

Index

- A**
- Actin, staining with fluorescent phalloidin
 - conjugates
 - cell preparation and staining, 214–215
 - materials, 213–214
 - recipes, 216
 - troubleshooting, 218
 - ade6*, 16
 - ADS. *See* Antidiploid selection
 - Ago1, 9
 - Ags1, 390
 - Antidiploid selection (ADS)
 - Pombe Epistasis Mapper system, 419, 426–427
 - selectable markers, 406–407
 - APC/C, 254
- B**
- Bgs, 390
- C**
- Camptothecin, hyphal growth induction
 - in *Schizosaccharomyces japonicus*, 473
 - Cdc2*, 4–6, 254
 - Cdc13*, 5, 254
 - Cdc45*, 9
 - CDK, 4
 - Cell cycle
 - cdc* mutants
 - cell cycle stage enrichment in culture, 273
 - execution point analysis, 272–273
 - induction synchrony and reciprocal shift analysis, 273, 278
 - pheromone response, 278
 - reciprocal shift experiments, 273
 - types, 274–275
 - cell size at division, 272
 - flow cytometry of DNA content
 - barcoding, 311
 - data analysis
 - cell doublet exclusion, 313
 - DNA quantification, 314
 - mononuclear versus binuclear cells, 313
 - fixation of cells, 311
 - materials, 310–311
 - recipes, 314
 - staining, 311–312
 - troubleshooting, 312–313
 - growth media, 271–272
 - markers, 276–279
 - meiotic cell cycle. *See* Meiosis
 - overview in *Schizosaccharomyces pombe*, 4–6, 271
 - Schizosaccharomyces japonicus*, 456–457
 - stationary phase, 7
 - synchronization with centrifugal elutriation
 - elutriation
 - cell preparation and setup, 284–286
 - elutriation, 286–287
 - 4-mL chamber, 287–288
 - materials, 283–284
 - recipes, 289–290
 - troubleshooting, 288
 - synchronization with lactose gradient centrifugation
 - gradient generation, 292
 - gradient running, 293
 - materials, 291–292
 - recipes, 294
 - staining, 293
 - synchronization with M-factor pheromone
 - incubation conditions, 306
 - materials, 305
 - overview, 306–308
 - recipes, 308
 - synchronization with *nda3-KM311*
 - arrest release
 - G₂ through repeated rounds of mitosis and S phase
 - incubation conditions, 301
 - materials, 300–301
 - overview, 301–302
 - recipes, 302–303
 - prophase through mitosis into S phase
 - incubation conditions, 296
 - materials, 295–296
 - overview, 297
 - recipes, 297–298
 - troubleshooting, 296–297
 - Cell wall
 - digestion, 201
 - fluorescence staining
 - materials, 208–209
 - recipes, 211
 - staining
 - Calcofluor, 210
 - DAPI, 210
 - DAPI/Calcofluor dual staining, 209
 - troubleshooting, 211
 - disruption, 119–120, 136–137
 - components
 - α-glucan, 389
 - B-BG, 388–389
 - β-glucans, 387–389
 - galactomannan, 389
 - L-BG, 388
 - overview, 387
 - organization, 389–390
 - biosynthetic and remodeling enzymes, 390
 - polysaccharide analysis
 - electron microscopy, 391
 - enzymatic digestion, 391
 - fluorescence microscopy, 391
 - radioactive labeling and quantitative analysis, 392
 - synthesis inhibitors, 391
 - protein analysis, 392
 - radioactive labeling and fractionation
 - data analysis, 401
 - enzymatic digestion, 399–400
 - galactomannan chemical
 - fractionation, 400–401
 - isolation of cell wall, 397–399
 - labeling, 397
 - materials, 395–396
 - recipes, 401
 - Centromere, fission yeast, 8
 - ChIP. *See* Chromatin immunoprecipitation
 - Chromatin. *See also* Heterochromatin
 - fluorescence staining
 - materials, 208–209
 - recipes, 211
 - staining
 - Calcofluor, 210
 - DAPI, 210
 - DAPI/Calcofluor dual staining, 209
 - troubleshooting, 211
 - modification overview in fission yeast, 9

- Chromatin immunoprecipitation (ChIP),
heterochromatin proteins and
modifications
cell culture, 382
immunoprecipitation, 383–384
materials, 381–382
overview, 363–364
recipes, 384–385
- Chromosomal DNA extraction. *See* DNA
