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- ☐ (17) Nucleic acid sequencing
- ☐ (18) Nucleic acid synthesis
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- ☐ (22) Pulsed field electrophoresis
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PCR Primer: A Laboratory Manual

Edited by Carl Dieffenbach, *National Institute of Allergy and Infectious Diseases*, Gabriela Dveksler, *Uniformed Services University of the Health Sciences*

From its first-published account in 1985, the polymerase chain reaction has become a standard research tool in a wide range of laboratories. Its impact has been felt in basic molecular biological research, clinical research, forensics, evolutionary studies, and the Human Genome Project. The PCR technique originally conceived by Nobel laureate Kary Mullis has proven to be exceptionally adaptable and has been transformed into a myriad array of methods, each with different applications.

PCR Primer: A Laboratory Manual introduces the complex world of PCR by beginning at an accessible level and then moving to more advanced levels of application. First, the practical requirements for performing PCR and other amplification techniques in the lab are introduced and then the basic aspects of the technique are explained by exploring important issues such as sample preparation, primer design, efficiency, detection of products, and quantitation. Protocols for a wide range of PCR and amplification techniques—each written by an expert investigator—are presented for cloning, sequencing, mutagenesis, library construction and screening, exon trapping, differential display, and expression, and these include RT-PCR, RNA PCR, LCR, multiplex PCR, panhandle PCR, capture PCR, expression PCR, 3' and 5' RACE, in situ PCR, and ligation-mediated PCR. Each protocol is augmented by analysis and troubleshooting sections and complete references.

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Introduction to PCR

Setting Up a PCR Laboratory (C.W. Dieffenbach et al.); A Standard PCR Protocol: Rapid Isolation of DNA and PCR Assay for β -Globin (M.T. Vahey et al.); Enzymatic Control of Carryover Contamination in PCR (J.L. Hartley, A. Rashchian); Ultraviolet Irradiation of Surfaces to Reduce PCR Contamination (R.W. Cone, M.R. Fairfax); Specificity, Efficiency, and Fidelity of the PCR (R.S. Cha, W.G. Thilly); Optimization and Troubleshooting in PCR (K.H. Roux); Long-Distance PCR (O.S. Foord, E.A. Rose)

Sample Preparation

Rapid Preparation of DNA for PCR Amplification with Gene ReleaserTM (E.P. Dawson et al.); PCR Amplification from Paraffin-

embedded Tissues: Sample Preparation and the Effects of Fixation (C.E. Greer et al.); RNA Purification (J.J. Adamovitz, W.C. Gause)

Primer Design

General Concepts for PCR Primer Design (C.W. Dieffenbach et al.); Design and Use of Mismatched and Degenerate Primers (S. Kwok et al.); Multiplex PCR (M.C. Edwards, R.A. Gibbs)

Detection of PCR Products: Quantitation and Analysis

Immunological Detection of PCR Products (J.G. Lazar); Quantitative PCR Using the AmpliSensor Assay (C.N. Wang); DNA Fingerprinting Using Arbitrarily Primed PCR (M. McClelland, J. Welsh); RNA Fingerprinting Using Arbitrarily Primed PCR (M. McClelland, J. Welsh); In Situ PCR (G.J. Nuovo); Single-strand Conformational Polymorphism (K. Fujita, J. Silver); Genetic Subtyping of Human Immunodeficiency Virus Using a Heteroduplex Mobility Assay (E.L. Delwart et al.); Sensitive and Fast Mutation Detection by Solid-phase Chemical Cleavage (L.L. Hansen et al.)

