

# Index

## A

- Affinity capture
  - binding reaction, 391
  - density gradient ultracentrifugation of protein complexes
    - centrifugation, 398–399
    - fraction analysis, 399–400
    - gradient preparation, 398
    - materials, 397–398
  - elution under denaturing conditions
    - antibody-conjugated bead equilibration, 391
    - cryogenic disruption, 389–390
    - extract preparation, 390–391
    - materials, 388–389
  - native elution
    - cleavable tags, 394–395
    - competitive elution with PEGylOx, 395–396
    - materials, 393–394
    - troubleshooting, 396
  - optimization, 384–386
  - principles, 383–384
- Agar medium, 211
- $\alpha$ -factor,  $G_1$  synchronization, 243–244
- Amyloid-prion buffers, 506–507
- Auxotrophic mutants, 12, 15

## B

- Biofilm
  - assays
    - culture and photography, 58–59
    - materials, 57–58
    - overview, 50–51
    - recipes, 59
  - induction, 50
- BioGRID
  - curation statistics, 578
  - feedback from users, 588
  - interaction network visualization
    - BioGRID viewer, 586
    - Cytoscape, 586–587
    - download options and formats, 587–588
  - overview, 577–578
  - scope, 579–580
  - searching for gene or protein of interest, 583–586
  - user interface, 578
- BRB80, 292, 297

- BSA. *See* Bulk segregant analysis
- Bulk segregant analysis (BSA)
  - candidate gene and variant identification, 659
  - materials, 656–657
  - overview, 653–654
  - phenotypically extreme segregant acquisition, 658–659
  - recipes, 659–660
  - recombinant cross progeny, 657–658
  - segregant generation, 657
  - sequencing and causal loci detection, 659

## C

- Calling card analysis
  - advantages and limitations, 533, 535
  - cloning strain, 538–540
  - DNA extraction, 541
  - genomic digestion, 541–542
  - inverse polymerase chain reaction, 542–543
  - materials, 536–538
  - overview, 533–534
  - plasmid transformation and induction, 540–541
  - recipes, 544–545
  - self-ligation, 542
  - troubleshooting, 543
- CalMorph. *See* High-throughput microscopy
- cdc15-2*,  $G_1$  synchronization, 245–246
- Cell cycle
  - drug-induced arrest, 246
  - position determination
    - flow cytometry
      - data analysis, 261
      - DNA staining, 260
      - enzymatic digestion, 260
      - ethanol fixation, 259
      - flow cytometry, 260–261
      - high-throughput staining, 262
      - materials, 258–259
      - recipes, 263
      - rehydration of fixed cells, 259
      - troubleshooting, 262–263
    - overview, 240–241
  - synchronization
    - centrifugal elutriation
      - cleanup and preparation for storage, 255

- Coulter counting, 252
  - culture pregrowth and inoculation volume calculation, 250
  - elutriation setup and sterilization, 251
  - exhaustive fractionation, 254–255
  - fraction collection and monitoring, 253–254
  - $G_1$  cell collection for sampling, 252–253
  - loading, 253
  - materials, 248–249
  - sample preparation, 252
  - troubleshooting, 255–256
  - chemical and genetic approaches
    - $G_1$  synchronization using  $\alpha$ -factor mating pheromone, 243–244
    - $M/G_1$  synchronization using *cdc15-2*, 245–246
    - materials, 242–243
    - overview, 239–240
    - selection of technique, 256–257
  - Cell wall
    - components, 199–200
    - disruption
      - disruptors, 201–202
      - imaging, 214–215
      - materials, 213–214
      - recipes, 215
    - fluorescent labeling
      - materials, 205–206
    - staining
      - 1,3- $\beta$ -glucan, 206–207
      - cell preparation, 206
      - chitin, 207–208
      - mannoproteins, 207–208
  - function, 199
  - spore wall integrity testing
    - fly feces analysis, 210–211
    - materials, 209–210
    - prey yeast cells
      - Drosophila* feeding, 210
      - preparation, 210
      - recipes, 211–212
    - synthesis and assembly, 200–201
- Centrifugal elutriation. *See* Cell cycle
- CgIs. *See* Chemical–genetic interactions

