American Association for the Advancement of Science

SCIENCE

15 March 1991 $6.00
Vol. 251 ■ Pages 1281–1396

Southerns/Northerns: Electrophoresis, Blotting, and Crosslinking in 2.5 Hours Instead of 30.

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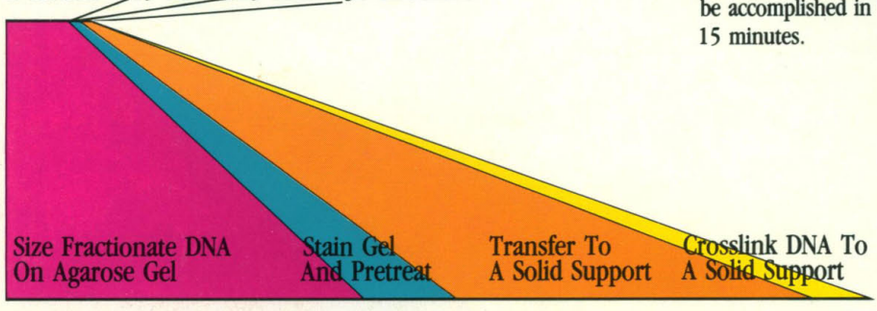
The VAGE™ vertical agarose/acrylamide gel electrophoresis system allows the casting of agarose or acrylamide gels in the unit. Nucleic acids can be electrophoresed through a 3 mm, 0.8% vertical agarose gel in less than two hours with excellent resolution (Figure 1). Because the gels are thin, staining,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

STRATAGENE METHOD—TIME 2.5 HOURS



12 HOURS 4 HOURS 12 HOURS 2 HOURS

CONVENTIONAL METHOD—TOTAL TIME 30 HOURS

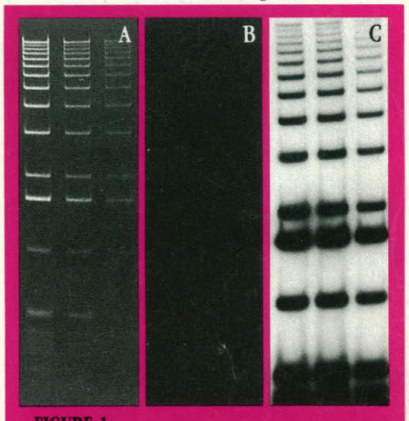
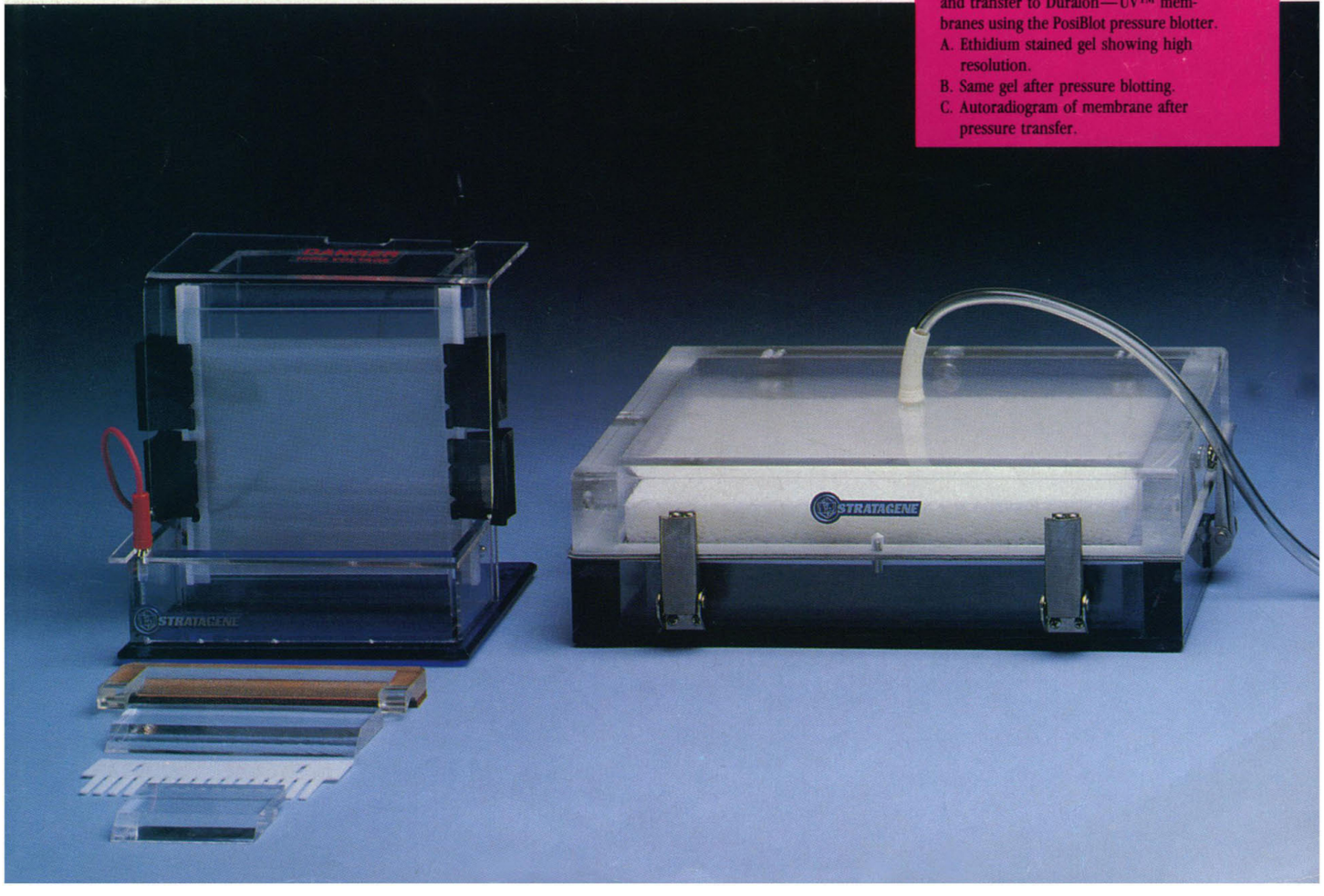