extraction
- Chromosome segregation. *See* Meiosis
- Chromosome stability. *See* Genomic
stability
- Cig2, 254
- Citrate-phosphate buffer, 76, 90, 401
- Clr4, 9, 359
- Cnp3, 254
- Colony polymerase chain reaction
boiling of cells, 93
enzymatic digestion, 93
materials, 92–93
recipes, 94
spheroplast buffer, 94
- Cps1, 390
- Cryoelectron microscopy. *See* Electron
microscopy
- Culture. *See* Growth
- D**
- Dcr1, 9
- Diploid selection
heteroallelic complementation, 48–49
materials, 48
nonsporulating diploid selection, 49
overview, 37
recipes, 49–50
- DNA extraction
cell culture and lysis, 89
epistasis mapping samples
long preparation, 414
materials, 413–414
quick preparation, 414
recipes, 414
isolation for two-dimensional gel
electrophoresis of DNA
replication intermediates,
326–328
materials, 88–89
overview, 68
polymerase chain reaction sample
preparation, 90
recipes, 90
Southern blot sample preparation, 90
- DNA repair
double-strand break repair assay
calculations, 353–354
cell culture and plating, 351
genetic analysis, 352
materials, 348–349
- mutant crossing into
minichromosome strain,
349–350
principles, 349
principles, 355
recipes, 356–357
transformation of mutant strains,
350–351
troubleshooting, 354–355
viability assay, 352, 354
- overview, 318–319
- pulsed-field gel electrophoresis of
chromosomal
rearrangements arising from
misrepair
agarose-embedded DNA
preparation, 343–344
cell culture, 343
gel electrophoresis, 344–345
materials, 342–343
recipes, 345–347
staining and imaging, 345
- DNA replication
combing assay
combing, 337–338
coverslip silanization, 337
labeling with nucleoside analogs
double labeling, 335
single labeling, 335
materials, 333–335
plug melting, 336–337
plug preparation and cell wall
digestion, 335–336
proteinase K treatment, 336
recipes, 339–340
staining
double staining, 338
triple staining, 338
Tris-EDTA washes, 336
- overview, 317–318
- two-dimensional gel electrophoresis of
replication intermediates
digestion of DNA, 328
DNA isolation, 326–328
electrophoresis
first dimension, 328–329
second dimension, 329
materials, 325–326
principles, 329–330
recipes, 330–332
- Double-mutant selection
overview, 407
Pombe Epistasis Mapper, 419, 427
- Double-strand break repair. *See* DNA
repair
- E**
- Edinburgh minimal media (EMM),
13–15, 23
- EMM2, 24, 49, 59, 76, 82, 161, 234,
240, 262, 265, 269, 289, 297,
302, 346, 356, 368, 466, 473
- EMM2-LowAmm, 161
- EMM2L, 82
- EMMG, 24, 323, 368, 420
- EMM-N, 25, 262, 270, 466
- EMMP, 25
- EMMSer, 25
- EMMUr, 26
- Electron microscopy (EM)
advantages, 173
cryoelectron microscopy
materials, 191–192
sample preparation and imaging,
192
immunolocalization of proteins
embedding, 186
freeze-substitution for
immunolabeling, 185–186
imaging of expressed fluorescent
protein tags, 187
materials, 184–185
recipes, 188–190
section immunolabeling, 186
three-dimensional reconstruction
from serial sections, 188
interpretation of images, 177–178
limitations, 174
scanning electron microscopy
freezing
high-pressure freezing, 180–181
plunge freezing, 181–182
materials, 179–180
principles, 176
recipes, 183
troubleshooting, 182
three-dimensional imaging, 177
transmission electron microscopy
principles, 177
yeast sample preparation
cell fixation, 175
cell fractionation, 176
freeze-substitution fixation,
175–176, 182
rapid freezing, 175
- Electroporation
Schizosaccharomyces pombe
buffer, 72
materials, 71–72
technique, 72
Schizosaccharomyces japonicus, 469
- EM. *See* Electron microscopy
- EMAP. *See* Epistasis
- EMM. *See* Edinburgh minimal media
- Epistasis
DNA preparation for mapping
long preparation, 414
materials, 413–414

