
Index

A

Albumins, seed protein extraction, 20, 21, 23

Antibody screening, *see* Protein microarray

Arabidopsis thaliana, *see* Chloroplast; Plasma membrane

B

Blue-native polyacrylamide gel electrophoresis (BN-PAGE), applications, 344 gel casting, 347 materials, 344, 345 principles, 343, 344 running conditions, 347, 348 sample preparation, membrane fractions, 345–347, 349 soluble fractions, 347

SDS-polyacrylamide gel electrophoresis in second dimension, 348–350 staining, 349, 350

BN-PAGE, *see* Blue-native polyacrylamide gel electrophoresis

C

C16 fluorescein, gel staining, 147, 148, 152–154

Cell wall protein extraction, Medicago sativa stem protein extraction, clean-up and concentration determination, 87, 90

concentration and desalting in spin filter units, 87

extraction, 85–87, 89, 90

materials, 83–85, 87

principles, 83

tissue disruption and washing, 85, 87, 88

overview of approaches, 79–82

proteomics studies, 82, 83

Chloroplast,

functions, 43

protein composition, 43, 44

protein isolation from *Arabidopsis thaliana*,

chloroplast isolation, 45, 46

leaf harvesting and processing, 44–46

materials, 44, 46

spin filtration, 45, 46

thylakoid proteins, 46

Cleveland peptide mapping,

electroelution from gels, 213

gel electrophoresis, 213, 214

materials, 212

Coomassie blue,

blue-native gel electrophoresis, *see* Blue-native polyacrylamide gel electrophoresis

gel staining, 145, 148–150, 152, 153

CyDyes, *see* Differential in-gel electrophoresis

D

Deep Purple, gel staining, 146, 148, 152–154

Denaturing gel electrophoresis, *see*
Two-dimensional gel
electrophoresis

Differential in-gel electrophoresis
(DIGE),
applications, 158, 159
limitations, 158, 159
tomato leaf and root protein analysis,
CyDye preparation and
fluorescent protein labeling,
162, 163, 171, 172
imaging,
analysis, 165, 166, 172
scanning, 165
isoelectric focusing, 163
materials, 160, 161
overview, 159, 160
post-staining for further
processing, 166, 172
salt stress studies, 166, 167, 170,
171
SDS-polyacrylamide gel
electrophoresis,
casting of gels, 163, 164
running conditions, 164, 165
solubilization, 162, 171
tissue harvesting and protein
precipitation, 161, 162, 171,
167
DIGE, *see* Differential in-gel
electrophoresis

E

Edman sequencing,
blocked amino-terminal,
causes, 212
deblocking,
acetyl group, 215, 216
formyl group, 215, 216
pyroglutamic acid, 216
principles, 211
sequencing and analysis, 215

Electroelution,

advantages for matrix-assisted laser
desorption ionization mass
spectrometry, 356
Cleveland peptide mapping, 213
electroblotting comparison, 355, 356
ElectroBlue dye staining of gels,
359–361
endoglucanase isoform analysis, 357
materials, 357, 359
nano liquid chromatography-tandem
mass spectrometry samples,
238, 239, 244, 246
passive elution comparison, 354–356
principles, 356, 357
recovery efficiency, 356, 357
technique, 359–362

Electrospray ionization, *see* Nano liquid
chromatography-tandem mass
spectrometry; Phosphoproteins

G

Globulins, seed protein extraction, 20,
21, 23

Glycoproteins,

detection kit, 324
glycan release for protein analysis,
chemical treatment, 328, 329, 338
endoglycosidase H treatment, 329
peptide *N*-glycosidase A
treatment, 331
peptide *N*-glycosidase F
treatment, 329, 331, 338
glycan structure analysis, 331, 333
glycoproteomics,
high-mannose *N*-glycan
glycoprotein identification,
334, 335, 338
O-*N*-acetyl glucosamine
glycoprotein identification,
335, 336, 338
overview, 334

heterogeneity, 320, 321
materials for analysis, 321–324, 337
matrix-assisted laser desorption
 ionization mass spectrometry
 of peptides, 331, 333
monosaccharide analysis, 327, 328,
 338
N-glycosylation, 317–319
O-glycosylation,
 N-acetyl glucosamine, 320
 secreted proteins, 319, 320
prospects for study, 336, 337
Western blot,
 antibody detection, 327, 337, 338
 lectin affinodetection, 325, 326, 337

H

High-performance liquid
 chromatography, *see* Mudpit
 analysis; Nano liquid
 chromatography-tandem mass
 spectrometry