FIGURE 1:
Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.
A. Ethidium stained gel showing high resolution.
B. Same gel after pressure blotting.
C. Autoradiogram of membrane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



PosiBlot™* Pressure Blotter

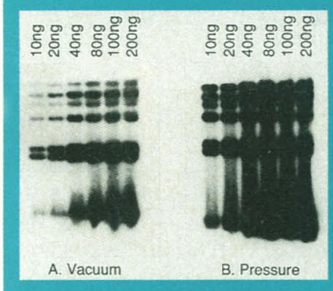


FIGURE 2:
Figure Lengend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried 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.

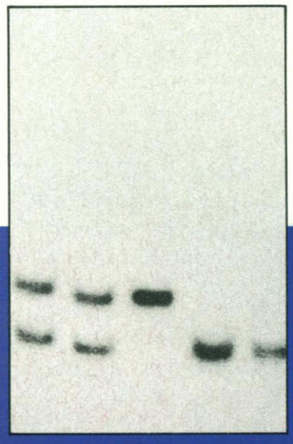


FIGURE 3:
Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

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.

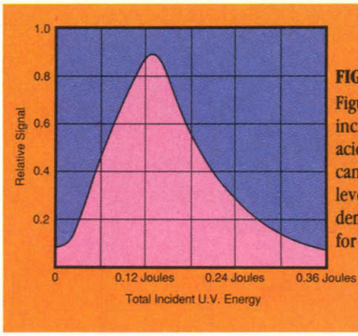


FIGURE 4:
Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

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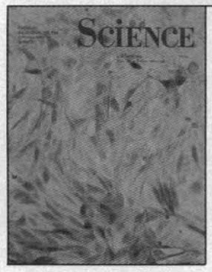

COVER Site-specific recombination in mammalian cells. Transient expression of a yeast recombinase (FLP) in mammalian cells will either excise specific chromosomal sequences or target integration of extrachromosomal DNA to specific chromosomal sites. In this photomicrograph, excisional recombination permanently activated a chromosomally integrated β-galactosidase construct in the cells that contain a blue histochemical reaction product. See page 1351. [Photomicrograph by Stephen O'Gorman and Geoffrey Wahl]