- Epistasis (*Continued*)
 quick preparation, 414
 recipes, 414
 mapping
 computational analysis, 405
 double-mutant selection, 407
 high-throughput mapping, 405
 overview, 403–405
 PEM system. *See* Pombe Epistasis Mapper
 S-score calculation, clustering, and visual interaction profiles
 colony size measurement, 431
 EMAP toolbox for data clustering and visualization, 431–432
 materials, 429–430
 plate imaging, 430–431
 transformation of *Schizosaccharomyces pombe* in 96-well format for mapping
 cell culture and heat shock, 411
 materials, 410–411
 recipes, 411–412
- Ethane methanesulfonate mutagenesis
 growth, 59
 materials, 58–59
 recipes, 59–60
- F**
- Fehlings reagent, 401
- Flow cytometry, DNA content in cell cycle
 barcoding, 311
 data analysis
 cell doublet exclusion, 313
 DNA quantification, 314
 mononuclear versus binuclear cells, 313
 fixation of cells, 311
 materials, 310–311
 recipes, 314
 staining, 311–312
 troubleshooting, 312–313
- Fluorescence microscopy
 actin staining with fluorescent phalloidin conjugates
 cell preparation and staining, 214–215
 materials, 213–214
 recipes, 216
 troubleshooting, 218
 chromatin and cell wall staining
 materials, 208–209
 recipes, 211
 staining
 Calcofluor, 210
 DAPI, 210
 DAPI/Calcofluor dual staining, 209
 troubleshooting, 211
- filters, 196
- imaging platform, 194–195
- immunofluorescence microscopy
 antibody application
 primary, 202, 204
 secondary, 202, 204
 cell wall digestion, 201
 DAPI staining, 202–203
 fixation
 chemical fixation, 205
 formaldehyde, 201, 203–204
 solvent fixation, 205–206
 materials, 199–200
 mounting, 203–205
 permeabilization and quenching, 202
 recipes, 206
 troubleshooting, 203
- light sources and detectors, 196–197
- live cell imaging. *See* Live cell imaging
- objective selection, 194, 196
- overview for fixed fission yeast, 193–194
- 5-Fluoroorotic acid
 counterselection, 66
 selection for *ura4* deletion, 17, 28
 YES medium, 84
- G**
- GALI*, 100
- Galactomannan, chemical fractionation, 400–401
- Gas, 390
- Gene Ontology, growth over time in fission yeast, 1–2
- Generation time. *See* Growth
- Genetic interaction mapping. *See* Epistasis; Pombe Epistasis Mapper
- Genomic stability
 minichromosome loss assay
 cell growth and plating, 322
 materials, 321–322
 principles, 322–323
 recipes, 323–324
 overview of assays, 315–317
- Growth
 efficient mating growth conditions, 23–24
 generation times, 13–14, 20
 growth state
 auxotrophic/prototrophic status and amino acid provision, 17–18
 liquid culture physiological experiments, 19
 starvation-induced memory from starters to main cultures, 19
- halo assay, 22
- media
- carbon sources, 14
- minimal media supplementation
 with amino acids and nucleobases, 16
- nitrogen sources, 14–15
- sporulation media, 15–16, 46
- minimal sporulating media, 26–27
- nitrogen starvation
 phenotype penetrance enhancement in deletion spores, 21
 starvation-induced pseudohyphal growth, 22
- overview in *Schizosaccharomyces pombe*, 6
- rate measurements, 21
- starter culture volume calculation, 19–20
- storage of fission yeast, 17
- stress
 response studies
 DNA damage, 23
 heavy metals, 22–23
 nutrient depletion, 22
 osmotic stress, 22
 oxidative stress, 23
 temperature, 22
 unwanted sources in liquid culture, 20–21
 temperature, 20
- H**
- Halo assay, 22
- hENT1, 318
- Hermes, 39
- Heterochromatin
 chromatin immunoprecipitation of heterochromatin proteins and modifications
 cell culture, 382
 immunoprecipitation, 383–384
 materials, 381–382
 overview, 363–364
 recipes, 384–385
- micrococcal nuclease digestion analysis
 agarose gel electrophoresis, 378
