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SCIENCE

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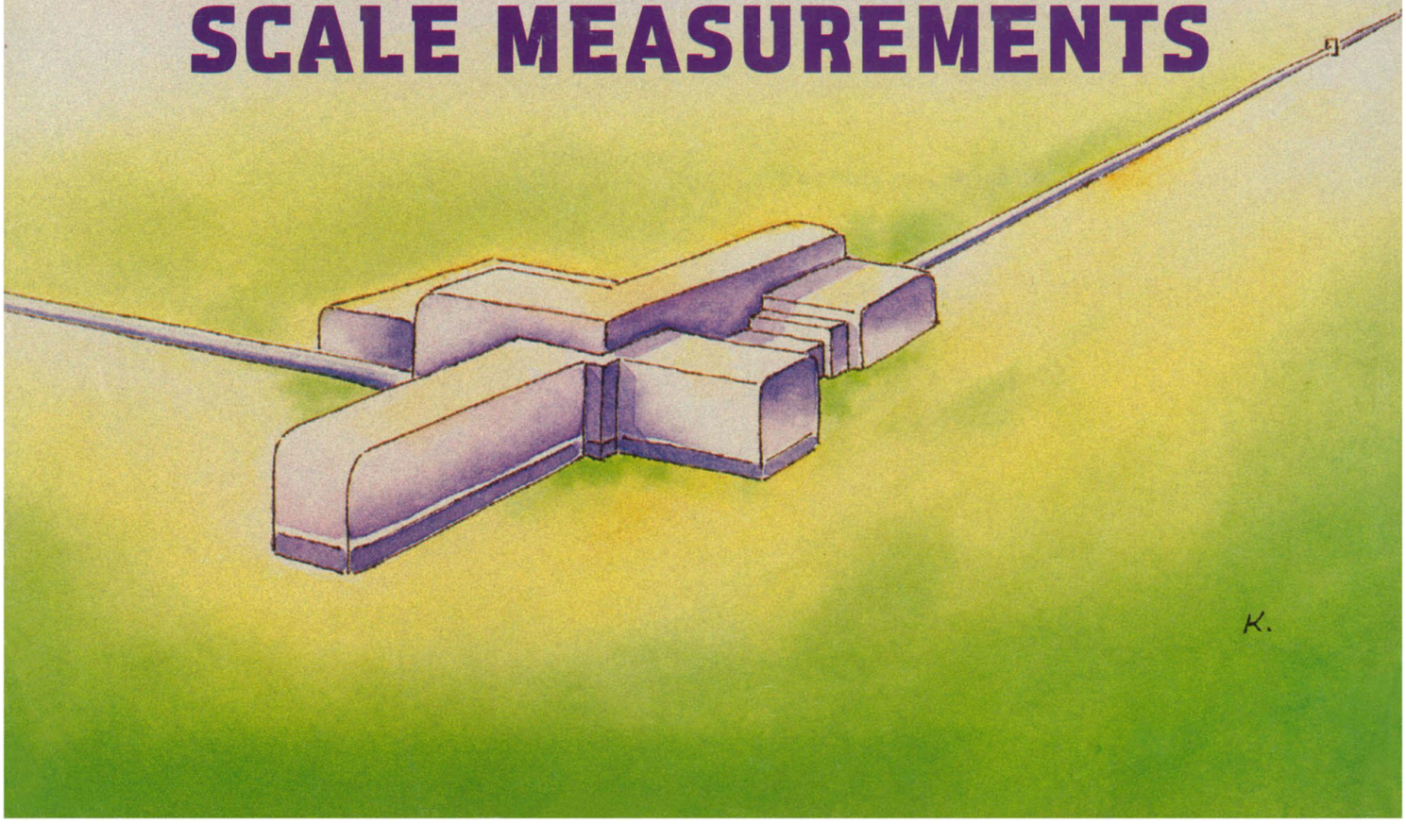
LARGE SCALE MEASUREMENTS