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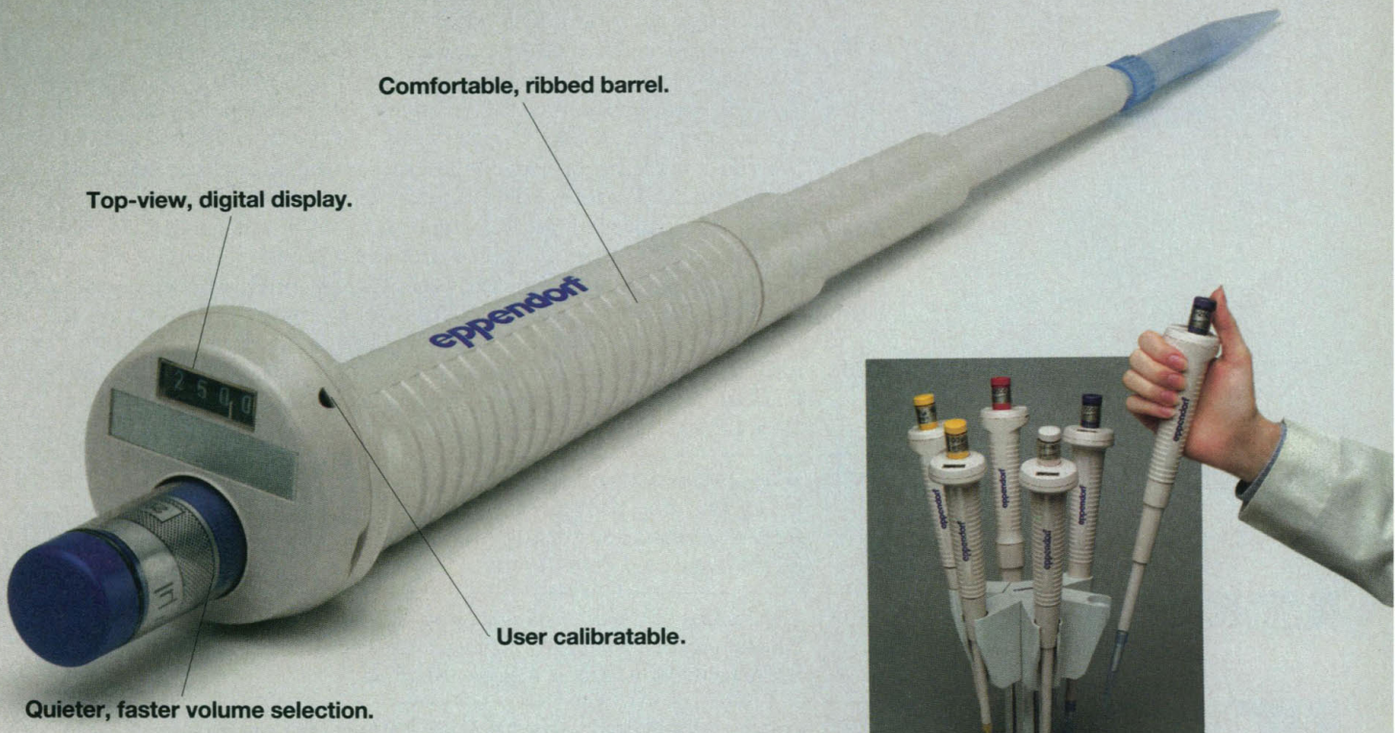

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This Week in
SCIENCE

Future fuels

THE production of ethanol as a transportation fuel could be carried out in the United States on a large scale with "cellulosic biomaterials"—wood, grasses, and wastes—as starting materials (page 1318). Interest in developing alternative fuel sources comes both because of the uncertainties surrounding importation of petroleum products and because currently available fuels are highly polluting. As explained by Lynd *et al.*, the conversion of cellulosic materials into ethanol is not just a variation of the procedure by which food crops (such as sugar cane and corn) are converted into ethanol; cellulose ethanol technology depends on biomaterials grown on marginal cropland, exacts a smaller environmental toll than does the cultivation of row crops, and is estimated to contribute one-fifth or less the amount of carbon dioxide that gasoline releases into the atmosphere, thus having a negligible impact on global climate changes. Although the current costs of production of ethanol from cellulosic materials are high, they are expected to drop to acceptable levels as the technology for large-scale production is developed.

