

METHODS IN YEAST GENETICS

A COLD SPRING HARBOR LABORATORY COURSE MANUAL

2000 EDITION

DAN BURKE

DEAN DAWSON

TIM STEARNS

Suranaree University of Technology



31051000622470



COLD SPRING HARBOR LABORATORY PRESS

Contents

Preface	vii
Introduction	ix
Genetic Nomenclature	xiii
Experiments	
I. Looking at Yeast Cells	1
II. Isolation and Characterization of Auxotrophic, Temperature-sensitive, and UV-sensitive Mutants	7
III. Meiotic Mapping	17
IV. Mitotic Recombination and Random Spore Analysis	29
V. Transformation of Yeast	39
VI. Synthetic Lethal Mutants	49
VII. Gene Replacement	55
VIII. Isolation of <i>ras2</i> Suppressors	73
IX. Manipulating Cell Types	79
X. Isolating Mutants by Insertional Mutagenesis	89
XI. Two-hybrid Protein Interaction Method	97
Techniques and Protocols	
1. High-efficiency Transformation of Yeast	103
2. "Lazy Bones" Plasmid Transformation of Yeast Colonies	107
3. Yeast DNA Isolations	109
A. Yeast DNA Miniprep (40 ml)	109
B. Yeast DNA Miniprep (5 ml)	110
C. A Ten-minute DNA Preparation from Yeast	111
D. Yeast Genomic DNA: Glass Bead Preparation	113
4. Yeast Protein Extracts	115
5. Yeast RNA Isolation	117

6. Hydroxylamine Mutagenesis of Plasmid DNA	121
7. Assay of β -Galactosidase in Yeast	123
8. Plate Assay for Carboxypeptidase Y	127
9. Random Spore Analysis	129
10. Yeast Vital Stains	131
11. Yeast Immunofluorescence	133
12. Actin Staining in Fixed Cells	137
13. PCR Protocol for PCR-mediated Gene Disruption	139
14. Yeast Colony PCR Protocol	141
15. Measuring Yeast Cell Density by Spectrophotometry	143
16. Cell Synchrony	145
17. Chromatin Immunoprecipitation	149
18. Flow Cytometry of Yeast DNA	155
19. Logarithmic Growth	157
20. EMS Mutagenesis	159
21. Tetrad Dissection	161
22. Making a Tetrad Dissection Needle	165
23. Picking Zygotes	167
24. Determining Plating Efficiency	169
Appendices	
A. Media	171
B. Stock Preservation	183
C. Yeast Genetic and Physical Maps	185
D. Templates for Making Streak Plates	193
E. Electrophoretic Karyotypes of Strains for Southern Blot Mapping	195
F. Strains	197
G. Counting Yeast Cells with a Standard Hemocytometer Chamber	199
H. Tetrad Scoring Sheet	201
I. Trademarks	203
J. Suppliers	205