## Index

- Chemical–genetic interactions (CGIs)  
challenges in screening, 465–466  
halo high-throughput assay  
incubation and analysis, 469–470  
materials, 468–469  
plate preparation, 469  
recipes, 470  
overview, 463–465  
parallel analysis of barcoded yeast strains  
array-based barcode quantification, 473–474  
competitive growth, 473  
data analysis, 474–475, 477  
materials, 471–472  
recipes, 477–478  
sequencing-based barcode quantification, 475–477
- Chemogenomics. *See* Chemical–genetic interactions
- Chemostat culture  
chemostat design, 662–663  
continuous culture overview, 662  
experimental evolution studies  
culture, 680–681  
inoculation, 680  
materials, 679–680  
sampling  
chemostat, 681  
effluent, 681–682  
troubleshooting, 682  
mini-chemostat array assembly  
carboy assembly with chemostats and effluent collection, 669–670  
cleanup and sterilization, 668  
culture chamber assembly, 666–668  
hydrated aeration, 668  
materials, 665–666  
media  
carboy preparation, 668–669  
filtration, 669  
recipes, 670–672  
physiology studies  
harvesting for analysis  
metabolites, 677  
microscopy, 676–677  
protein, 677  
RNA, 675–676  
materials, 673–674  
recipes, 678  
steady state establishment, 674–675
- Chitin, staining in cell wall, 207–208
- Chromatin  
conformation studies. *See* Chromosome conformation capture  
organization in yeast, 104–105
- Chromosome conformation capture (3C)  
chromosome conformation capture  
carbon copy  
advantages, 125  
ligating fragments, 124  
materials, 121–122  
polymerase chain reaction, 124–125  
probes  
annealing, 123–124  
design, 125–126  
preparation, 122  
troubleshooting, 125  
cross-linking chromatin, 110  
digestion, 110–111  
end-point polymerase chain reaction, 112–113
- Hi-C  
biotin removal, 134  
biotinylation of digested ends, 131  
cross-link reversal and ligation  
product purification, 132–133  
cross-linking chromatin, 129–131  
digestion, 131  
DNA end repair and A-tailing, 134–135  
efficiency estimation, 133–134  
library  
aired-end polymerase chain reaction amplification, 137–138  
fractionation, 135–136p  
sonication, 134  
ligating cross-linked fragments, 131  
ligation product enrichment, 136–137  
materials, 127–129  
recipes, 138–139  
troubleshooting, 138  
ligation of cross-linked chromatin fragments, 111  
materials, 108–110  
overview, 103–106  
randomized ligation control  
chromosomal DNA isolation, 116–117  
genomic DNA digestion, 117–118  
ligating digested fragments, 118–119  
materials, 115–116  
recipes, 119–120  
recipes, 113  
reverse cross-linking, 111–112
- Chromosome replication. *See also* Meiosis  
overview, 87  
single-fiber analysis  
DNA combing  
agarose plug preparation and digestion, 96–97
- cell synchronization and bromodeoxyuridine labeling, 95–96  
glass surface preparation, 92–93  
imaging, 98–99  
immunodetection, 97–98  
materials, 90–92  
plug melting and DNA combing, 97  
recipes, 100–101  
simple machine preparation, 93–95  
troubleshooting, 99  
overview, 88  
techniques for study, 88
- CM medium. *See* Complete minimal medium
- COM drop-out powder, 639, 644
- Complete minimal (CM) medium, 169–170, 215
- Complete synthetic medium, 497–498
- Complex traits, genetic dissection  
bulk segregant analysis  
candidate gene and variant identification, 659  
materials, 656–657  
overview, 653–654  
phenotypically extreme segregant acquisition, 658–659  
recipes, 659–660  
recombinant cross progeny, 657–658  
segregant generation, 657  
sequencing and causal loci detection, 659  
causal gene and genetic variant dissection, 654  
challenges, 651–652  
genome-wide association study, 652–653  
linkage mapping, 653
- Concanavalin A. *See* High-throughput microscopy
- Congenetic strain  
conditional effects of mutations, 2–4  
overview, 1–2
- Continuous culture. *See* Chemostat culture
- Culture  
batch culture, 661–662  
continuous culture. *See* Chemostat culture  
optimal growth conditions, 11–12  
propagating culture, 12–13  
synchronous meiotic cultures  
assessment of efficiency and synchrony, 34  
liquid medium culture, 33–34  
materials, 32–33  
overview, 23  
recipes, 35–36