 cell culture, 377
 digestion, 378
 materials, 376–377
 overview, 363, 378
 recipes, 379
- overview in fission yeast, 359–360
- reporter gene silencing analysis
 cell culture and analysis, 368
 materials, 367
 overview, 360–362
 recipes, 368–369
- short interfering RNA detection
 gel electrophoresis, 373
 materials, 370–371

- Heterochromatin (*Continued*)
 northern blot, 373–374
 overview, 362–363
 probe preparation, 372–373
 recipes, 374–375
 RNA extraction, 372
- Historical perspective, fission yeast as
 model system, 1–3
- Homologous recombination, 318
- HP1, 359–360
- Hypthal growth. *See Schizosaccharomyces japonicus*
- I**
- Immunofluorescence microscopy. *See*
 Fluorescence microscopy
- Immunoprecipitation. *See* Protein extracts
- K**
- KEGG. *See* Kyoto Encyclopedia of Genes
 and Genomes
- Kyoto Encyclopedia of Genes and
 Genomes (KEGG), 435
- L**
- Lead citrate, 190
- Life cycle, fission yeast, 3–4
- Linear DNA, genomic integration
 mutant production, 67
 overview, 66
 tools, 66–67
 transformation. *See* Transformation,
 Schizosaccharomyces pombe
- Live cell imaging
 cell cycle markers, 277
 cell growth and media, 220
 chromosome segregation during meiosis
 cell culture and microscopy, 261
 materials, 260–261
 overview, 254–255
 recipes, 262–263
 fluorescent probes
 dyes, 217–218
 fluorescent proteins, 218, 220
 fluorescent protein fusion protein
 imaging in fission yeast
 cell culture, 231
 Hoescht 33342 staining, 231–232
 image acquisition, 233
 lectin coating of coverslips, 231
 materials, 230–231
 recipes, 234–235
 sample preparation
 long-term observation, 233
 short-term observation,
 232–233
 troubleshooting, 233–234
 strain testing, 57
 mating-type region in fission yeast
 genetics, 32–36
 overview in *Schizosaccharomyces pombe*,
 36–37
 recipes, 46–47
 Schizosaccharomyces japonicus
 diploid cell selection, 465–466
 materials, 464–465
 mating-type switching, 457
 nitrogen starvation, 465
 recipes, 466–467
 selectable markers for mating-type
 selection, 406–407
 troubleshooting, 46
- MEA. *See* Malt extract agar
- Mei2, 7, 252
- Mei3, 252
- Meiosis
 cell cycle regulation, 254
 chromosome segregation
 cell culture and microscopy, 261
 materials, 260–261
 overview, 254–255
 recipes, 262–263
 gene expression regulation, 252–253
 initiation, 252
 live cell imaging of sexual life cycle. *See*
 Live cell imaging
- overview, 7, 251–252, 256–257
- sporulation
 overview, 256
 synchronization
 cell culture and incubation, 269
 materials, 268
 recipes, 269–270
- synchronous induction
 incubation conditions, 265
 materials, 264
 recipes, 265–266
- Messenger RNA (mRNA)
 high-throughput sequencing data
 analysis, 99
 polyadenylated tail measurement,
 98–99
 polysome profile analysis and RNA
 purification
 cell growth and harvesting, 109
 fractionation, 110
 materials, 108–109
 overview, 100
 polypeptide chain termination
 induction, 109
 recipes, 111–112
 ribosomal subunit dissociation, 110
 RNA cleavage between ribosomes,
 110
 RNA preparation, 111
 protein interactions, 99–100
- image analysis
 deconvolution, 227
 maximum projection, 226
- instrumentation
 confocal microscopy, 223
 detectors, 225–226
 filters, 225
 image acquisition, 226
 light source, 225
 objective lenses, 223–224
 wide-field microscopy, 223
- lacO/LacI*-GFP system for imaging
 specific genome locus
 cassette product integration into
 genome, 239
 lacO repeat integration into genome,
 239
 materials, 236–238
 polymerase chain reaction primer
 design and cassette
 amplification, 238–239
 recipes, 240–241
- mounting, 220–222
- overview, 217
- sexual life cycle
 cell culture, 243–245
