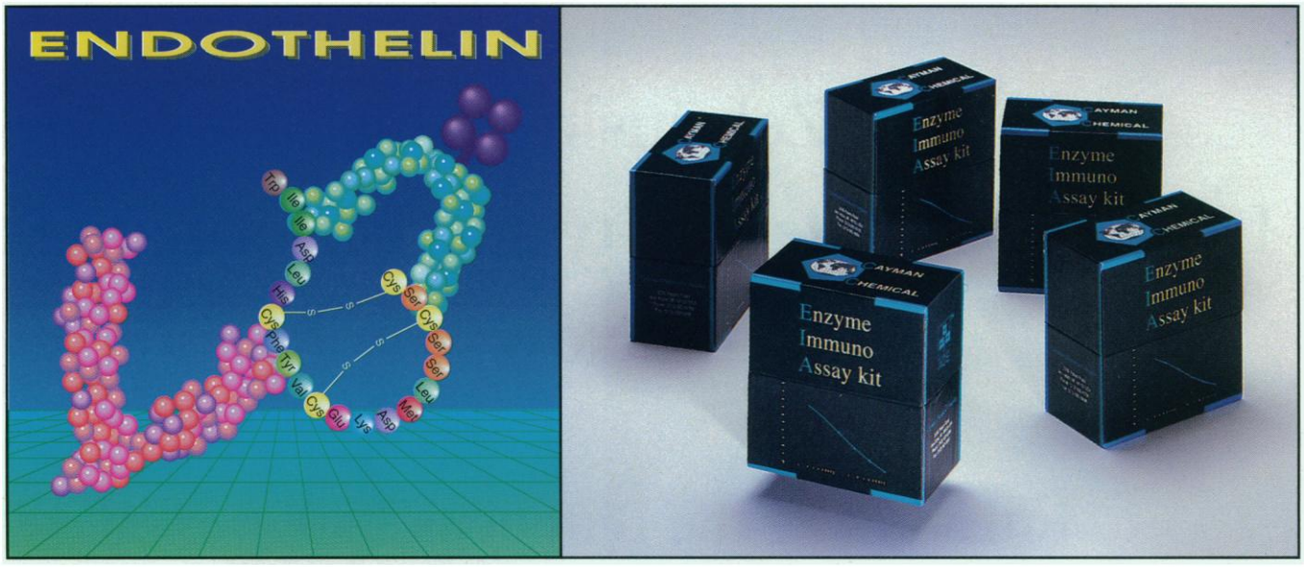

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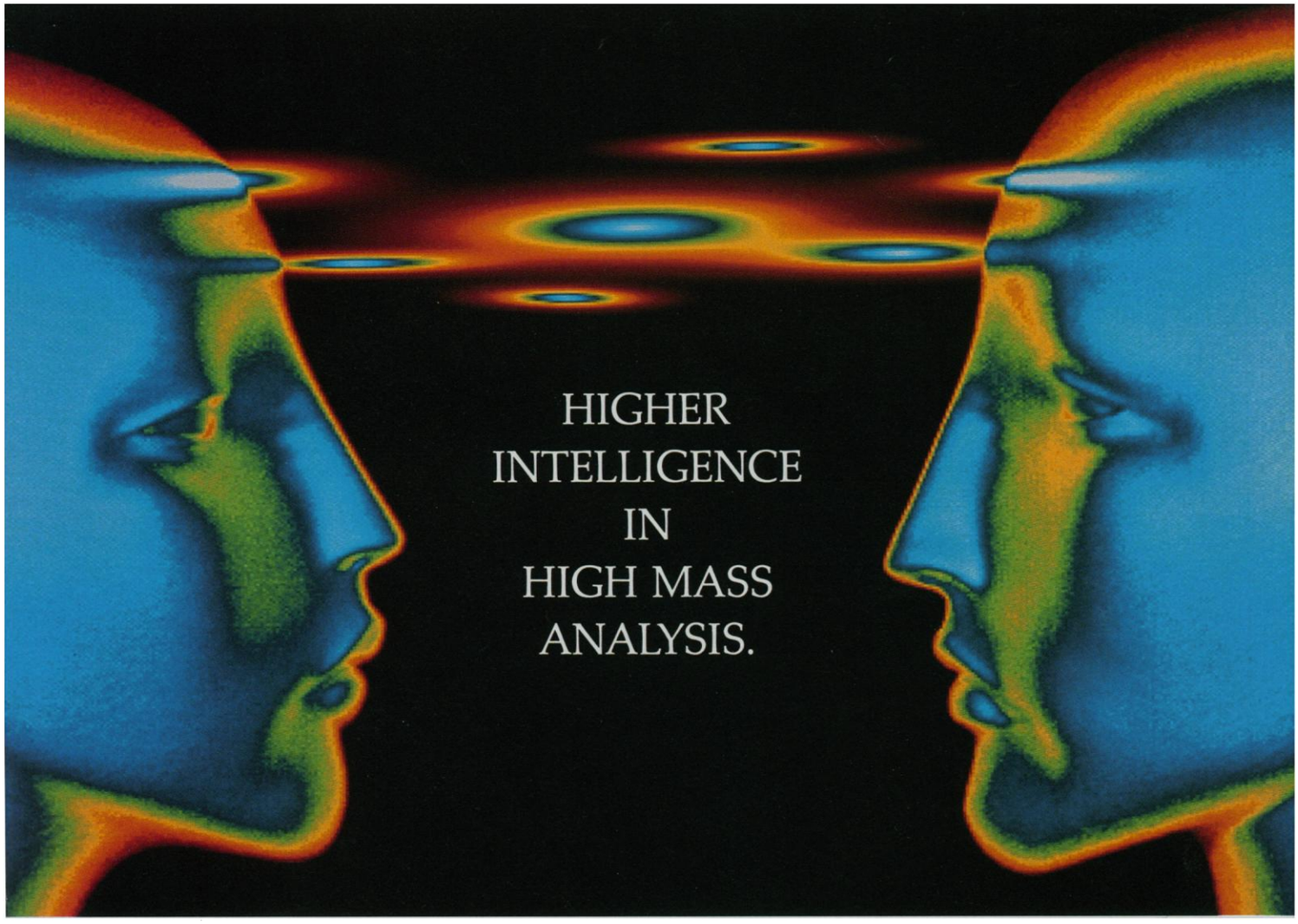

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