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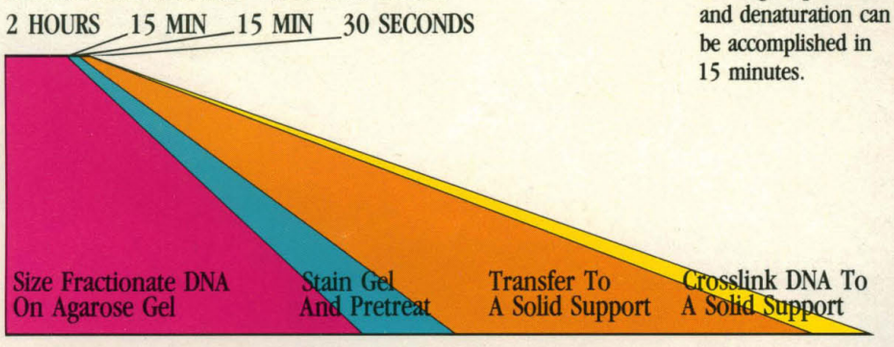


12 HOURS 4 HOURS 12 HOURS 2 HOURS

CONVENTIONAL METHOD—TOTAL TIME 30 HOURS

VAGE™ System

The VAGE™ vertical agarose/acrylamide gel electrophoresis system allows the casting of agarose or acrylamide gels in the unit. Nucleic acids can be electrophoresed through a 3 mm, 0.8% vertical agarose gel in less than two hours with excellent resolution (Figure 1). Because the gels are thin, staining, depurination, and denaturation can be accomplished in 15 minutes.

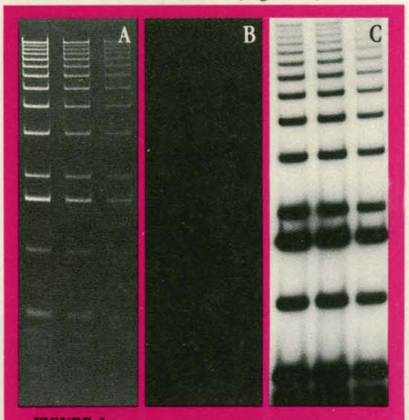
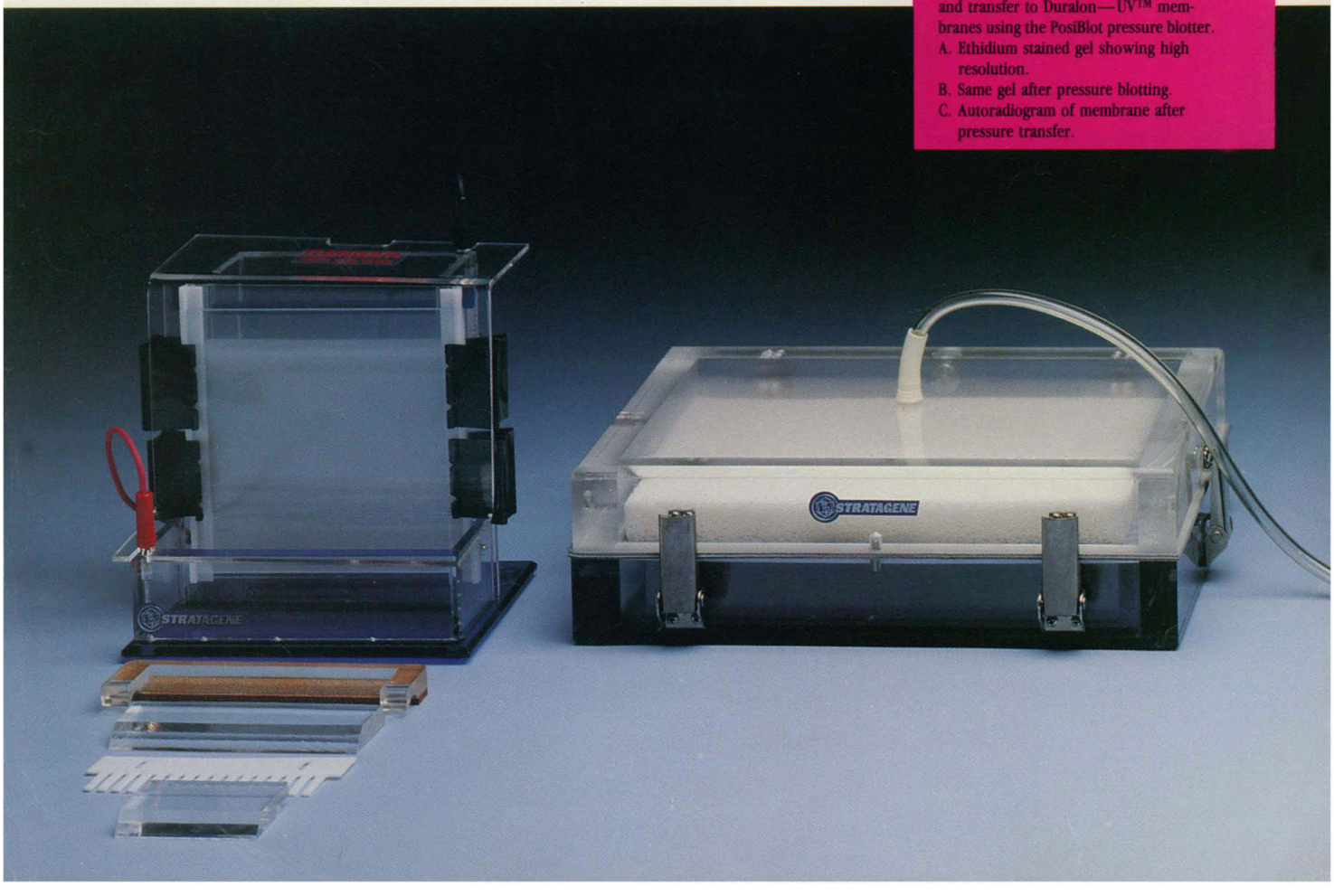