- Culture (*Continued*)  
  sample collection  
    meiotic recombination analysis, 34–35  
    surface spreading of nuclei for immunofluorescence analysis, 35  
    western blot analysis, 35
- Cytoscape  
  BioGRID interaction network  
    visualization, 586–587  
  data preparation for import, 592  
  installation, 591–592  
  networks  
    annotation, 595–598  
    loading, 592–594  
    organization, 594, 596–597  
    visualization, 594
- Cytosine deaminase protein-fragment  
  complementation assay. *See* Protein-fragment complementation assay
- D**
- DAPI. *See* 4',6-Diamidino-2-phenylindole
- Deep mutational scanning  
  doped synthetic oligonucleotides, 193–194  
  enrichment score calculation from DNA sequencing output files  
    enrich output file analysis, 196  
    materials, 195  
    troubleshooting, 197  
  functional selection, 192  
  high-throughput sequencing, 192–193  
  library construction, 192  
  principles, 187–189  
  sequence-function map analysis, 189
- Deletion collections. *See* *Saccharomyces* Genome Deletion Project
- Density gradient ultracentrifugation. *See* Affinity capture; Prions
- 4',6-Diamidino-2-phenylindole (DAPI),  
  assessment of efficiency and synchrony of meiotic cultures, 34
- Dihydrofolate reductase protein-fragment  
  complementation assay. *See* Protein-fragment complementation assay
- Diploids, applications, 13–14
- DNA binding motifs. *See* Transcription factor–DNA binding motifs
- DNA combing. *See* Chromosome replication
- DNA sequencing  
  bulk segregant analysis, 659  
  high throughput strain sequencing  
    platforms, 621–623  
    prospects, 623  
  library preparation  
    fragmentation, 627  
    genomic DNA extraction, 626–627  
    materials, 625–626  
    polymerase chain reaction, 628–629  
    recipes, 629  
    tailing, 627–628  
    troubleshooting, 629  
  DNA synthesis. *See* Synthetic genome synthesis  
  Drop-out medium, 169–170, 215, 275, 338, 343, 458, 544
- E**
- Electron microscopy, prion amyloids, 484–485
- Electron tomography  
  grid preparation, 310  
  high-pressure freezing/freeze substitution, 305, 309–310  
  materials, 308–309  
  prospects for study, 307  
  recipes, 311–312  
  three-dimensional reconstruction, 305–307, 311  
  tilt series acquisition, 310–311  
  troubleshooting, 311  
  yeast specimen preparation for transmission electron microscopy, 303–305
- Evaporative light-scattering detection.  
  *See* Lipids, yeast
- F**
- Fatty acids. *See* Lipids, yeast
- Filamentous growth  
  assays  
    mitogen-activated protein kinase pathway  
      materials, 65–66  
      mucin secretion profiling, 69–70  
      pectinase assay, 69  
      recipes, 70–72  
      western blot, 67–69  
    overview, 50–51  
  plate-washing assay  
    agar invasion, 54–55  
    materials, 53–54  
    recipes, 55–56  
  single-cell analysis  
    culture and microscopy, 62–63  
    materials, 61–62  
    recipes, 64  
  induction, 49–50
- Flow cytometry. *See* Cell cycle
- 5-Fluorocytosine solution, 371
- 5-Fluoroorotic acid plates, 170
- Forward genetics  
  mutant identification and selection, 16–17  
  overview, 13–14
- Freeze-substitution fixative, 311
- G**
- Galactose-Ura plates, 544
- Gas chromatography. *See* Lipids, yeast
- GenFlex tags, 477–478
- Genome synthesis. *See* Synthetic genome synthesis
- Genome-wide association study (GWAS),  
  complex trait dissection, 652–653
- Genotype–phenotype mapping  
  causality confirmation of genotype–phenotype links  
    materials, 646  
    overview, 634  
    recipes, 649–650  
    reciprocal hemizyosity, 647–648  
    troubleshooting, 648–649  
  overview, 631–632  
  phenomics, 633  
  quantitative trait loci mapping  
    F<sub>1</sub> segregant generation, 642  
    mapping, 642–643  
    materials, 641–642  
    overview, 633–634  
    recipes, 644–645  
    troubleshooting, 643  
  *Saccharomyces cerevisiae* ecology and population genetics, 632  
  strain isolation and domestication  
    enrichment and isolation, 637  
    materials, 636  
    recipes, 639–640  
    sampling, 637  
    species identification, 637  
    strain preparation for laboratory work, 637–638  
    troubleshooting, 638
- Glucose-His plates, 545
- Glucose-limited chemostat medium, 670
- Glucose-Ura Medium, 545
- Glycerophospholipids. *See* Lipids, yeast
- GWAS. *See* Genome-wide association study
- H**
- Halo assay. *See* Chemical–genetic interactions
- Hi-C. *See* Chromosome conformation capture