 image acquisition, 245–246
 materials, 242–243
 mounting, 245
 overview, 246–248
 recipes, 248–249
 troubleshooting, 246
 temperature control, 222–223
- M**
- Malt extract agar (MEA), 23, 26
- Mass spectrometry
 metabolomic analysis with liquid
 chromatography–mass
 spectrometry
 high-performance liquid
 chromatography, 448
 mass spectrometry, 448–452
 materials, 447
 troubleshooting, 452
 workflow, 448
- SILAC. *See* Stable isotope labeling by
 amino acids in cell culture
- Mating
 cross setup
 materials, 44–45
 protoplast fusion, 45–46
 standard crosses, 45
 diploid selection. *See* Diploid selection
 live cell imaging of sexual life cycle. *See*
 Live cell imaging
 mating-type determination
 materials, 56–57
 polymerase chain reaction, 57

- Messenger RNA (mRNA) (*Continued*)
 splicing in fission yeast, 7–8, 97–98
 terminus mapping, 97
 total RNA preparation
 cell growth and harvesting, 104
 extraction, 105
 materials, 103–104
 overview, 96
 purification, 105–106
 recipes, 107
 troubleshooting, 106
 transcript synthesis measurement, 96–97
 turnover analysis
 biotinylation of RNA, 114–115
 extraction of RNA, 114
 materials, 113–114
 overview, 100–101
 purification of biotinylated RNA, 116
 recipes, 117
 streptavidin bead preparation, 115–116
 4-thiouridine labeling, 114
 total RNA purification, 115
 troubleshooting, 117
- Metabolomics
 databases, 435
 liquid chromatography–mass spectrometry
 high-performance liquid chromatography, 448
 mass spectrometry, 448–452
 materials, 447
 troubleshooting, 452
 workflow, 448
 metabolite extraction from liquid *Schizosaccharomyces pombe* cultures
 cell harvesting and quenching, 445
 materials, 443–444
 metabolite extraction, 445
 sample preparation, 444–445
 troubleshooting, 446
 metabolome in yeast, 433–435
 prospects, 440–441
 sample preparation
 cell culture, 436
 data management and processing, 439–440
 metabolites
 detection and quantification, 438–439
 extraction, 437–438
 quenching, 436–437
 workflow, 435–436
 METLIN database, 435
 M-factor. *See* Cell cycle
 Micrococcal nuclease digestion. *See* Heterochromatin
- Microscopy. *See* Electron microscopy; Fluorescence microscopy; Live cell imaging
- Mineral stock, 26, 50, 60, 77, 83, 161, 234, 240, 248, 262, 266, 270, 289, 298, 303, 308, 323, 331, 346, 356, 369, 420, 467, 473
- Minichromosome loss. *See* Genomic stability
- Mitosis-promoting factor (MPF), 254
- Mitotic cell cycle. *See* Cell cycle
- Mmi1, 7
- Moa1, 254
- Morphology, overview in *Schizosaccharomyces pombe*, 6
- MPF. *See* Mitosis-promoting factor
- mRNA. *See* Messenger RNA
- Mutant screening
 arrays, 39
 general considerations, 38–39
 overexpression screens, 40
 spontaneous or chemically induced mutants, 39–40
 synthetic genetic interactions, 40
 transposon mutagenesis with Hermes, 39
- N**
- NETO. *See* Newend takeoff
- Newend takeoff (NETO), 6
- NHEJ. *See* Nonhomologous end joining
- Nitrogen-deprived minimal sporulation liquid medium, 248
- Nitrogen-rich minimal sporulation liquid medium, 249
- Nonhomologous end joining (NHEJ), 318
- Northern blot, short interfering RNAs, 373–374
- O**
- Origin of replication, fission yeast, 8–9
- P**
- Pat1, 252
- PBS. *See* Phosphate-buffered saline
- PCR. *See* Polymerase chain reaction
- PEM. *See* Pombe Epistasis Mapper
- PFGE. *See* Pulsed-field gel electrophoresis
- Phalloidin. *See* Actin, staining with fluorescent phalloidin conjugates
- Pheromone. *See* Cell cycle
- Phloxin B, staining, 16–17, 28
- Phosphate-buffered saline (PBS), 190, 211, 216, 289, 294, 303, 314, 356, 385
- Plasmids
 genome integration, 64–66