FIGURE 1:
Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.
A. Ethidium stained gel showing high resolution.
B. Same gel after pressure blotting.
C. Autoradiogram of membrane after pressure transfer.



PosiBlot™ * Pressure Blotter

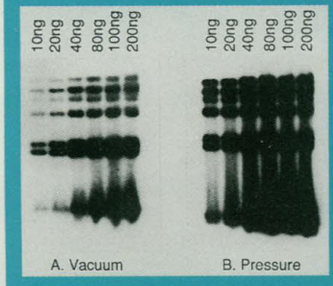


FIGURE 2:
Figure Lengend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

The PosiBlot™ positive pressure blotter permits the transfer of nucleic acids in 1/3 the time of vacuum blotters and 1/50 the time of capillary blotting (Figure 2). Pressure blotting does not dehydrate gels as do other methods. This allows the use of substantially higher pressure differentials, compared with vacuum blotting, without gel collapse. The PosiBlot apparatus reduces blotting time to 15 minutes.

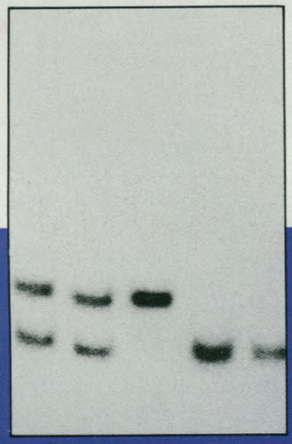


FIGURE 3:
Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

Stratalinker™ * UV Crosslinker

The Stratalinker™ UV Crosslinker fixes nucleic acids to solid supports such as nitrocellulose or nylon membranes, in less than one minute. This compares favorably to vacuum baking, which requires 2 hours. The Stratalinker actually monitors the ultra violet energy flux and deactivates the light source upon reaching the user-programmed energy level (Figure 4). Figure 3 shows an autoradiogram of a human genomic Southern blot performed using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

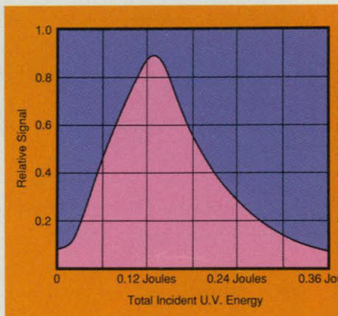


FIGURE 4:
Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

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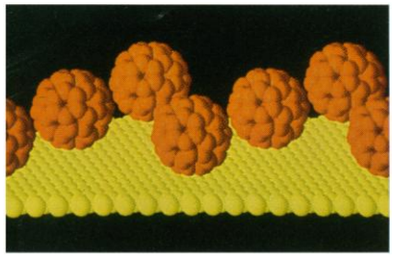
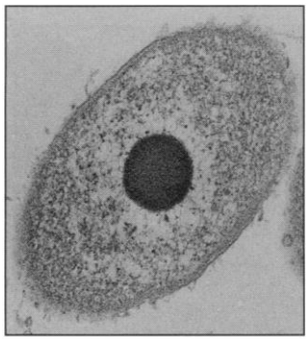