## Index

- High-performance liquid chromatography.  
  See Lipids, yeast
- High-throughput microscopy  
  automated image analysis, 268  
  imaging pipelines, 266–267  
  morphology studies with CalMorph  
    concanavalin A coating of  
      microplates, 279  
  fixation, 278  
  image acquisition and processing,  
    279–281  
  materials, 277–278  
  recipes, 281  
  specimen preparation, 279  
  staining, 278–279
- overview, 265–266
- synthetic genetic array for fluorescent  
  tagging  
    drug treatment and medium switch,  
      273–274  
  imaging, 274  
  materials, 271–273  
  recipes, 275–276  
  subculture preparation, 273
- Homologous recombination-based cloning  
  applications, 76  
  overview, 73–75  
  plasmid construction  
    competent cell preparation  
      *Escherichia coli*, 83  
      yeast, 81–82  
    DNA fragment preparation, 81  
    genomic DNA preparation, 82  
    materials, 78–80  
    overview, 80, 83–84  
    plasmid recovery from bacteria, 83  
    recipes, 84–86  
  polymerase chain reaction-free  
    recombination, 75
- Hydrogen–deuterium exchange, prion  
  amyloids, 485
- Hydrophilic interaction chromatography–  
  tandem mass spectrometry.  
  See Metabolomics
- I**
- Immobilized metal affinity chromatogra-  
  phy. See Proteomics
- Immunoaffinity precipitation. See  
  Proteomics
- Intragenic complementation, 13–14
- ISO buffer, 691
- Isogenic strain, overview, 1–2, 15
- Isothermal reaction master mix, 69
- K**
- Knockout marker cassettes. See MX  
  cassettes
- L**
- LB media, 338, 343, 691
- LB medium plus ampicillin, 84
- LB plates, 338, 343, 591
- Lead citrate solution, 312
- Linkage mapping, complex trait dissection,  
  653
- Lipids, yeast  
  challenges in study, 217–218  
  composition by strain, 218  
  extraction  
    cell growth and harvesting, 224  
    materials, 223  
    organic extraction, 224–225  
    troubleshooting, 225  
  fatty acids, 218–219  
  gas chromatography  
    fatty acid methyl ester derivatization,  
      232–233  
    materials, 231–232  
    running conditions, 233–234  
    troubleshooting, 234  
  glycerophospholipids, 219–220  
  high-performance liquid  
    chromatography/evaporative  
    light-scattering detection  
    materials, 235–236  
    running conditions, 236–237  
    troubleshooting, 238  
  minor components, 220–221  
  overview of analytical techniques, 221  
  sphingolipids, 220  
  sterols, 220  
  thin-layer chromatography  
    materials, 227–228  
    running and development, 228–229  
    troubleshooting, 229
- Lipid–protein interactions. See Protein  
  microarray
- Lyticase solution, 498
- M**
- Mass spectrometry (MS). See also  
  Proteomics
- metabolomics  
    amino acid analysis with hydrophilic  
      interaction chromatography–  
      tandem mass spectrometry  
    conditioning samples, 611  
    culture, 610  
    materials, 608–610  
    recipes, 612–613  
    running conditions, 611–612  
    sample collection and extraction,  
      610–611  
  cell growth and extraction of  
    metabolites, 604–606  
  materials, 603–604
- overview, 601  
  technique for amyloid purification and  
    identification, 505–506
- Mat formation  
  assays  
    culture and photography, 58–59  
    materials, 57–58  
    overview, 50–51  
    recipes, 59  
  induction, 50
- Meiosis  
  chromosomes  
    segregation, 21  
    structure, 22–23  
    visualization, 24  
  progression regulation, 23  
  recombination, 22  
  recombination analysis  
    chromosome visualization  
      fluorescence microscopy, 42  
      immunodecoration, 41–42  
      surface spreading of nuclei,  
        40–41  
  materials, 38–40  
  overview, 24  
  physical analysis  
    DNA extraction and purification,  
      42, 44  
    restriction enzyme digestion, 44  
    Southern blot, 45–46  
  recipes, 47–48
- S phase, 22
- spore formation and viability, 24,  
  26–30
- strain selection for studies, 23
- synchronous cultures  
  assessment of efficiency and syn-  
    chrony, 34  
  liquid medium culture, 33–34  
  materials, 32–33  
  overview, 23  
  recipes, 35–36  
  sample collection  
    meiotic recombination analysis,  
      34–35  
    surface spreading of nuclei for  
      immunofluorescence analysis,  
      35  
    western blot analysis, 35
- Membrane yeast two-hybrid system  
  (MYTH)  
  bait generation and validation  
    integrated MYTH bait generation,  
      337  
  materials, 334–336  
  NubGI test for validation, 337–338  
  recipes, 338–339  
  subcellular localization verification,  
    338