Suspension feeding

SUSPENSION-FEEDING blackfish extract their food, tiny plankton, from large volumes of water that they suck into their oral cavities. It had been thought that cartilaginous structures on the gill arches of the oral cavity acted as mechanical sieves to physically trap plankton particles. However, videos of the flow of fluid inside the oral cavity, made by Sanderson *et al.*, indicate that the blackfish gill-arch structures are not sieves separating water and particles but are barriers that alter the direction of flow of incoming materials (page 1346). Particle-laden water was directed by the arches to the mucus-lined roof of the oral cavity (the palatal organ) where large clumps formed as small particles were brought together by mucus. Later, these clumps progressed down the esophagus. The measuring and visualization devices used in these studies can be adapted for the study of filtration strategies in other suspension-feeding fishes, bivalves, and birds.

Pain and the brain

THE cortex of the brain has been thought to play a minor part in the perception of pain. But studies of changes in blood flow in the cortex in awake healthy human volunteers subjected to painful stimuli indicate that some areas of the cortex are actively involved in pain perception (page 1355). Subtractive positron emission tomography was combined with magnetic resonance imaging head scans to identify which cortical areas were activated in response to painful pulses of heat on the forearm. Four areas of the cortex were activated; all were on the side of the brain contralateral to the arm that was subjected to pain. The most notable response was in the limbic cortex in Brodmann's area 24; a second stimulated area was more posterior in area 24. In the parietal lobe, both primary and secondary somatosensory areas were activated. Talbot *et al.* note that localized regions rather than broad areas of the cortex are activated in response to pain; they propose that both the parietal and limbic cortical areas respond to the location and intensity of the pain and that the limbic region may then regulate the subject's emotional reactions to the painful sensation.

Gene therapy experiments

THE first clinical trial of gene therapy is being carried out on a patient with severe combined immunodeficiency caused by a defect in the gene for the enzyme adenosine deaminase (ADA). The strategy for treating this disease is to complement the defective gene with one that encodes functional ADA molecules. Some of the preliminary indications that gene replacement might succeed in this and similar human patients were obtained in mixed-species experiments (page 1363). Blood cells were taken from patients with an ADA deficiency. A retroviral vector was used to carry a normal ADA gene into the cells where it complemented the defective gene. The altered cells were then injected into immunodeficient mice. The cells survived in the animals, ADA was produced and appeared to be a requirement for the survival of altered cells, and some of the immune functions of the animals were restored. Among these were the production of human immunoglobulins and the appearance of antigen-specific human T cells and human B cells in the animals. Ferrari *et al.* note that because this form of gene replacement involves somatic cells that are not stem cells long-term restoration of immune function may require repeated injections of altered cells.

Cancer gene candidate

MANY types of human cancers, including colorectal cancers, are thought to develop after a series of mutations or deletions have occurred in specific tumor suppressor genes and proto-oncogenes. A candidate for one of the tumor suppressor genes that may be inactivated in patients with colorectal cancer has been identified by Kinzler *et al.* (page 1366). The gene, named MCC (mutated in colorectal cancer), is found in human chromosomal region 5q21, a chromosomal region that has been linked to familial adenomatous polyposis, which is a precursor condition for some cases of colorectal cancer. Most normal tissues examined in rats and humans were found to express an MCC-like gene; this is consistent with a role for the gene in growth regulation for several types of cells. The human gene is predicted to encode a large protein that has a small but possibly significant region of similarity to a G protein–coupled receptor. Previous studies have suggested associations of G proteins with various types of cancers. Marx provides additional information about the isolation of the gene and its possible significance (page 1317). ■ RUTH LEVY GUYER