Chemistry *in computero* **306**



377 Bacterial nucleoid condensation

NEWS & COMMENT

RESEARCH NEWS

SPECIAL SECTION

Large Scale Measurements

NEWS REPORTS

ARTICLES

DEPARTMENTS

COVER

Illustration of one site in the proposed Laser Interferometer Gravitational-Wave Observatory (LIGO). The LIGO facilities will consist of two such interferometers located at widely dispersed sites; scientists hope LIGO will be able to detect gravitational waves emanating from collisions of black holes and neutron stars. See page 325. A special section in this issue of *Science* focuses on large scale measurements; see the Editorial and pages 316 to 349. [Illustration: Ruth Sofair Ketler]

RESEARCH ARTICLE

REPORTS

333 Wide regions of deformation associated with plate boundaries

◩ Indicates accompanying News story or Perspective

■ **SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005.** Second-class postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Domestic individual membership and subscription (51 issues): $87 ($47 allocated to subscription). Domestic institutional subscription (51 issues): $195. Foreign postage extra: Mexico, Caribbean (surface mail) $50; other countries (air assist delivery) $95. First class, airmail, student and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. **Change of address:** allow 6 weeks, giving old and new addresses and 11-digit account number. **Postmaster:** Send change of address to *Science*, P.O. Box 2033, Marion, OH 43305-2033. **Single copy sales:** $6.00 per issue prepaid includes surface postage; Guide to Biotechnology Products and Instruments, $20. Bulk rates on request. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

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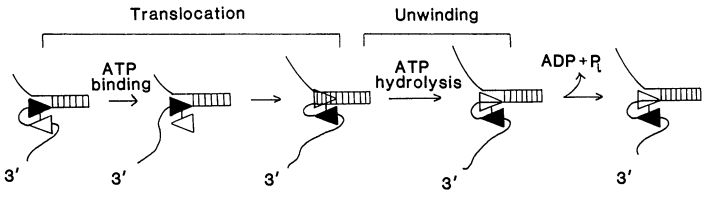
edited by PHIL SZUROMI

Rolling along

During replication double-stranded DNA unwinds to form segments of single-stranded DNA through the action of helicase enzymes. Wong and Lohman (p. 350) explored the mechanistic details of the *Escherichia coli* Rep helicase by comparing the energetics of DNA binding and DNA-induced Rep dimerization for different nucleotide cofactors, ADP (adenosine diphosphate) and AMPP(NH)P, a nonhydrolyzable analog of adenosine triphosphate (ATP). On the basis of these results they propose a rolling model for the unwinding of duplex DNA in which binding of ATP moves the helicase along the DNA; subsequent hydrolysis unwinds several base pairs.



Dating reversals

The record of reversals of the magnetic field preserved in sediments provides a valuable means for dating. However, earlier calibrations, based primarily on K-Ar dating, may be in error. The most recent major reversal, the Brunhes-Matuyama reversal, has also been dated by comparing climatic and paleomagnetic records preserved in sediments to predictions from orbital dynamics. This age is considerably older than the K-Ar age. Baksi *et al.* (p. 356) present a new Ar-Ar age determination of the boundary from lava flows in Hawaii. The new age of 783 ± 11 thousand years ago is in agreement with the astronomically calculated age (see also, *Science*, 8 Nov. 1991, p. 802).

■

Charges on contact

Contact or rubbing of dissimilar materials can cause electrical charging, which can be a nuisance (static cling) or a boon (as in photocopying). Despite its importance, this effect has been difficult to understand and study. Horn and Smith (p. 362) modified the surface forces apparatus, in which surfaces can be brought together and separated with subnanometer accuracy, so that charging and electrostatic forces could be measured simultaneously. The energies required to separate silica and mica after contact charging are nearly as high as their fracture energy. Gas discharges could be observed at distances of about 1 micrometer.

■

Water channel structure

Water rapidly enters red blood cells (RBCs) and renal tubes through water channels, whose physiology is well studied but whose structure is unknown. Preston *et al.* (p. 385) microinjected *Xenopus* oocytes with RNA for the CHIP28 protein, an abundant integral membrane protein found in RBCs. The expressed protein conferred increased osmotic water permeability on the oocytes, an activity that could be inhibited with mercuric chloride, a water channel inhibitor. The CHIP28 protein is probably a functional part of the water channel.