- Membrane yeast two-hybrid system  
(MYTH) (*Continued*)  
transitional MYTH bait generation,  
336–337  
integrated versus transitional MYTH,  
332–333  
overview, 331–332  
screening  
bait-dependency testing, 343  
materials, 340–341  
recipes, 343–345  
secondary screening and prey identification, 342–343  
transformation, 341–342
- MES wash buffer, 47
- Metabolomics  
amino acid analysis with hydrophilic interaction chromatography–tandem mass spectrometry  
conditioning samples, 611  
culture, 610  
materials, 608–610  
recipes, 612–613  
running conditions, 611–612  
sample collection and extraction, 610–611  
ethanol and glucose analysis in culture media  
ethanol spectrophotometric assay, 617–618  
glucose spectrophotometric assay, 615–617  
materials, 614–615  
troubleshooting, 619  
mass spectrometry  
cell growth and extraction of metabolites, 604–606  
materials, 603–604  
overview, 601  
nuclear magnetic resonance, 601  
overview, 599–601
- Metal affinity chromatography. *See* Proteomics
- Metal stripping solution, 407
- Methotrexate medium, 364
- Microscopy. *See* High-throughput microscopy; Single-molecule total internal reflection fluorescence microscopy
- Mitogen-activated protein kinase. *See* Filamentous growth
- MS. *See* Mass spectrometry
- Mucins, secretion profiling in filamentous growth, 69–70
- MX cassettes  
collections for attainment, 144  
gene regulation cassettes, 142–143  
introduction into yeast  
materials, 146–147  
overview, 142  
recipes, 151–152  
transformation, incubation, and amplification, 148–149  
troubleshooting, 149–151  
multiple cassettes and selections, 143  
overview, 141  
polymerase chain reaction  
amplification, 141–142  
recycling  
confirmation of pop-out, 157  
materials, 153–154  
overview, 143–144  
pop out cassettes flanked by large MX3 or PR direct repeats with counterselection, 154  
without counterselection, 155–156  
pop out cassettes flanked by loxP direct repeats, 156  
recipes, 158–159  
troubleshooting, 157  
types and yeast strain genotypes, 147  
MYTH. *See* Membrane yeast two-hybrid system
- N**
- Nitrogen base agar plates, 659  
Nitrogen-limited chemostat medium, 671  
NMR. *See* Nuclear magnetic resonance  
Nonquenched fluorescent liposome. *See* Protein microarray
- Nuclear magnetic resonance (NMR)  
metabolomics, 601  
solid-state NMR of prion amyloids, 485–486
- O**
- One-hybrid assay. *See* Yeast one-hybrid assay
- P**
- PBS. *See* Phosphate-buffered saline  
PCA. *See* Protein-fragment complementation assay  
PCR. *See* Polymerase chain reaction  
Pectinase agar plates, 70–71  
Pectinase, filamentous growth assay, 69  
PEGylOx. *See* Affinity capture  
Phenol:chloroform, 113, 119–120, 138  
Phenomics. *See* Genotype–phenotype mapping  
Phosphate-buffered saline (PBS), 381, 413  
Phosphate-limited chemostat medium, 671  
Phosphopeptide binding solution, 407  
Phosphopeptide elution solution, 407  
Phosphopeptides. *See* Proteomics  
Plasmid construction. *See* Homologous recombination-based cloning  
PLATE solution, 357, 371, 381  
Polymerase chain reaction (PCR)  
calling card analysis and inverse polymerase chain reaction, 542–543  
chromosome conformation capture and end-point polymerase chain reaction, 112–113  
chromosome conformation capture carbon copy, 124–125  
DNA sequencing, 628–629  
Hi-C, 137–138  
MX cassette amplification, 141–142  
synthetic genome synthesis  
colony screening PCR, 688–689  
finish PCR, 687–688  
templateless PCR, 687  
transposon-insertion libraries, 167–168  
yeast one-hybrid assay genomic and plasmid templates  
amplification, 531  
materials, 530–531  
recipes, 532  
troubleshooting, 531–532
- Posttranslational modifications. *See* Protein microarray; Proteomics
- Potassium acetate medium, 639, 644  
Potassium phosphate buffer, 678  
Potassium phosphate-buffered solution, 71  
Presporulation medium, 36
- Prions  
approaches for study  
biochemical methods, 484  
cell biology, 484  
computational methods, 484  
genetics, 481–483, 488–492  
physical studies, 484–486  
curing, 482–483, 492  
cytoduction  
cyclohexamide resistance, 491–492  
guanidine curing, 493  
induction by overproduction, 492  
standard cytoduction, 491  
isolation and analysis  
agarose gel electrophoresis, 504  
density gradient sedimentation, 504  
lysate preparation, 502–503  
materials, 501–502  
recipes, 506–508  
technique for amyloid purification and identification  
amyloid protein isolation, 504–505  
digestion, 505–506  
mass spectrometry, 505–506