PCR Starting from RNA

Use of the PCR to Quantitate Relative Differences in Gene Expression (W.C. Gause, J.J. Adamovitz); Quantitative Liquid Hybridization PCR Method Employing Storage Phosphor Technology (M.T. Vahey, M.T. Wong); Use of the SNUPE Assay to Quantitate Allele-specific Sequences Differing by a Single Nucleotide (J. Singer-Sam); Trapping Internal and 3'-Terminal Exons (P.E. Nisson et al.); Expression-PCR (D.E. Lanar, K.C. Kain)

PCR-mediated Cloning

Rapid Amplification of cDNA Ends (M.A. Frohman); Panhandle PCR (D.H. Jones); Detection and Identification of Expressed Genes by Differential Display (P. Warthoe et al.); Construction of Subtractive cDNA Library Using Magnetic Beads and PCR (A. Lonneborg); PCR-based Method for Screening DNA Libraries (D.I. Israel); Screening of YAC Libraries with Robotic Support (M.M. Blanchard, V. Nowotny); Phagemid Display Libraries Derived from PCR-immortalized Rearranged Immunoglobulin Genes (H.H. Hogrefe, B. Shopes)

PCR Sequencing

Direct Sequencing of PCR-amplified DNA (V.B. Rao); Cycle Sequencing (K. Kretz et al.)

Cloning of PCR Products

Strategies for Cloning PCR Products (R. Levis); Cloning and Analysis of PCR-generated Fragments (G.L. Costa, M.P. Weiner)

Mutagenesis by PCR

Mutagenic PCR (R.C. Cadwell, G.F. Joyce); PCR Mutagenesis and Recombination In Vivo (D.H. Jones); Mutagenesis and Synthesis of Novel Recombinant Genes Using PCR (A.N. Vallejo et al.); Rapid PCR Site-directed Mutagenesis (M.P. Weiner, G.L. Costa)

Alternative Amplification Technologies

Ligase Chain Reaction (M. Weidmann et al.); Optimization and Characterization of 3SR-based Assays (T.R. Gingeras et al.); One-tube Quantitative HIV-1 RNA NASBA (B. van Gemen et al.)

Appendices

Computer Software for Selecting Primers; Reagents and Equipment

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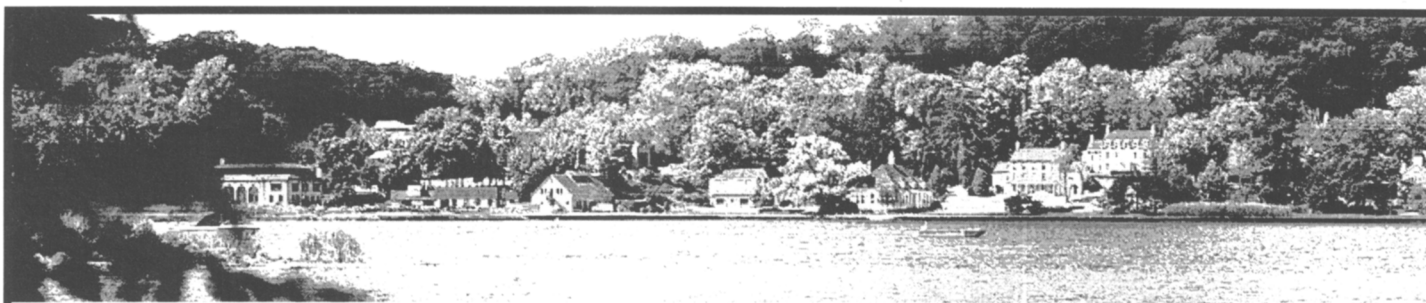
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W. Richard McCombie, Richard Wilson

Cloning & Analysis of Large DNA Molecules April 10 - 23

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Harold Riethman

Protein Purification and Characterization April 10 - 23

Richard Burgess, Al Courey,
Sue-Hwa Lin, Sheenah Mische

Early Development of *Xenopus Laevis* April 12 - 21

Robert Grainger, Hazel Sive

Summer Application
Deadline: March 15, 1996

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Bonnie Bassler, Colin Manoil, Nancy Trun

Molecular Approaches to Ion Channel Biology June 7 - 27

John Caldwell, Rock Levinson, Robert Maue

Molecular Embryology of the Mouse June 7 - 27

Richard Behringer, Virginia Papaioannou

Genetic-Epidemiologic Studies of Complex Diseases June 11 - 18

Neil Risch, Elizabeth Squires-Wheeler

Neurobiology of Human Neurological Disease: Mechanisms of Neurodegeneration June 20 - 26