A family affair

Some protein families contain transcription factors that are structurally related. However, each family member may respond to different regulatory signals and function in distinct ways in different cell types. The C/EBP family of proteins belongs to the bZip class of transcription factors. C/EBPα has been shown to function in the adipogenesis of 3T3-L1 cells. The sequence-specific DNA binding protein Myc is involved in the control of cellular proliferation and differentiation. Freytag and Geddes (p. 379) show that expression of Myc in 3T3-L1 adipoblasts inhibits the induction of adipogenesis (formation of fat cells) by C/EBPα. Changes in intracellular calcium levels can result in the activation of specific genes. Wegner *et al.* (p. 370) show that another member of the C/EBP family, C/EBPβ, is phosphorylated in pituitary cells in response to an increase in intracellular calcium. The calcium-calmodulin–dependent protein kinase II phosphorylates C/EBPβ in vitro at Ser^{276}. Mutation of this site in C/EBPβ prohibits calcium-regulated stimulation of a reporter gene containing C/EBPβ binding sites.

■

Chlamydia chromosome

During the biphasic life cycle of *Chlamydia trachomatis*, a sexually transmitted parasite that can cause blindness, the chromosome undergoes organizational changes that appear to be regulated by the expression of Hc1, a protein related to eukaryotic histones. In its extracellular phase, the chromosome is unusually condensed for a prokaryote and does not undergo transcription, but after entering host cells the chromosome is loosely organized and transcription occurs. Barry *et al.* (p. 377) expressed the gene for Hc1 in *Escherichia coli* and observed by microscopy the formation of a condensed nucleoid structure that is similar to that in *Chlamydia*.

Altered states

One way to increase both the specificity and the repertoire of genes whose expression can be regulated is through interaction among distinct transcription factors. Another means of expanding regulatory possibilities is to generate functionally distinct forms of transcription factors through alternate splicing. Narayanan *et al.* (p. 367) identified a naturally occurring variant of p65, one of the two constituent proteins of the transcription factor NF-κB, that contains a deletion in the transcriptional activation domain (p65Δ). A member of the Rel family of proteins, p65 participates in the transcriptional regulation of viral and cellular genes. Expression of p65Δ but not p65 in Rat-1 fibroblasts resulted in focus formation in culture and tumor formation by injection of transformed Rat-1 cells into nude mice. In vitro assays showed that p65Δ interfered with DNA binding by p65. Myc is a transcription factor that is involved in the control of cell proliferation, and its binding to DNA is enhanced by the protein Max. Mäkelä *et al.* (p. 373) identified an alternate form of Max (ΔMax) that can still bind to DNA in a complex with Myc, but lacks a nuclear localization signal. Expression of Max in rat embryo fibroblasts suppressed transformation, while ΔMax enhanced this process.

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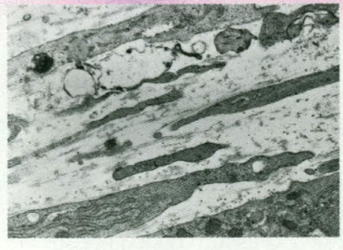
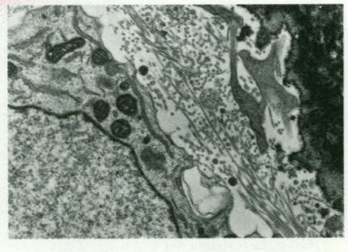


Electron micrograph of chick calvarial cells cultured in 10% chicken serum-supplemented medium. These cells remain undifferentiated, depositing a matrix characterized by a sparse fibrous component and no mineralization.



Chick calvarial cells grown for 10 days in 10% Nu-Serum IV-containing medium. Note the extracellular matrix showing collagen fibers characterized by the typical banding pattern.

Photos courtesy of Drs. Warren Ramp and Richard Dillaman.