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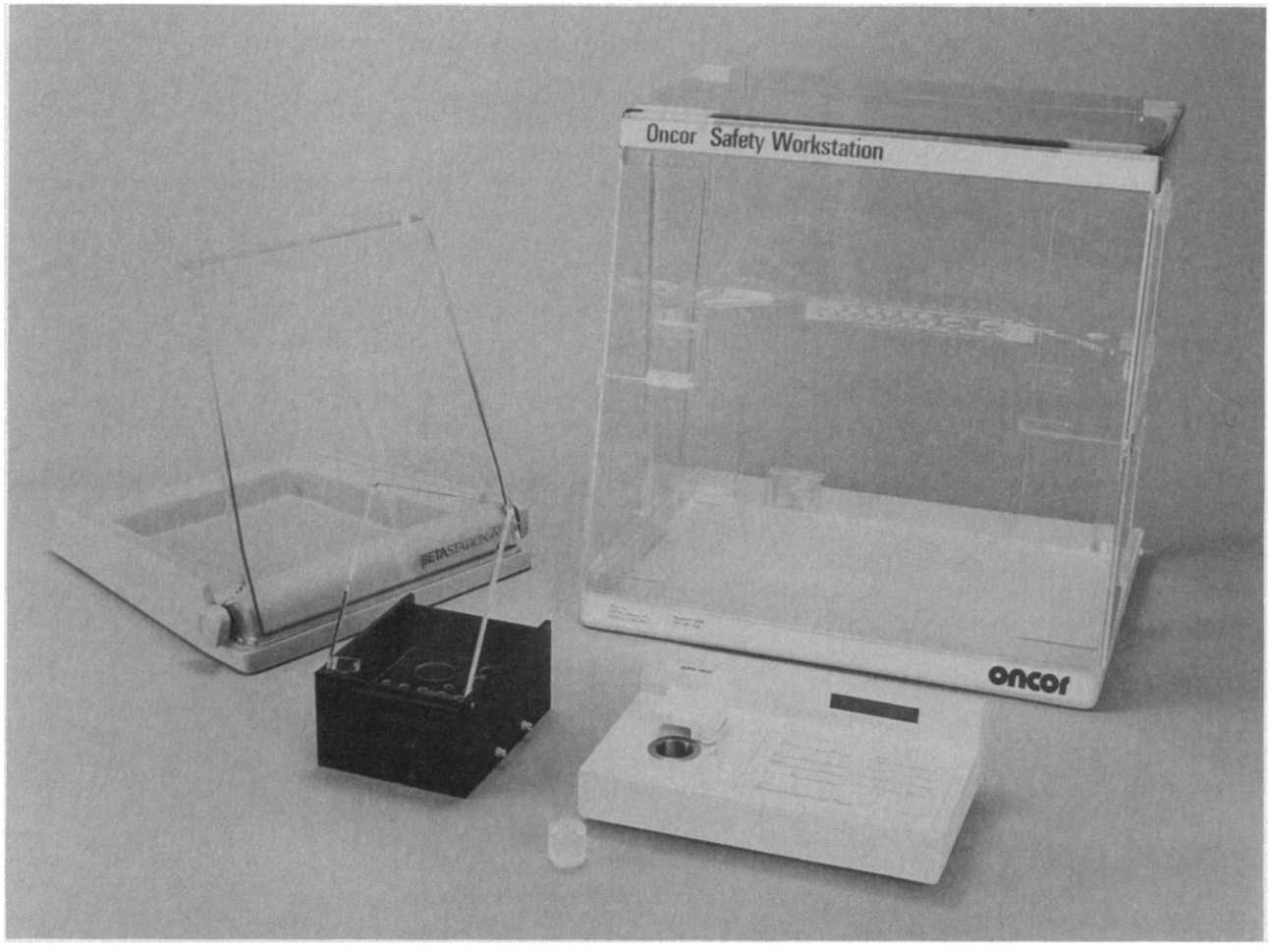

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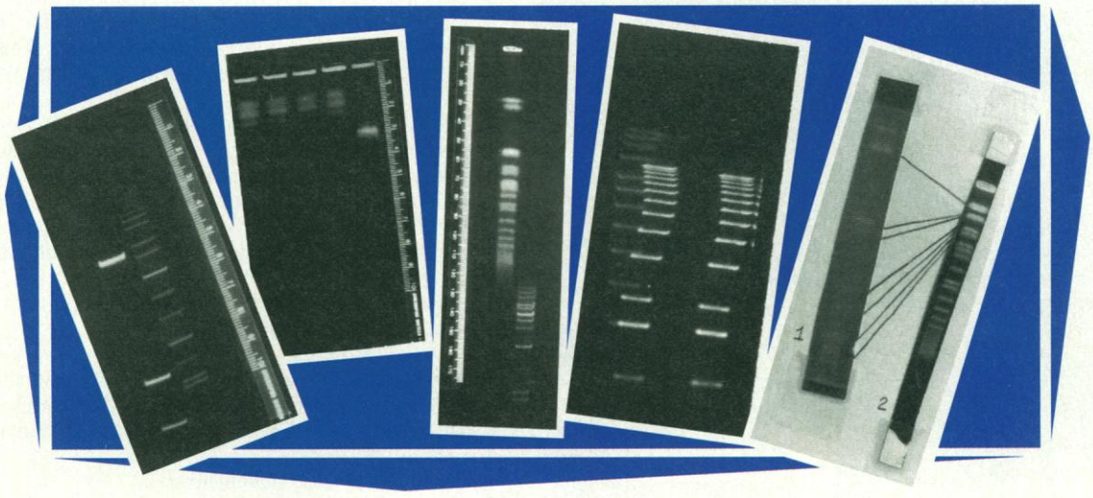
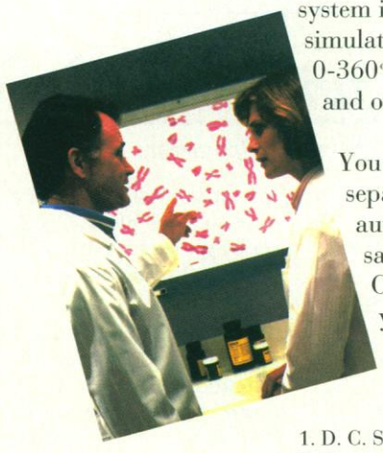