Sam Gandy, William Mobley, Stan Prusiner

Computational Neuroscience: Vision

June 28 - July 11

David Heeger, Michael Shadlen,
Eero Simoncelli

Arabidopsis Molecular Genetics July 1 - 21

Xing-Wang Deng, Robert Last, Daphne Preuss

Molecular Cloning of Neural Genes July 1 - 21

Robert Darnell, Nathaniel Heintz

Genetics of Behavior and Neurobiology of *Drosophila* July 1 - 21

Nipam Patel, Barbara Taylor, Tim Tully

Neurobiology: Brain Development & Function July 14 - 27

Ronald McKay, Erin Schuman

Eukaryotic Gene Expression July 24 - August 13

Kenneth Burtis, Michael Carey,
Stephen Smale

Imaging Structure & Function in the Nervous System July 24 - August 13

Arthur Konnerth, Fred Lanni, Rafael Yuste

Yeast Genetics July 24 - August 13

Allison Adams, Daniel Gottschling,
Chris Kaiser

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Michael Ashburner, Scott Hawley

Tentative 1996 Fall Courses
Dates and Instructors
to be announced

Macromolecular Crystallography

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Advanced *In Situ* Hybridization & Immunocytochemistry

Computational Genomics

Monoclonal Antibodies from Combinatorial Libraries

1996 Meetings at Cold Spring Harbor

Zebrafish Development Genetics April 24 - 28

Nigel Holder, Nancy Hopkins,
Philip Ingham, Christiane Nusslein-
Volhard, Monte Westerfield
Abstract Deadline, February 7, 1996

Molecular Chaperones and the Heat Shock Response May 1 - 5

Costa Georgopoulos, Susan Lindquist
Rick Morimoto
Abstract Deadline, February 14, 1996

Genome Mapping & Sequencing May 8 - 12

David Bentley, Eric Green,
Philip Hieter
Abstract Deadline, February 21, 1996

The Cell Cycle May 15 - 19

Fred Cross, Jim Roberts
Abstract Deadline, February 28, 1996

Retroviruses May 21 - 26

Ron Desrosiers, Anna Marie Skalka
Abstract Deadline, March 6, 1996

61st Symposium: Function & Dysfunction in the Nervous System

May 29 - June 5
Bruce Stillman

Abstract Deadline, March 13, 1996

Cancer Genetics & Tumor Suppressor Genes August 14 - 18

Anton Berns, Terri Grodzicker,
Ed Harlow, David Livingston,
Carol Prives, Bert Vogelstein
Abstract Deadline, May 29, 1996

Molecular Genetics of Bacteria & Phages August 20 - 25

Carol Gross, Jeff Roberts,
Marjorie Russel
Abstract Deadline, June 5, 1996

Mouse Molecular Genetics August 28 - September 1

Rosa Beddington, Allan Bradley, Robb
Krumlauf, Liz Robertson
Abstract Deadline, June 12, 1996

Translational Control September 4 - 8

Richard J. Jackson,
Michael Mathews, Marvin Wickens
Abstract Deadline, June 19, 1996

Molecular Approaches to the Control of Infectious Diseases September 9 - 13

Fred Brown, Dennis Burton,
John Mekalanos, Erling Norrby
Abstract Deadline, June 26, 1996

Molecular Biology of Hepatitis B Viruses September 18 - 22

Robert Lanford, Michael Nassal
Abstract Deadline, July 3, 1996

Gene Therapy September 25 - 29

Theodore Friedmann,
Richard Mulligan, Gary Nabel,
David Weatherall
Abstract Deadline, July 10, 1996

Learning & Memory October 2 - 6

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Richard F. Thompson,
Susumu Tonegawa
Abstract Deadline, July 17, 1996

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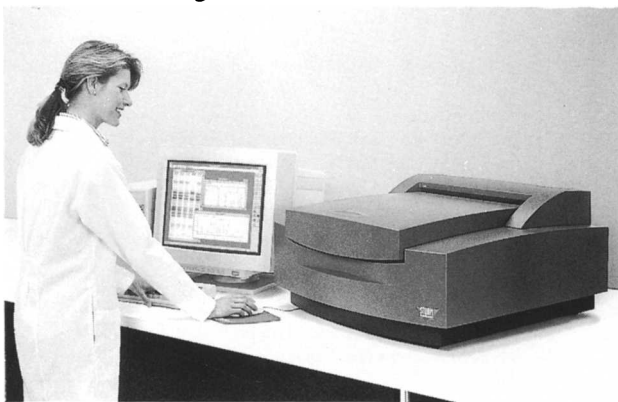
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Pre-Mixed PCR Reagents