## Index

- Prions (*Continued*)  
troubleshooting, 506  
nomenclature, 482  
overproduction and generation,  
482, 492  
phenotype assays, 488–491  
phenotype relationship, 482–483  
transfection  
incubation and growth conditions,  
496–497  
materials, 495–496  
overview, 483  
recipes, 497–499  
types in yeast, 481, 483
- Protein localization. *See* Transposon-  
insertion libraries
- Protein microarray  
applications, 417  
lipid–protein interaction analysis  
liposome applying to microarray,  
430–431  
materials, 428–429  
nonquenched fluorescent liposome  
preparation, 429–430  
recipes, 432  
troubleshooting, 431  
overview, 415–416  
posttranslational modification assays  
blocking, 435  
detection and processing, 437–438  
materials, 433–435  
posttranslational modification  
reactions, 436  
reaction buffer preparation  
acetylation, 435, 438–439  
phosphorylation, 435, 439  
SUMOylation, 436, 440  
ubiquitylation, 436, 440  
recipes, 438–440  
troubleshooting, 438  
washing, 436–437  
protein–protein interaction analysis  
antibody incubations  
primary antibody, 419–420  
secondary antibody, 420  
blocking, 419  
materials, 418–419  
probing, 419  
recipes, 420–421  
troubleshooting, 420  
RNA-binding protein characterization  
Cy5 labeling of RNA probe,  
424–425  
materials, 422–423  
recipes, 427  
RNA-binding assay, 425–426  
troubleshooting, 426
- Protein-fragment complementation assay  
(PCA)  
cytosine deaminase protein-fragment  
complementation assay  
Cdk1 protein interaction detection,  
368–370  
expression plasmid construction,  
368  
FCY1 gene deletion, 368  
image analysis, 370  
materials, 366–367  
protein–protein interaction detec-  
tion in different cyclin  
deletion strains, 370  
recipes, 371–372  
troubleshooting, 370
- dihydrofolate reductase protein-  
fragment complementation  
assay  
homologous recombination of frag-  
ments, 352  
large-scale screening  
bait strain preparation, 353  
image analysis, 354  
overview, 352–353  
prey strain preparation,  
353–354  
statistical analysis, 355  
tray incubation, 354  
materials, 350–352  
recipes, 357–358  
troubleshooting, 355–356  
general considerations, 348–349  
genotype-to-phenotype mapping of  
protein complexes and  
interaction networks  
diploid strain construction, 362  
gene deletion introgression into  
DHFR PCA strains, 361  
image and statistical analysis,  
362–363  
materials, 359–360  
recipes, 364–365  
sporulation and recombinant hap-  
loid strain selection, 361–362  
troubleshooting, 363  
principles, 347–348
- real-time assay  
applications, 378–379  
cell preparation  
fluorescence microscopy,  
376–378  
fluorometric analysis using  
infrared fluorescence protein,  
377  
homologous recombination of frag-  
ments, 375–376  
materials, 373–375  
recipes, 380–381  
transformation of expression  
plasmid pairs, 375  
troubleshooting, 378
- Proteinase K solution,  
100, 263
- Protein–protein interactions. *See* Affinity  
capture; BioGRID;  
Membrane yeast two-hybrid  
system; Protein-fragment  
complementation assay;  
Protein microarray;  
Proteomics; Yeast two-hybrid  
system
- Proteomics  
immobilized metal affinity  
chromatography of  
phosphopeptides  
binding conditions, 406  
filtration tip preparation, 406  
materials, 404–406  
recipes, 407  
resin preparation, 405–406  
troubleshooting, 407  
washing, elution, and filtration,  
406–407  
immunoaffinity precipitation of  
modified peptides  
antibody conjugation to agarose  
beads, 410  
incubation conditions, 411  
materials, 409–410  
peptide washes and elution,  
411–412  
recipes, 413  
sample preparation for liquid  
chromatography-tandem  
mass spectrometry, 412  
troubleshooting, 412–413  
washing and storage of beads,  
410–411  
posttranslational modification types,  
401–402  
protein microarray. *See* Protein  
microarray  
techniques, 402–403  
PTC buffer, 498
- Q**  
Quantitative trait loci. *See* Genotype–  
phenotype mapping
- R**  
Reciprocal hemizygoty. *See* Genotype–  
phenotype mapping  
Recombination. *See* Homologous recom-  
bination-based cloning;  
Meiosis  
RNA-binding proteins. *See* Protein  
microarray  
RNase A, boiled, 363