Left to right: Asymmetric voltage FIGE, 5-50 kb; *S. pombe* 24 hrs, 106° angle; 8kb - 2.2 mb;2-D separation of linear and supercoiled DNA; 8 state high resolution separation of *S. cerevisiae*.



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1. D. C. Schwartz and C. R. Cantor. *Cell*, **67** (1984).
2. Programmed Autonomously Controlled Electrodes. See S. M. Clark, E. Lai, B. W. Birren and L. Hood. *Science*, **241**, 1203 (1988).

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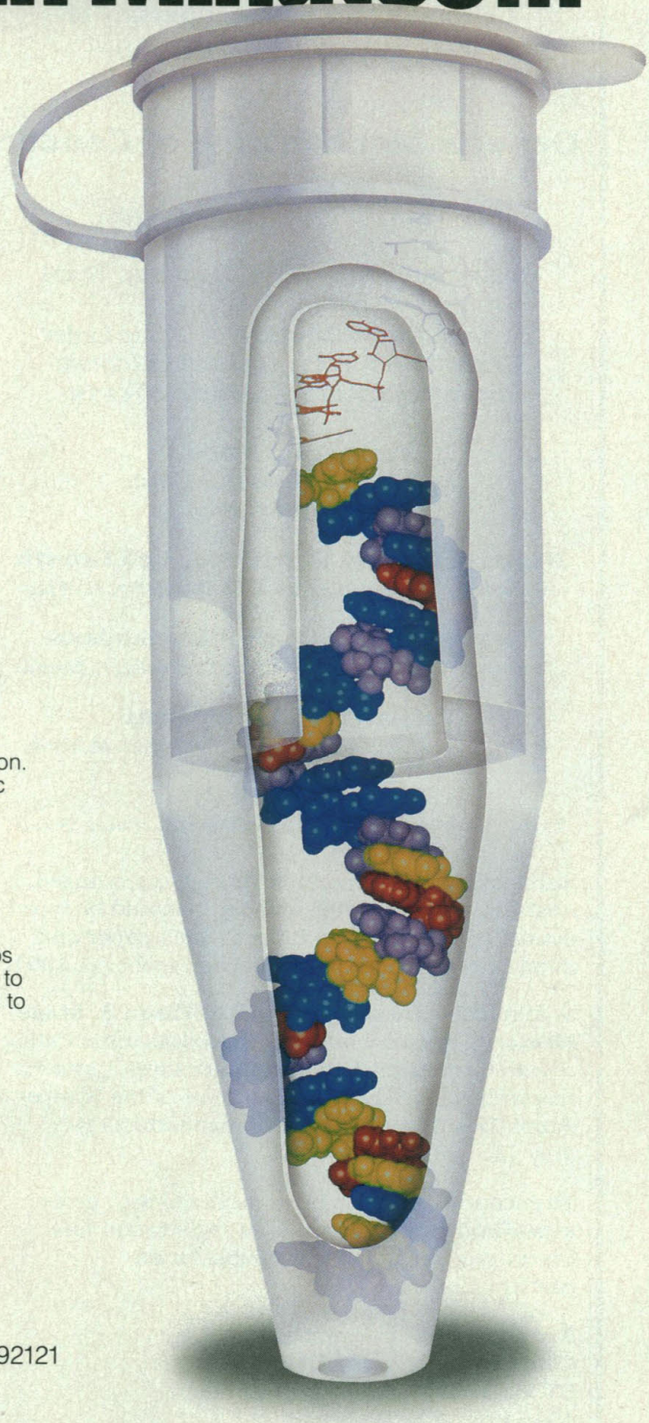

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Nominations are solicited for the **Wilson S. Stone Memorial Award** which acknowledges outstanding research accomplished by a junior investigator for research consistent with the theme of the Symposium. Deadline for receipt of nominations is July 15, 1991.