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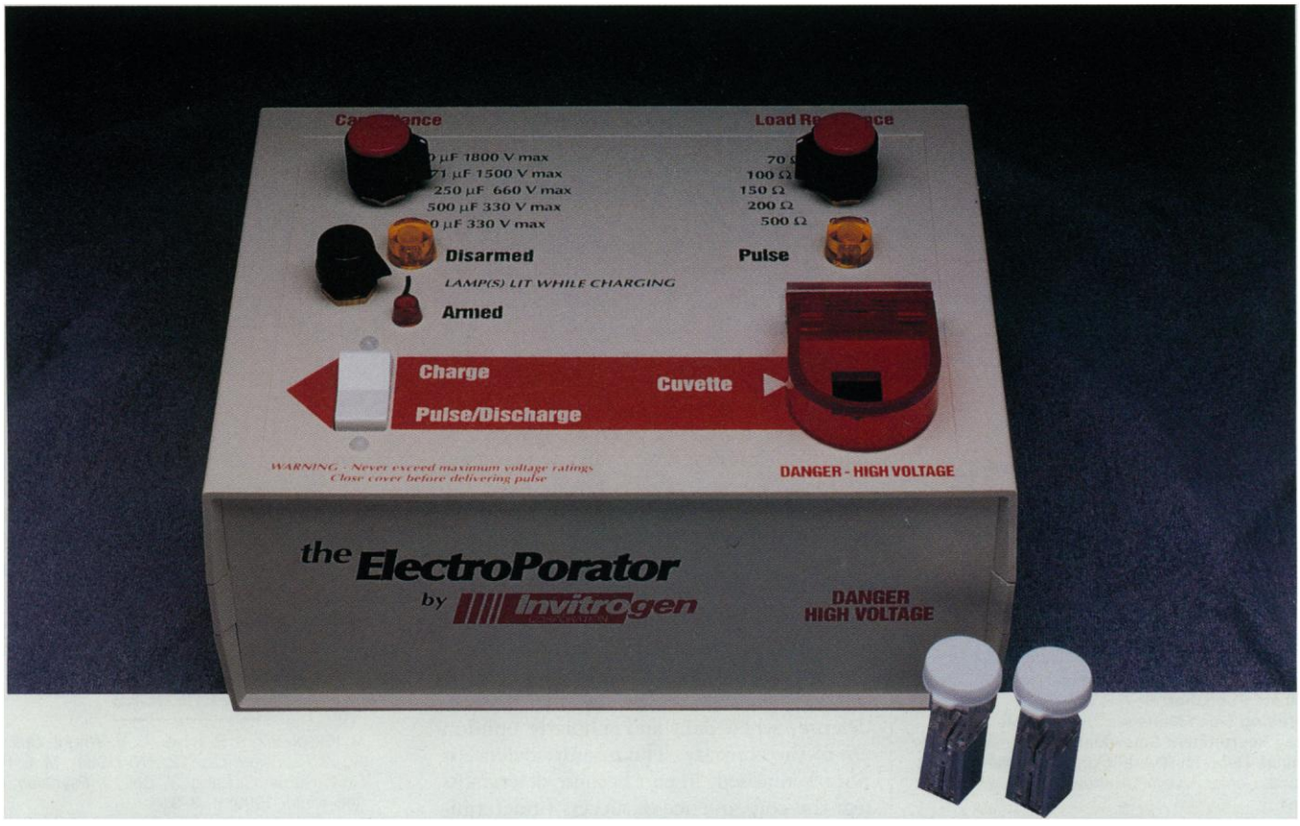

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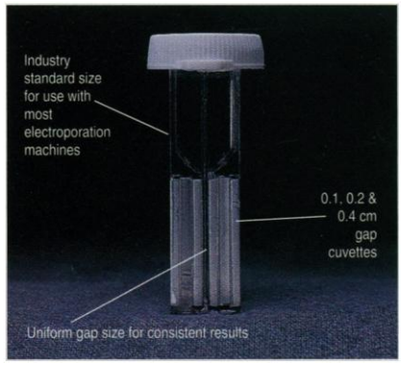

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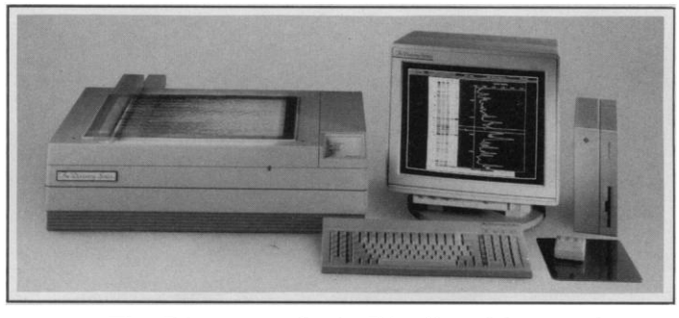