- S**
- Saccharomyces* Genome Database (SGD)  
 annotations, 558, 570–572  
 biochemical pathway analysis, 561–562  
 data mining  
 microarray data exploration,  
 568–569  
 YeastMine, 566–568  
 genome feature exploration, 574–576  
 mutant phenotype analysis, 562–564  
 ontology, 558, 570–572  
 overview, 557–558  
 reference genome sequence, 558  
 user interface, 558–559
- Saccharomyces* Genome Deletion Project  
 applications, 176  
 collection attainment, 184  
 functional profiling of collections  
 fitness measurements  
 liquid medium culture, 182  
 pool construction and growth,  
 182–183  
 solid medium culture, 181–182  
 inoculation of collections, 180  
 materials, 179–180  
 principles, 175–176  
 recipe, 184  
 troubleshooting, 183  
 overview, 173–175
- Saccharomyces sensu stricto* enrichment  
 medium, 639
- Salmon sperm DNA solution, 343
- SC medium. *See* Synthetic complete  
 medium
- SCE buffer, 100
- SDE plates, 151
- SDS gel loading buffer, 71
- SGA. *See* Synthetic genetic array
- SGD. *See* *Saccharomyces* Genome Database
- Single-molecule total internal reflection  
 fluorescence microscopy  
 applications, 284–285  
 coverslip cleaning and functionalization  
 lipid passivation, 290–292  
 materials, 287–289  
 recipes, 292  
 silanization, 289–290  
 data analysis, 299–301  
 principles, 283–284  
 reaction preparation for imaging  
 flow chamber assembly, 295–296  
 materials, 294–295  
 microtubule binding interactions  
 dynamic microtubules, 297  
 paclitaxel-stabilized microtu-  
 bules, 296–297  
 recipes, 297
- Site-directed mutagenesis, 17–18
- SLAHD plates, 64
- Sodium acetate buffer, 113, 120, 138
- Sodium phosphate solution, 281, 338, 344
- Sorbitol solution, 629
- SOS medium, 499
- Southern blot, meiotic recombination  
 analysis, 45–46
- Spheroplast fixative solution, 47
- Spheroplast lysis buffer, 47
- Spheroplast storage buffer, 35
- Sphingolipids. *See* Lipids, yeast
- SPM plates, 30
- SPO agar, 212
- Spore  
 mutation effects on formation and  
 viability, 24  
 sporulation efficiency and viability  
 analysis from tetrad  
 dissection, 27–29
- Sporulation medium, 36, 459–460, 660
- SSC, 47
- ST buffer, 499
- STC buffer, 499
- Sterols. *See* Lipids, yeast
- Strains, *Saccharomyces cerevisiae*  
 choice, 6–7  
 conditional effects of mutations, 2–4  
 congenic versus isogenic strains, 1–2  
 expansion, 4–5  
 genotype–phenotype mapping. *See*  
 Genotype–phenotype  
 mapping  
 high throughput sequencing. *See* DNA  
 sequencing  
 isolation and domestication  
 enrichment and isolation, 637  
 materials, 636  
 recipes, 639–640  
 sampling, 637  
 species identification, 637  
 strain preparation for laboratory  
 work, 637–638  
 troubleshooting, 638  
 lipid composition, 218  
 meiosis studies, 23  
 prospects, 7  
 resources, 4–6  
 spore wall integrity testing. *See* Cell wall  
 table, 3  
 yeast two-hybrid system, 316
- Sulfate-limited chemostat medium, 671
- Synthetic amino acid dropout medium, 85
- Synthetic complete (SC) medium,  
 169–170, 215, 357, 365,  
 371–372, 381
- Synthetic defined medium, 55–56, 64, 381,  
 644, 649–650
- Synthetic dextrose medium, 492
- Synthetic dextrose plates, 151–152
- Synthetic drop-out medium, 338–339, 344
- Synthetic genetic array (SGA)  
 alternative techniques, 442–443  
 analysis and imaging, 454, 456–457  
 applications, 443, 445, 457–458  
 deletion mutant array construction,  
 454–455  
 genetic interaction quantification, 443  
 high-throughput microscopy, synthetic  
 genetic arrays for fluorescent  
 tagging  
 drug treatment and medium switch,  
 273–274  
 imaging, 274  
 materials, 271–273  
 recipes, 275–276  
 subculture preparation, 273  
 materials, 448–451  
 mutant strain collections, 442  
 pin tool sterilization,  
 453–454  
 principles, 441–442, 444  
 query strain construction, 450,  
 452–453  
 recipes, 458–461
- Synthetic genome synthesis  
 building block synthesis from  
 oligonucleotides  
 assembly, 689  
 cloning, 688  
 overlapping oligonucleotide  
 preparation, 687  
 polymerase chain reaction  
 colony screening PCR,  
 688–689  
 finish PCR, 687–688  
 templateless PCR, 687  
 recipes, 691  
 transformation, 688  
 troubleshooting, 689–690  
 materials, 685–686
- Synthetic lysine dropout medium, 85
- Synthetic minimal medium, 612–613
- T**
- TAPI. *See* Technique for amyloid purifica-  
 tion and identification
- TB medium. *See* Terrific broth medium
- TBE buffer, 139, 519, 525–528, 532
- TBS. *See* Tris-buffered saline
- TBST, 71, 432
- TCA buffer, 71
- TE buffer, 48, 113, 120, 139, 171, 629
- Technique for amyloid purification and  
 identification (TAPI)  
 amyloid protein isolation,  
 504–505

