

*Methods in Enzymology*

*Volume 283*

*Cell Cycle Control*

EDITED BY

*William G. Dunphy*

DIVISION OF BIOLOGY  
HOWARD HUGHES MEDICAL INSTITUTE  
CALIFORNIA INSTITUTE OF TECHNOLOGY  
PASADENA, CALIFORNIA



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

## Table of Contents

CONTRIBUTORS TO VOLUME 283 . . . . .	ix
PREFACE . . . . .	xv
VOLUMES IN SERIES . . . . .	xvii

### Section I. General Methodologies

1. Purification and Reconstitution of Cyclin-Dependent Kinase 2 in Four States of Activity	ALICIA A. RUSSO	3
2. Purification of Recombinant Cyclin B1/cdc2 Kinase from <i>Xenopus</i> Egg Extracts	HEIKE WILHELM, SØREN S. L. ANDERSEN, AND ERIC KARSENTI	12
3. Protein Phosphorylation Analysis by Electrospray Ionization-Mass Spectrometry	KATHERYN A. RESING AND NATALIE G. AHN	29
4. Cloning Cell Cycle Regulatory Genes by Transcomplementation in Yeast	CHRIS NORBURY AND SERGIO MORENO	44
5. Fluorescence-Activated Cell Sorting of Transfected Cells	PETER D. ADAMS, PETER LOPEZ, WILLIAM R. SELLERS, AND WILLIAM G. KAEHLIN, JR.	59
6. Microinjection of Antibodies into Mammalian Cells	NED J. C. LAMB AND ANNE FERNANDEZ	72
7. Small Pool Expression Screening: Identification of Genes Involved in Cell Cycle Control, Apoptosis, and Early Development	KEVIN D. LUSTIG, P. TODD STUKENBERG, THOMAS J. MCGARRY, RANDALL W. KING, VINCENT L. CRYNS, PAUL E. MEAD, LEONARD I. ZON, JUNYING YUAN, AND MARC W. KIRSCHNER	83
8. Localization of Cell Cycle Regulators by Immunofluorescence	JONATHON PINES	99
9. Chemical Inhibitors of Cyclin-Dependent Kinases	LAURENT MEIJER AND SUNG-HOU KIM	113

10. Identifying Human Homologs of Cell Cycle Genes Using dbEST and XREFdb	DOUGLAS E. BASSETT, JR., MARK BOGUSKI, AND PHILIP HIETER	128
11. Gene Identification Using the Yeast Two-Hybrid System	CHANG BAI AND STEPHEN J. ELLEDGE	141
<b>Section II. Cell Cycle Regulators in Mammalian Systems</b>		
12. Use of Tetracycline-Controlled Gene Expression Systems to Study Mammalian Cell Cycle	SABINE FREUNDLIEB, UDO BARON, ANGELIKA L. BONIN, MANFRED GOSSEN, AND HERMANN BUJARD	159
13. Regulation of Mammalian Cyclin-Dependent Kinase 2	ROBERT J. SHEAFF	173
14. Assay for Activity of Mammalian Cyclin D-Dependent Kinases CDK4 and CDK6	DAWN E. PHELPS AND YUE XIONG	194
15. Functional Analysis of E2F Transcription Factor	JOSEPH R. NEVINS, JAMES DEGREGORI, LASZLO JAKOI, AND GUSTAVO LEONE	205
16. Analysis of Mammalian Cdc37, a Protein Kinase Targeting Subunit of Heat Shock Protein 90	LILIA STEPANOVA, XIAOHONG LENG, AND J. WADE HARPER	220
17. Purification and Analysis of CIP/KIP Proteins	B. D. DYNLACHT, C. NGWU, J. WINSTON, E. C. SWINDELL, S. J. ELLEDGE, E. HARLOW, AND J. W. HARPER	230
18. Functional Dissection of p53 Tumor Suppressor Protein	LATA JAYARAMAN, ELLA FREULICH, AND CAROL PRIVES	245
19. Reconstitution of Mammalian CDK-Activating Kinase	ROBERT P. FISHER	256
20. Characterization of Mammalian NIMA-Related Kinases	ANDREW M. FRY AND ERICH A. NIGG	270
21. Generation of Phosphorylated Cyclin-Dependent Kinase 2 and Functional Characterization of Threonine-160-Specific Phosphatase KAP	RANDY Y. C. POON	283
22. Isolation and Characterization of Temperature-Sensitive Mammalian Cell Cycle Mutants	TAKEHARU NISHIMOTO	292