Life Technologies, producer of Gibco-BRL products, introduces PCR SuperMix, a ready-to-use reaction mix for amplification of DNA templates by PCR. Formulated in a 1.1 × mix, PCR SuperMix contains the rTaq DNA Polymerase, dNTPs, Mg^{2+} , and salts needed for a broad range of PCR applications. Typically, the user prepares a template-primer mix in 5 ml, adds 45 μ l of PCR SuperMix (stored at 4°C, so no thawing is required), and the reaction is ready for cycling; reactions can be scaled according to the particular experimental requirements. Thus, reaction set-up time is reduced, fewer pipette tips are used, and the risk of contamination is reduced. PCR SuperMix is priced at \$89 per 100 reactions.

For additional information, contact Life Technologies at 1-(800)-828-6686. Reader Service No. 165

Filmless Autoradiography Plus Fluorescent and Chemifluorescent Gel and Blot Analysis



Molecular Dynamics' StormTM 840 system offers quantitative chemifluorescence detection plus storage autoradiography in a single instrument, allowing investigators to use non-radioactive chemifluorescence and blue-excited fluorescence, as well as traditional radioisotope-based methods, for DNA and protein studies on blots and gels (e.g., western blotting, membrane-based ELISA assays, Southern and Northern blots, gel-shift assays, multiplex DNA sequencing, and membrane-based DNA dot-blot hybridization assays). In addition, the system's quantitative, filmless autoradiography capability can be used for any application traditionally visualized with autoradiography film. The Storm sys-

tem has a 35 × 43 cm scan area that accommodates sequencing gels and other large samples; ImageQuantTM software for Macintosh or PC platforms enables system control and data analysis.

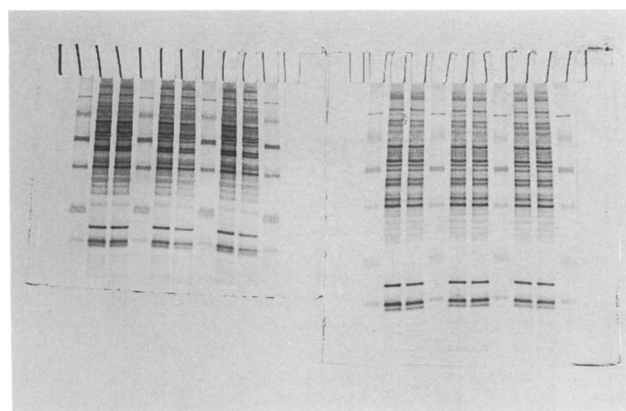
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Pipetting System Eliminates Aerosol Contamination in PCR Reactions

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For more information, contact Tri-Continent at (800)-937-4738. Reader Service No. 167

Expanded Line of Precast Mini and MiniPlus SepraGelsTM



Integrated Separation Systems offers an expanded line of Mini and MiniPlus SepraGels. ISS SepraGels, made with highest quality reagents, are produced in glass cassettes for the most efficient heat transfer and are available in a variety of gradients and single percentages. SepraGels are produced with a basic Laemmli formulation and are compatible with Tris-Glycine-SDS, Tris-Glycine and TBE-Urea, and Tricine compatible gels are also available.

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Molecular Probes of the Nervous System

Volume 1 Selected Methods for Antibody and Nucleic Acid Probes

By Susan Hockfield, *Yale University School of Medicine*; Steve Carlson, *University of Washington*; Chris Evans, *University of California, Los Angeles*; Pat Levitt, *Medical College of Pennsylvania*; John Pintar, *Columbia University College of Physicians & Surgeons*; Laura Silberstein, *San Jose State University*

Over the past ten years, new techniques have transformed most branches of neuroscience. The most powerful of them have been adapted from cell and molecular biology and immunology to permit analysis of nerve cell structure and function at a level of detail never before possible. With this volume, CSHL Press introduces a new series of manuals designed specifically for neuroscientists who wish to acquire the skills of molecular analysis. A laboratory course taught each year at Cold Spring Harbor demonstrates the potential and pitfalls of antibody and nucleic acid probes as biochemical and anatomical reagents. This first volume in the series, designed for ease of use at the lab bench, is a distillation by the instructors of much of the material covered in several years of the course. As an aid to understanding the strategy and rationale of the protocols, brief supplementary explanations are included. The manual is a valuable resource for established investigators and for anyone working with nervous system tissue for the first time.