## Index

- Technique for amyloid purification and identification (TAPI)  
(*Continued*)  
digestion, 505–506  
mass spectrometry, 505–506
- Terrific broth (TB) medium, 86
- Tetrad genetics  
crossing over and gene conversion  
analysis in meiosis, 29–30  
overview, 14–15
- Thin-layer chromatography. *See* Lipids, yeast
- 3C. *See* Chromosome conformation capture
- TLE buffer, 139
- Total internal reflection fluorescence microscopy. *See* Single-molecule total internal reflection fluorescence microscopy
- Transcription factor–DNA binding motifs  
consensus sequences, 550  
enrichment computation, 554–555  
generation, 550–552  
overview, 547  
putative binding site identification, 553–554  
repositories, 552–553  
scoring, 547–549  
visualization, 549
- Transmission electron microscopy. *See* Electron tomography
- Transposon calling cards. *See* Calling card analysis
- Transposon-insertion libraries  
advantages and limitations, 163  
applications, 163–164  
features, 162–163  
overview, 161  
phenotypic screening and protein localization  
Cre-*lox* recombination to  
generate epitope-tagged alleles, 168  
insertion site identification with  
inverse polymerase chain reaction, 167–168  
materials, 165–166  
recipes, 169–171  
screening of transformants, 167  
transformation, 166–167  
resources, 162
- Tris-buffered saline (TBS), 48, 423, 427, 432, 507
- Tween wash buffer, 139
- Two-hybrid system. *See* Membrane yeast two-hybrid system; Yeast two-hybrid system
- V**
- Vitamin stock solution, 672
- W**
- Western blot  
mitogen-activated protein kinases in filamentous growth, 67–69  
synchronous meiotic cultures, 35
- X**
- X-Gal plates, 171
- X-ray fiber diffraction, prion amyloids, 485
- Y**
- YAPD medium, 519
- YAPD plates, 520, 526, 529, 532
- Yeast one-hybrid assay  
advantages and limitations, 511–512  
bait strain generation  
autoactivity testing, 517  
integrated baits  
glycerol stock preparation, 518  
identity confirmation, 517–518  
materials, 514–515  
recipes, 519–520  
reporter construct linearization, 515–516  
transformation, 516–517  
troubleshooting, 518  
colony lift  $\beta$ -galactosidase assay  
culture and incubation, 528  
materials, 527–528  
recipes, 529  
troubleshooting, 528  
library screening  
double-positive yeast identification, 523  
gap-repair for interaction retesting, 524–525  
materials, 521–522  
recipes, 525–526
- transformation, 522–523  
polymerase chain reaction of genomic and plasmid templates  
amplification, 531  
materials, 530–531  
recipes, 532  
troubleshooting, 531–532  
principles, 509–511
- Yeast two-hybrid system. *See also* Membrane yeast two-hybrid system  
array-based screening, 316–317, 325–327  
bait self-activation testing, 315, 323–324  
false negatives, 317  
false positives, 317–318  
host strain selection, 316  
library screening  
advantages and disadvantages, 316  
mating, 325  
prey and bait culture preparation, 324  
materials, 319–322  
overview, 313  
pooled array screening, 317  
rationale, 313–315  
recipes, 328–329  
transformation, 322–323  
troubleshooting, 327–328  
vector choice, 315, 320
- YeastMine. *See* *Saccharomyces* Genome Database
- YEP-GAL medium, 72
- YEPA medium, 56, 59, 71, 86, 101, 152, 159, 171, 184, 358, 365, 372, 381, 470, 478, 492, 499, 545, 640, 645, 650, 660
- YEPD plates, 30, 36, 56, 59, 71, 86, 152, 212, 329, 372, 461, 640, 645, 650
- YPA agar, 212
- YPAD medium, 339, 344–345
- YPG medium, 493
- YPG plates, 30, 36
- YPGal medium, 159
- Z**
- Z-buffer, 529
- Zymolase 100-T, 210
- Zymolase buffer, 48
- Zymolase suspension, 532