

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
- α -factor, G₁ 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*, G₁ 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
 - G₁ cell collection for sampling, 252–253
 - loading, 253
 - materials, 248–249
 - sample preparation, 252
 - troubleshooting, 255–256
 - chemical and genetic approaches
 - G₁ synchronization using α -factor mating pheromone, 243–244
 - M/G₁ 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- β -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 hemizygoty, 647–648
 troubleshooting, 648–649
 overview, 631–632
 phenomics, 633
 quantitative trait loci mapping
 F₁ 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 microtubules, 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 purification 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 β -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