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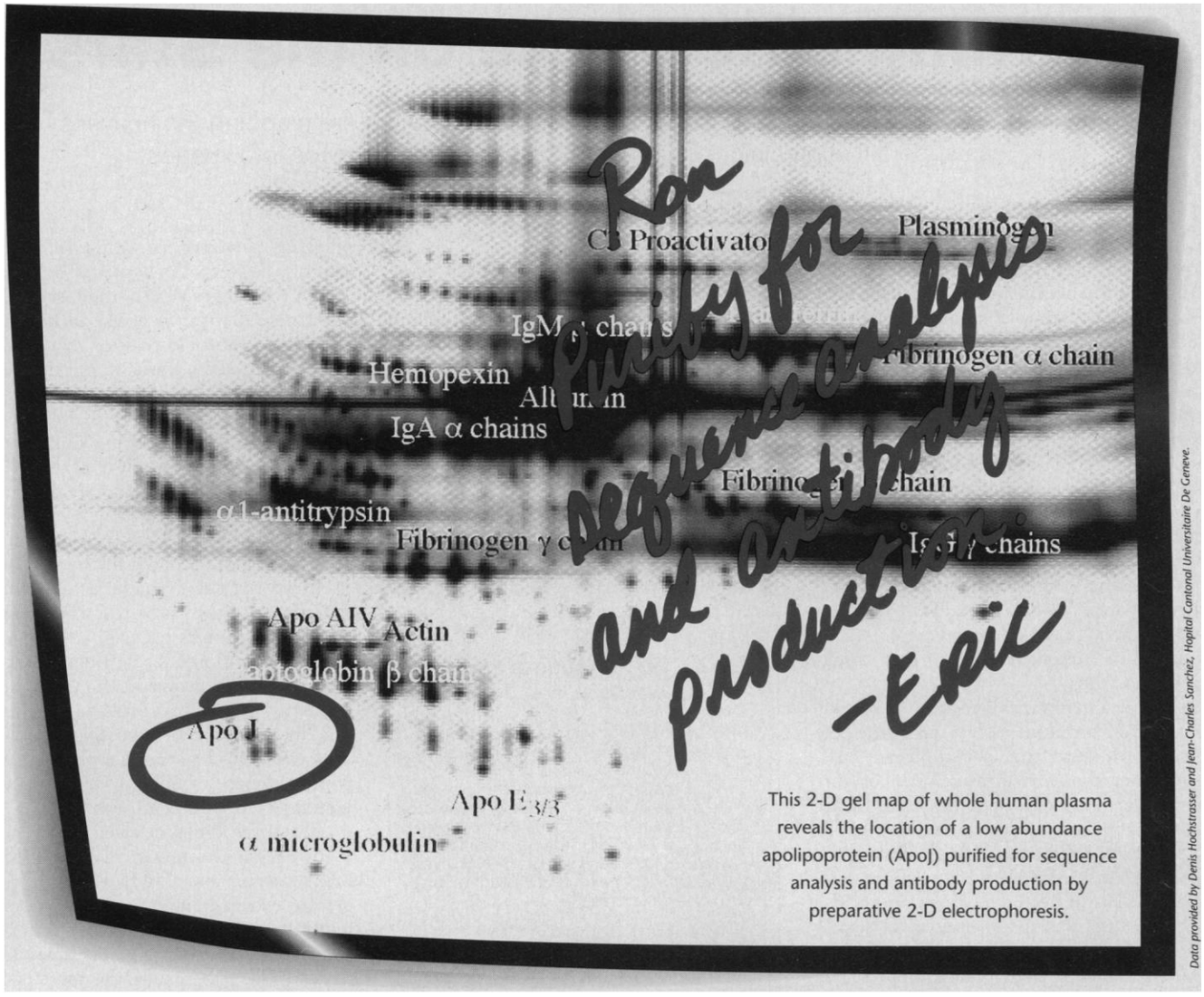

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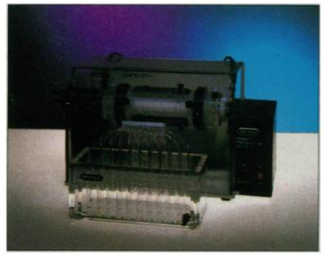

First dimension purification with the Rotofor provides initial fractionation of any crude protein sample, up to 500-fold. The second dimension, and final purification step, preparative-PAGE, is then used to isolate individual proteins within Rotofor fractions, proteins differing in MW by as little as 2%.

For more information on the new Model 491 Prep Cell, the Rotofor cell and Preparative 2-D electrophoresis, call 1-800-4BIORAD.

BIO-RAD **Life Science Group**

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