CONTENTS

Abbreviations

Cell Culture

Sterile Technique; Growing Myelomas and Hybridomas; Counting Cells Using a Hemocytometer; Cell Viability Determinations; Freezing and Thawing Myelomas and Hybridomas; Macrophage Feeder Layers; Ascites-producing Tumors; Contamination with *Mycoplasma*; Troubleshooting Guide for Dying Cells; Primary Cultures from the Central Nervous System

Generating Monoclonal Antibodies

The Immune System; Creating Hybridomas; Use of Selection Media in Hybridoma Production; Isolating Clones Producing a Desirable Antibody; Fusion Partners for Hybridomas; Immunization; Conjugating the Antigen to a Carrier; Fusion; Designing Assays for Antibodies of the Desired Specificity; Immunosuppression to Generate Monoclonal Antibodies with Restricted Specificities; In Vitro Immunization for Hybridoma Production; Subcloning

Immunocytochemistry

Preparing Tissues and Cells for Immunocytochemistry; Anesthesia; Fixation; Freezing Tissue for Sectioning; Noninfiltrating Matrices for Embedding Fixed Tissue for Sectioning; Subbed Slides; Sectioning; Conditions for Immunocytochemistry; Light-microscopy Immunocytochemistry Using Labeled Secondary Antibody (Single-label Immunocytochemistry); Light-microscopy Immunocytochemistry Using Double Labeling; Reducing Nonspecific Background Labeling; Electron-microscopy Immunocytochemistry

In Situ Hybridization

Tissue and Section Preparation; Hybridization Probes; Controls for Specificity of Hybridization; In Situ Hybridization Using cRNA Probes; In Situ Hybridization Using Digoxigenin-labeled RNA Probes; Combining Immunocytochemistry with In Situ Hybridization

Immunochemical Identification and Characterization of Antigens

SDS-polyacrylamide Gel Electrophoresis; Preparing and Running SDS-polyacrylamide Gels; Visualizing Proteins on SDS-polyacrylamide Gels; Western Blotting; Immunoprecipitation; Dot Blotting; Screening cDNA Libraries Using Antibodies

Immunochemical Characterization, Purification, and Derivatization of Monoclonal Antibodies

Microwell Assays; Isotyping; Purifying Immunoglobulins and Concentrating Monoclonal Antibodies in Culture Supernatants; Generation of Proteolytic Subfragments from Purified IgG; Conjugating Purified Antibodies to Marker Molecules

Purification of Antigens

Extracting and Solubilizing the Antigen of Interest; Determining the Concentrations of the Antigen and the Total Protein; Monitoring the Specific Activity of the Antigen; Monitoring the Recovery of the Antigen; Evaluating the Purity of the Antigen; Immunoaffinity Purification of Antigens; Additional Protein Purification Methods

Radioimmunoassay

Principles of the Radioimmunoassay; Plotting Standard Curves; Antibodies for the Radioimmunoassay; Generating Radiolabeled Antigens for Use as Tracers; Methods for Separating Antibody-bound Antigen from Free Antigen; Optimizing the Sensitivity of the Radioimmunoassay; Ensuring the Specificity of Tracer Displacement

Peptide Synthesis

Solid-phase Peptide Synthesis; Attaching the Carboxy-terminal Amino Acid to Wang Resin or Peptide Amide Linker Polystyrene Resin; Synthesis Cycle; Cleavage of the Peptide from the Resin and Removal of the Side-chain-protecting Groups; Purification of the Peptide; Validation of the Structure and Purity of the Peptide Product

Appendices

References

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