 transformation. *See* Transformation, *Schizosaccharomyces pombe*
 use and propagation, 63–64
- Polymerase chain reaction (PCR). *See also* Colony polymerase chain reaction
 DNA extraction and sample preparation, 90
 mating-type determination, 57
- Polysome. *See* Messenger RNA
- Pombe Epistasis Mapper (PEM). *See also* Epistasis
 DNA preparation for mapping
 long preparation, 414
 materials, 413–414
 quick preparation, 414
 recipes, 414
 manual colony replicator for mapping
 antidiploid selection, 419
 double-mutant selection, 419
 library preparation and query strains for mating, 418
 materials, 416–417
 mating and sporulation, 418
 mating-type selection, 419
 principles, 419
 recipes, 420–421
 overview, 406–407
- ROTOR HDA colony replicating robot
 in 1536 array format for mapping
 antidiploid selection and mating-type selection
 round 1, 426
 round 2, 426–427
 double-mutant selection, 427
 L-array preparation, 425
 materials, 422–424
 mating and sporulation, 425–426
 Q-array preparation, 424–425
 recipes, 427–428
- S-score calculation, clustering, and visual interaction profiles
 colony size measurement, 431
 EMAP toolbox for data
 clustering and visualization, 431–432
 materials, 429–430
 plate imaging, 430–431
 transformation in 96-well format for mapping
 cell culture and heat shock, 411
 materials, 410–411
 recipes, 411–412
- PP2A, 254–255
- Promoters, systems for transcriptional regulation, 68–69

- Protein extracts
barriers to analysis
cell wall, 119–120
vacuolar proteases, 120
immunoprecipitation, large-scale
cell disruption, 136–137
cell extraction
denaturing conditions, 137
native conditions, 137
cell harvesting, 135–136
immunoprecipitation, 138
magnetic bead preparation, 137
materials, 134–135
overview, 138–139
pombe popcorn preparation, 136
recipes, 139–140
troubleshooting, 138
immunoprecipitation, small-scale
cell extraction
denaturing conditions, 129–130
native conditions, 129
cell pelleting, 128–129
immunoprecipitation, 130
magnetic bead preparation, 130
materials, 127–128
overview, 130–131
recipes, 132
troubleshooting, 130
SILAC. *See* Stable isotope labeling by amino acids in cell culture
SUMOylation analysis. *See* SUMOylation analysis
tandem affinity purification. *See* Tandem affinity purification
trichloroacetic acid precipitation
cell extraction, 124
cell pelleting, 123
glass bead preparation, 124
materials, 122–123
protein quantification on gels, 125
recipes, 126
troubleshooting, 125
Protoplast transformation. *See* Transformation, *Schizosaccharomyces pombe*
Pulsed-field gel electrophoresis (PFGE), chromosomal rearrangements arising from misrepair
agarose-embedded DNA preparation, 343–344
cell culture, 343
gel electrophoresis, 344–345
materials, 342–343
recipes, 345–347
staining and imaging, 345
- R**
Rdp1, 9
Rec8, 254–255
- Recombinase-mediated cassette exchange (RMCE), *Schizosaccharomyces pombe*
carboxy-terminal tagging and gene transfer detection in *loxP-ura4⁺-loxM3* base strains, 80
insertion detection at *urg1* locus, 80–81
materials, 79–80
principles, 81–82
recipes, 82–84
Replica plating, genotyping, 17
Replication protein A (RPA), 318
Rho1, 390
RMCE. *See* Recombinase-mediated cassette exchange
RNA polymerase II, 95
RNA splicing, overview in fission yeast, 7–8, 97–98
RPA. *See* Replication protein A
- S**
Salt stock, 27, 50, 60, 77, 83, 162, 235, 240, 263, 266, 270, 290, 298, 303, 323, 331, 347, 356, 369, 421, 467, 473
Schizosaccharomyces japonicus
antibiotics for positive and negative selection, 35
comparative genomics, 455–456
culture, 458–459
differentiation triggers, 454
evolution, 453–454
fission yeast genetics, 31–34
genetic map, 462
