Advances With
In Situ PCR

“Current Protocols” Publishes
A Successful Methodology

Over the past five years, several investigators have advanced development of in situ PCR & hybridization methodologies. However, the resulting heterogeneity of protocols has often led to confusion among researchers, as others tried to adapt the technique to their own applications.

Now, however, a well-tested protocol has been published by John Wiley & Sons in Current Protocols in Molecular Biology (Vol. 2, Sect. 14.8, Supp. 31). The protocol was contributed by Omar Bagasra et al., and this exact methodology has been used for many published papers. Furthermore, its validity has recently been demonstrated at a “bake-off” of various protocols conducted in September 1995 at Uppsala, Sweden, where a group of over fifty scientists found the Bagasra method to be the most successful and reproducible of those tried.

ENGINES REVVING FOR
LARGE-SCALE SEQUENCING

Human Genome Project Advances
Towards Actual Sequencing Phase

The Human Genome Project has now reached a crossroads where existing technology—based upon thermal cycle sequencing of M13 templates—has achieved a level of development where the actual sequencing of the three-billion bases in human DNA can now begin. As reported in Science (267 783-784, 1995), two leaders of the project have proposed a plan to begin the sequencing phase immediately. They argue that existing technology could allow the project to be completed as soon as 2001—a full five years ahead of schedule.

MJ Research has been working with teams—both in the U.S. and the U.K.—to use the DNA Engine system to help drive this accelerated schedule. The extremely speedy nature of its cycling—combined with the ease with which the instrument integrates into automated robotic systems—has made the DNA Engine the cyclical choice for thermal cycle sequencing. Higher-density formats are in development, as are pre-engineered systems that will feature robotic fluid handling.

PCR Process Patents—
How to Get License

Licenses to conduct PCR for human and veterinary diagnostic use are available from Roche Molecular Systems in the U.S. and F. Hoffmann-La Roche Ltd., worldwide. Standard terms exist that are independent of the thermal cycle to be used, and hundreds of licenses have been granted to date. For more information, contact: Director of Licensing; Roche Molecular Systems; 1145 Atlantic Avenue; Alameda, CA 94501 U.S.A.

License for other research and forensic uses can be obtained by use of reagents carrying a label license, which currently states that they must be used in conjunction with an “authorized” thermal cycler. Any MJ Research cycler can be “authorized” contact: Director of Licensing; P.E.ABD; 850 Lincoln Drive; Foster City, CA 94404 U.S.A.

FIRST THERMAL CYCLER TO
OFFER CRUCIAL ABILITIES

Excellent Platform Upon
Which Tests Can Be Developed

Perhaps the most powerful tool yet invented for the diagnosis of infectious and genetic disease—the polymerase chain reaction (PCR)—is now poised to enter the clinical arena. A decade has passed since the original conception of this enormously specific and sensitive technique, and now PCR promises to be helpful not only for the diagnosis of disease, but also for the treatment of cancer, heart disease, and other conditions as well.

Many companies have been working to bring PCR to clinical pathology laboratories, but limitations had lain with the thermal cyclers that drive the reaction, which had not yet fully met the special needs of the clinical market. Now, MJ Research has used its eight years of manufacturing experience to construct the PTC-200 DNA Engine, the highest-performance thermal cycling system ever. Its Peltier-Joule heat pumps outperform every competing technology, its adjustable Hot Bonnet® heated lids make oil-free operation easy and reproducible, and its networking and digital output capabilities can make documentation automatic. The various methods of control are compatible with existing systems, easy-to-use software allows protected protocols, and swappable blocks can accommodate virtually any vessel (soon even slides for in situ).

In short, this system is the perfect clinical platform.

The PTC-200 DNA Engine

Critical Issue: Achieving
Annealing Temperatures Precisely

For any test involved in human diagnosis, a very important consideration is the precision with which annealing temperatures are achieved and reproduced. Only high-fidelity annealing leads to the needed specificity—no “slip” can be tolerated here.