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MOLECULAR INTERACTIONS IN PLANT DEVELOPMENT

The Thomas J. Watson, Jr. Lecture in Molecular Biology
J. Schell, Max-Planck-Institut (Germany)

Regulation of Morphogenesis
Ian Sussex, Elliot Meyerowitz, Renee Sung, Keith Roberts

Regulation of Biochemical Processes
Christopher Somerville, John Mullet, Gerald Fink, Christopher Lamb

Hormonal and Environmental Regulation of Development
Michael Freeling, June Medford, Klaus Palme, Stephen Howell

Plants, Microbes, and Their Interactions
Roger Beachy, Brian Staskawicz, Frederick Ausubel, Eugene Nester

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Poster/Abstract deadline: May 25, 1991.
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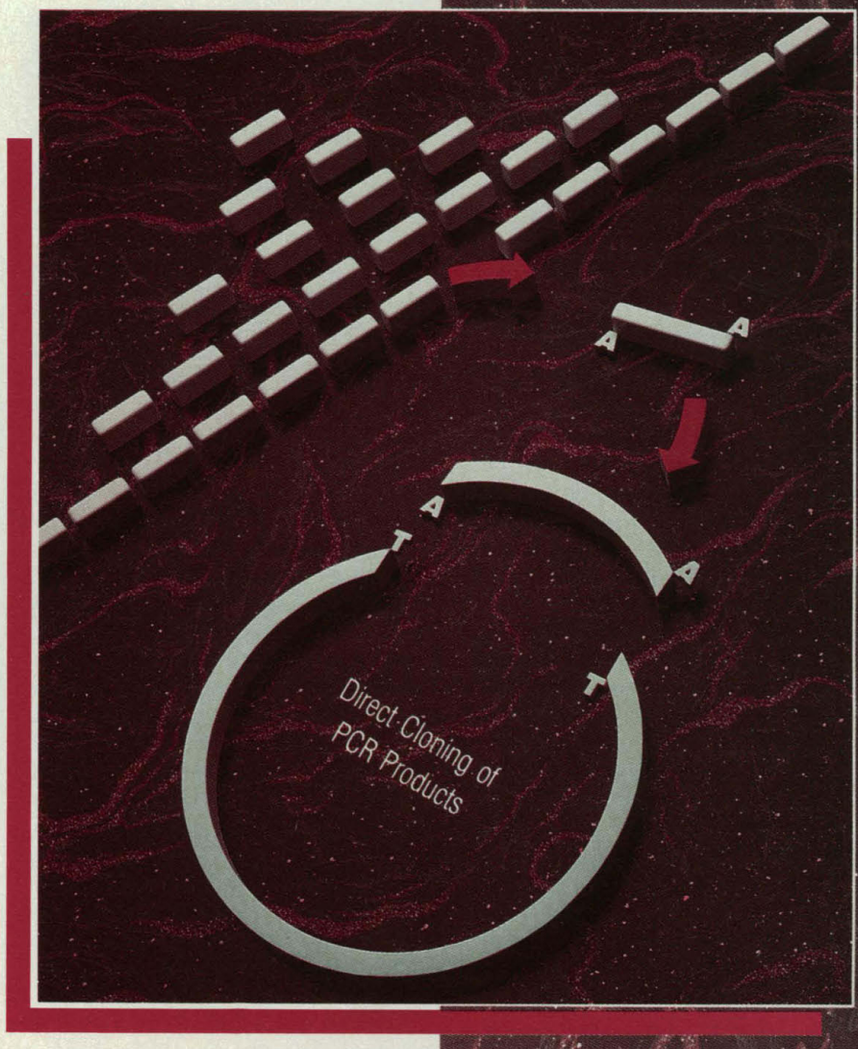

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