hyphal growth
induction
camptothecin induction, 473
materials, 471
nutrient deficiency on agar plates, 472
recipes, 473–474
overview, 454, 458
light response, 454, 458
manipulation, 460–461
mating
diploid cell selection, 465–466
materials, 464–465
mating-type switching, 457
nitrogen starvation, 465
recipes, 466–467
mitosis, 456–457
prospects for study, 461
Selectable markers
sporulation
overview, 457
spore dissection, 464
strains, 454–455
transformation
cell culture, 469
electroporation, 469
materials, 468–469
recipes, 470
Sgo1, 254
SILAC. *See* Stable isotope labeling by amino acids in cell culture
Small interfering RNA, fission yeast, 95
Small Molecule Pathway Database (SMPDP), 435
SMPDP. *See* Small Molecule Pathway Database
SOS buffer, 78
Southern blot, DNA extraction and sample preparation, 90
SPAS medium, 357
SPD. *See* Spindle pole body
SpGSA system, 406–407
Spindle pole body (SPD), 5
Splicing. *See* RNA splicing
Spore analysis
materials, 52–53
overview, 37–38
random spore analysis, 53
Sporulation. *See also* Meiosis
medium, 420
minimal sporulating media, 26–27
Schizosaccharomyces japonicus
overview, 457
spore dissection, 464
selectable markers
overview, 457
spore dissection, 464
SSC, 374
S-score. *See* Epistasis
Stable isotope labeling by amino acids in cell culture (SILAC)
challenges in fission yeast, 152–153
principles, 151–152
quantitative proteomics
high-performance liquid chromatography, 168–169
mass spectrometry and data processing, 169
materials, 164–166
protein digestion in-solution, 166
recipes, 169–170
strong cation exchange chromatography, 166–167
titanium oxide chromatography, 167–168
yeast strain generation
growth of cells, 157–158
harvesting of cells, 158
materials, 155–157
mutant construction, 157
overview, 160–161
protein extraction, 159–160
recipes, 161–162
START, 271–273

- Ste11, 252
- SUMOylation analysis
- cell disruption, 142
 - materials, 141–142
 - metal affinity chromatography, 142–143
 - overview, 143
 - recipes, 144–145
 - streptavidin bead binding and elution, 143
- Swi6p, 359–360
- T**
- Tandem affinity purification (TAP)
- cell growth, harvesting, and storage, 147
 - FLAG tag capture, 138–149
 - lysate preparation, 148
 - materials, 146–147
 - protein A capture, 148
 - recipes, 149–150
 - TEV protease cleavage, 148
- TAP. *See* Tandem affinity purification
- Telomere, fission yeast, 8
- TES buffer, 107
- Tetrad dissection
- growth and microscopy, 51
 - materials, 52–53
 - overview, 37–38
- Transformation, *Schizosaccharomyces japonicus*
- cell culture, 469
 - electroporation, 469
 - materials, 468–469
 - recipes, 470
- Transformation, *Schizosaccharomyces pombe*
- electroporation. *See* Electroporation, *Schizosaccharomyces pombe*
 - epistasis mapping samples in 96-well format
 - cell culture and heat shock, 411
 - materials, 410–411
 - recipes, 411–412 - lithium acetate/dimethyl sulfoxide transformation
 - incubation conditions, 86
 - materials, 85–86
 - recipes, 87 - overview, 68
 - protoplast transformation
 - cell culture and treatment, 75–76
 - materials, 74–75
 - recipes, 76–77
- Tris-acetate-EDTA, 347
- Tris-borate-EDTA buffer, 331, 374
- Tris-EDTA buffer, 94, 331, 340, 385
- V**
- Valap, 249
- Vitamin stock, 27, 47, 50, 60, 78, 83, 162, 235, 240, 262, 266, 270, 290, 298, 303, 308, 324, 332, 347, 357, 369, 428, 467, 473
- Y**
- Yeast extract with supplements (YES), 13, 16, 27–28, 47, 50, 54, 60, 83–84, 149, 162, 235, 241, 266, 294, 298, 332, 340, 347, 357, 369, 375, 379, 385, 401, 412, 421, 428
- Yeast Metabolome Database (YMDB), 435
- YES. *See* Yeast extract with supplements
- YMDB. *See* Yeast Metabolome Database