Unlike most non-Peltier systems, DNA Engine cyclers hit and maintain annealing temperatures with remarkable precision. NIST-traceable accuracy helps make every profile the same—cycle-to-cycle, run-to-run, and machine-to-machine. This is monitored continuously by software, and it can be verified independently with NIST-traceable probes.

How a Peltier Heat Pump Works

As current passes through the P/N semiconductor couples, charge carriers (electrons or “holes”) absorb energy at one of the metal-semiconductor interfaces and deposit energy at the other, generating a thermal gradient of up to 70°C across the crystals. The rate of heat pumping is proportional to current, and the direction is reversible by switching polarity.
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0 10 30 60 180 time (min)

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- p42 MAPK

B. control MAPK Ab
- p44 MAPK
- p42 MAPK

Western Blotting: Quick and easy detection of MAP kinase activation (en Tyr) from SDS-hydrates of total PC12 cell extracts using A. phospho- and B. control-MAP kinase antibodies

NIH 3T3 CELLS/PHOSPHO-MAP KINASE Ab

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Immunohistochemistry: Phospho-antibody allows in situ-detection and subcellular resolution of Epidermal Growth Factor (EGF)-induced MAP kinase activation and nuclear translocation.

And cdc2 Kinase

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- p34/36

B. control-cdc2 Ab
- p34/36

Western Blotting of cell extracts from Saos cells treated with Hydroxyurea (G1/S) or Nocodazole (G2/M) with A. phospho-cdc2 (Tyr15) B. control cdc2 antibody.

For more information on Phospho-Specific Antibodies, call 1-800-NEB-LABS or via the internet: <info@neb.com> • NEB home page: <http://www.neb.com>

Stop by the NEB booth #649/651 at the ASCB Meeting for more information.
Paper of the Year Award

Each year, Molecular Biology of the Cell sponsors the MBC Paper of the Year Award. The winner of the award is the first author, who must be a student or postdoc of the paper judged by the Editorial Board to be the best among those published from July to June of each year. The winner will then be a speaker in an appropriate minisymposium at the next ASCB Annual Meeting. The award pays travel expenses for the winner to attend the Annual Meeting.

_Papers are currently being considered for the fifth MBC Paper of the Year Award, the winner of which will be featured at the ASCB Annual Meeting in San Francisco, California, December 7–11, 1996._

Submit papers to: MBC Managing Editor, The American Society for Cell Biology, 9650 Rockville Pike, Bethesda, MD 20814-3992. Phone: (301) 530-7153. Fax: (301) 571-8304.
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- Presently researching protein expression and DNA/RNA isolation and purification systems.
- Published in Gene, Journal of Immunology, and Molecular Biology of the Cell.
- Presented abstracts at Symposium for Protein Society and ASMB/DBC-ACS Joint Meeting.
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FEATURED IN THE NOVEMBER 1995 ISSUE:
Apical Topography and Modulation of ICAM-1 Expression on Activated Endothelium, *Angels Almenar-Queralt*, Alain Duperrey, Lindsey A. Miles, Jordi Felez, and Dario C. Altieri

Immunohistochemical Detection of Active Transforming Growth Factor-β in Situ Using Engineered Tissue, *Mary Helen Barcellos-Hoff*, E. J. Ehrhart, Manu Kalia, Randolph Jirtle, Kathleen Flanlers, and Monica Tsang


FEATURED IN THE DECEMBER 1995 ISSUE:
Evidence of Apoptotic Cell Death after Experimental Traumatic Brain Injury in the Rat, Andreas Rink, Kar-Ming Fung, John Q. Trojanowski, Virginia Lee, Edmund Neugebauer, and Tracy K. McIntosh

Immunolocalization of SPARC, Tenascin, and Thrombospondin in Pulmonary Fibrosis, Charles Kuhn and Robert J. Mason


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