## Section III. Cell Cycle Control in Yeast and Fungal Systems

23. Use of Conditional Promoters for Expression of Heterologous Proteins in <i>Saccharomyces cerevisiae</i>	VOLKER RÖNICKE, WOLFF GRAULICH, DOMINIK MUMBERG, ROLF MÜLLER, AND MARTIN FUNK	313
24. Flow Cytometric Analysis of DNA Content in Budding Yeast	STEVEN B. HAASE AND DANIEL J. LEW	322
25. $\alpha$ -Factor Synchronization of Budding Yeast	LINDA L. BREEDEN	332
26. Elutriation of Budding Yeast	LELAND H. JOHNSTON AND ANTHONY L. JOHNSON	342
27. Functional Analysis of <i>FAR1</i> in Yeast	NICOLE VALTZ AND MATTHIAS PETER	350
28. Cell-Free Ubiquitination of Cell Cycle Regulators in Budding Yeast Extracts	RATI VERMA, YONG CHI, AND RAYMOND J. DESHAIES	366
29. Genomic Footprinting of Budding Yeast Replication Origins during the Cell Cycle	CORRADO SANTOCANALE AND JOHN F. X. DIFFLEY	377
30. Preparation of Active Cdc7/Dbf4 Kinase from Yeast Cells	WENDY J. DIXON AND JUDITH L. CAMPBELL	390
31. Analysis of Budding Yeast Kinases Controlled by DNA Damage	YOLANDA SANCHEZ, ZHENG ZHOU, MINGXIA HUANG, BRUCE E. KEMP, AND STEPHEN J. ELLEDGE	398
32. Use of <i>cdc13-1</i> -Induced DNA Damage to Study Effects of Checkpoint Genes on DNA Damage Processing	DAVID LYDALL AND TED WEINERT	410
33. The Spindle Assembly Checkpoint in Budding Yeast	AARON F. STRAIGHT AND ANDREW W. MURRAY	425
34. Genetic and Physiological Analysis of DNA Replication in Fission Yeast	STUART A. MACNEILL AND PETER A. FANTES	440
35. Use of Green Fluorescent Protein for Intracellular Protein Localization in Living Fission Yeast Cells	KENTARO NABESHIMA, SHIGEAKI SAITOH, AND MITSUHIRO YANAGIDA	459
36. Analysis of Radiation-Sensitive Mutants of Fission Yeast	RHIAN J. EDWARDS AND ANTONY M. CARR	471
37. Cytokinesis in Fission Yeast <i>Schizosaccharomyces pombe</i>	MOHAN K. BALASUBRAMANIAN, DANNELL MCCOLLUM, AND KATHLEEN L. GOULD	494

38. Stress-Activated Protein Kinase Pathway in Cell Cycle Control of Fission Yeast	KAZUHIRO SHIOZAKI AND PAUL RUSSELL	506
39. Characterization of NIMA Protein Kinase in <i>Aspergillus nidulans</i>	XIANG S. YE, GANG XU, RUSSELL R. FINCHER, AND STEPHEN A. OSMANI	520
 Section IV. Analysis of Cell Cycle Regulators in Oocyte, Egg, and Embryonic Systems  		
40. <i>Xenopus</i> Replication Assays	MARK A. MADINE AND DAWN COVERLEY	535
41. Characterization of the <i>Xenopus</i> Replication Licensing System	JAMES P. J. CHONG, PIA THÖMMES, ALISON ROWLES, HIRO M. H. MAHBUBANI, AND J. JULIAN BLOW	549
42. Regulation of <i>Xenopus</i> Cdc25 Protein	AKIKO KUMAGAI AND WILLIAM G. DUNPHY	564
43. Characterization of Spindle Assembly Checkpoint in <i>Xenopus</i> Egg Extracts	REY-HUEI CHEN AND ANDREW MURRAY	572
44. Mechanisms of <i>Xenopus</i> Oocyte Maturation	MONICA S. MURAKAMI AND GEORGE F. VANDE WOUDE	584
45. Apoptosis in <i>Xenopus</i> Egg Extracts	SALLY KORNBLUTH	600
46. Preparation of Clam Oocyte Extracts for Cell Cycle Studies	JOAN V. RUDERMAN, VALERY SUDAKIN, AND AVRAM HERSHKO	614
47. Mitotic Protein Kinases in <i>Drosophila</i> Embryos	ÁLVARO TAVARES AND DAVID M. GLOVER	622
AUTHOR INDEX . . . . .		633
SUBJECT INDEX . . . . .		661