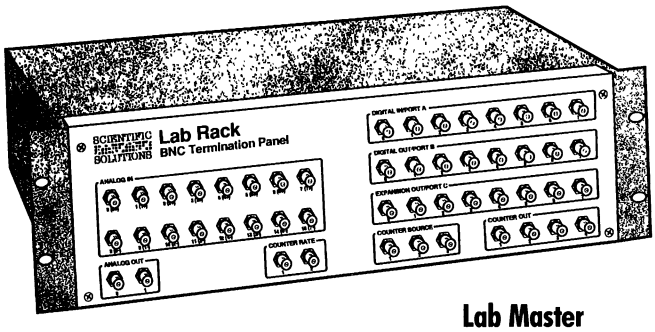

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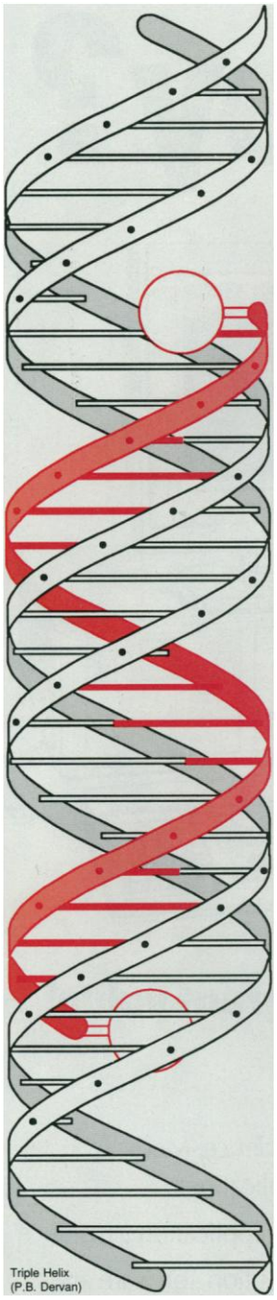

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