Strategies for Protein Purification and Characterization
A Laboratory Course Manual

By Daniel R. Marshak, Cold Spring Harbor Laboratory; James T. Kadonaga, Department of Biology, University of California, San Diego; Richard R. Burgess, University of Wisconsin, Madison, and Mark W. Knuth, Promega Corporation, Madison, Wisconsin; William A. Brennan, Jr., Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, and Sue-Hwa Lin, University of Texas M.D. Anderson Cancer Center

Investigators who have identified and cloned a gene of interest often want to isolate and characterize the protein product, yet the methods required are notoriously tricky for the inexperienced. For the past four years, a course has been held at Cold Spring Harbor Laboratory to teach scientists how to execute the major protein techniques by applying them to four distinct, representative types of molecule: a regulatory protein, a DNA-binding protein, a recombinant protein, and a membrane-bound receptor. This course has now been adapted in the form of a laboratory manual that covers a variety of bulk fractionation, electrophoretic, and chromatographic techniques. Step-by-step protocols are accompanied by troubleshooting advice and guidance on generalizing the techniques for other classes and types of protein. The emphasis throughout is on strategies for purification and characterization rather than automated instrumental analysis.

After years of rigorous testing, these techniques are robust and reliable, and are presented here with the clarity and completeness for which Cold Spring Harbor manuals are celebrated. The book is invaluable for specialists in genetics, microbiology, neuroscience, and cell biology who wish to develop expertise in working with proteins.

CONTENTS

Foreword by James E. Rothman
Introduction
How to Use This Manual

UNIT I: PURIFICATION OF CALMODULIN

Introduction
Experiment 1: Activity Assays: Assay of Calmodulin Fractions
Experiment 2: Preparation of a Tissue Extract
Experiment 3: Bulk Fractionation
Experiment 4: Ion-exchange Chromatography
Experiment 5: Hydrophobic Interaction Chromatography
Experiment 6: Characterization of Calmodulin: Calculation of Recovery
Experiment 7: Characterization of Calmodulin: Electrophoresis
Experiment 8: Proteolytic Digestion
Experiment 9: Reverse-phase HPLC
Experiment 10: Physical Analysis of Calmodulin
Preparation of Reagents
References

UNIT II: PURIFICATION OF TRANSCRIPTION FACTOR AP-1 FROM HELA CELLS

Introduction
Experiment 1: Preparation of a Nuclear Extract from HeLa Cells
Experiment 2: Gel Filtration Chromatography with Sephacryl S-300 HR
Experiment 3: Sequence-specific DNA Affinity Chromatography
Experiment 4: DNase I Footprinting
Experiment 5: Gel Mobility-shift Assay
Experiment 6: Preparation of Heparin-Sepharose CL-2B
Preparation of Reagents
References

UNIT III: PURIFICATION OF A RECOMBINANT PROTEIN OVERPRODUCED IN ESCHERICHIA COLI

Introduction
Experiment 1: Breakage of E. coli Cells and Preparation of Inclusion Bodies
Experiment 2: Solubilization, Refolding, and Ion-exchange Chromatography of the Inclusion Body Pellet (σ32)
Experiment 3: Polyethyleneimine Precipitation and Immunoaffinity Chromatography of the Soluble Extract (Core RNA Polymerase-σ32 Complex)
Experiment 4: Quantitation and Summary of Preparation
Experiment 5: Protein Characterization
Protocol Development Trials: Purification of σ32 from a Bacterial Overexpresser
Preparation of Reagents
References

UNIT IV: SOLUBILIZATION AND PURIFICATION OF THE RAT LIVER INSULIN RECEPTOR

Introduction
Experiment 1: Isolation of Plasma Membranes from Rat Liver
Experiment 2: Solubilization of Insulin Receptor from Membranes
Experiment 3: Lectin Affinity Chromatography of Solubilized Receptors
Experiment 4: Insulin Affinity Chromatography of Partially Purified Receptors
Experiment 5: Cross-linking of Insulin Receptors with [125I] Insulin
Experiment 6: Insulin-stimulated Insulin Receptor Autophosphorylation
Experiment 7: Analysis of Insulin Receptor Glycosylation
Preparation of Reagents
References
Appendices

1996, 396 pp., illus., appendices, indexes
Plastic comb binding $85 ISBN 0-87969-385-1
IN 1996 SEARCH THE CONTENTS OF GENES & DEVELOPMENT...ON-LINE!

http://www.cshl.org
Abstracts now available for 1996 research papers!
Now you can preview the latest issue before you receive it! Tables of contents are available two weeks before the issue mail date and abstracts are available the day the issue mails. Keyword searches can be done both on the tables of contents and 1996 abstracts.

Sample abstract:

A novel, mitogen-activated nuclear kinase is related to a Drosophila developmental regulator

Gerald V. Dens and Michael R. Green

Howard Hughes Medical Institute, Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, Massachusetts 01605 USA

Although the ultimate targets of many signal transduction pathways are nuclear transcription factors, the vast majority of known protein kinases are cytoplasmic. Here, we report on a novel nuclear kinase that is present exclusively in the nucleus. Kinase activity is increased upon cellular proliferation and is markedly elevated in patients with acute and chronic lymphocytic leukemia. We have identified a human gene that encodes this nuclear kinase and find that it is closely related to Drosophila, female sterile homolog (fs-h), a developmental regulator with no known biochemical activity. Collectively, these results suggest that this nuclear kinase is a component of a signal transduction pathway that plays a role in Drosophila development and human growth control.

[Key Words, Signal transduction, kinases, Drosophila, leukemias, leukosis]

Corresponding author.

The contents and abstracts of Genes & Development are available at your fingertips. Just log into our WorldWideWeb site at http://www.cshl.org

Subscribe Today!
Cold Spring Harbor Laboratory Press, 10 Skyline Drive, Plainview, N.Y. 11803-2500
Phone: 1-800-843-4388 or 516-349-1930 Fax: 516-349-1946
E-mail: cshpress@cshl.org or World Wide Web Site http://www.cshl.org/