I

IMAC, *see* Immobilized metal affinity
 chromatography
Image analysis, two-dimensional gel
 electrophoresis,
 differential in-gel electrophoresis,
 see Differential in-gel
 electrophoresis
 digitalization, 178, 198, 209
 experimental design considerations,
 equilibrated design, 176, 177
 reference gel, 178
 technical and biological
 replicates, 177, 178
 gray levels, 179
 multivariate data analysis,
 overview, 195–197
 partial least squares regression,
 principles, 196, 207, 208

 validation, 208, 209
 principal component analysis,
 biological interpretation, 203
 loading plot interpretation, 203
 principles, 196
 score plot interpretation, 203
 spot list analysis, 199–201
 validation method, 201, 210
 software, 197, 198
 spot list,
 generation, 198, 209, 210
 importing into software, 198,
 199, 210
 qualitative variations, 186
 quantitative variations, 186–191, 193
 relative intensity versus relative amount
 relation linearity, 184–186
 resolution, 178, 179, 193
 spot volume normalization, 181–184
 statistical analysis packages, 175, 176
 transmission data conversion to
 optical density, 179, 180
Immobilized metal affinity
 chromatography (IMAC),
 phosphoprotein enrichment,
 306–308, 314
Isoelectric focusing, *see* Two-
 dimensional gel electrophoresis

L

Lectin affinodetection, *see*
 Glycoproteins
Liquid chromatography-mass
 spectrometry, *see* Mudpit
 analysis; Nano liquid
 chromatography-tandem mass
 spectrometry; Phosphoproteins

M

MASCOT, peptide mass fingerprint
 database search, 225, 226, 229,
 232, 233, 309

- Mass spectrometry, *see* Membrane proteins; Mudpit analysis; Nano liquid chromatography-tandem mass spectrometry; Peptide mass fingerprinting; PROTIcDb database
- Matrix-assisted laser desorption ionization mass spectrometry, *see* Electroelution; Glycoproteins; Membrane proteins; Peptide mass fingerprinting
- Medicago sativa*, *see* Cell wall protein extraction
- Membrane proteins,
 gel filtration/ion-exchange chromatography-SDS polyacrylamide gel electrophoresis separation, chromatography and gel electrophoresis, 270–273, 276, 277
 materials, 268
 membrane stripping in sample preparation, 268, 269, 276
 overview, 267, 268
 solubilization of proteins, 270, 276
 trypsinization in gel, 273, 277
 matrix-assisted laser desorption ionization mass spectrometry following separation, database searching, 276
 mass spectrometry, 274–277
 materials, 268
 overview, 267, 268
 phosphoproteins, *see* Phosphoproteins
- Microarray, *see* Protein microarray
- Mitochondria,
 functions, 49
 isolation and fractionation, differential centrifugation, 53, 54, 59
 filtering, 53
 homogenization, 53
 integrity assays,
 cytochrome c oxidase activity, 56, 59
 oxygen consumption, 56
 marker enzyme assays, 54, 55, 59, 60
 materials, 50–52, 58, 59
 overview, 52, 53, 59
 Percoll gradient centrifugation, 54, 59
 storage, 56, 57
 subfractionation of compartments, 57, 58, 61
 protein composition, 49, 50
- Mudpit analysis,
 advantages, 250
 challenges in plant proteomics, 251
 principles, 249–252
 rice leaf and root protein analysis, column preparation, 255, 256
 database searching and protein identification, 257–263
 high-performance liquid chromatography, 256, 257, 264
 mass spectrometry, 257
 materials, 252, 253
 protein precipitation and resuspension, 254, 259, 263
 proteolytic digestion, 254, 255, 263
 tissue harvesting, 253, 254, 259
- Multidimensional protein identification technique, *see* Mudpit analysis
- Multivariate data analysis, *see* Partial least squares regression; Principal component analysis
- N**
- Nano liquid chromatography-tandem mass spectrometry,
 data processing and interpretation, 242–247
 electrospray ionization settings, 239, 240

- high-performance liquid chromatography, 239–241
 - liquid junction, 239, 240, 246
 - mass spectrometer settings, 241, 246
 - mass spectrometry modes, 236
 - materials, 237
 - principles, 235–237
 - protein digestion and peptide extraction from gels, 238, 239, 244, 246
 - two-dimensional nanoflow liquid chromatography-tandem mass spectrometry, *see* Mudpit analysis
- Nuclear protein extraction,
nucleus structure, 73
rice proteins,
 homogenization, 75
 materials, 74
 nuclei purification, 75
 overview, 74
 sucrose density gradient centrifugation, 75, 77
root meristem from onion,
 materials, 65–67, 70
 meristem preparation, 67, 68, 70, 71
 nuclei purification, 68, 69, 71
 overview, 63–65
 precipitation and resuspension, 69–71
- O, P**
- Onion, *see* Nuclear protein extraction
- Partial least squares regression (PLSR),
 two-dimensional gel data,
 principles, 196, 207, 208
 software, 197, 198
 spot list,
 generation, 198, 209, 210
 importing into software, 198, 199, 210
 validation, 208, 209
- PCA, *see* Principal component analysis
- Peptide mass fingerprinting (PMF),
 annotation of spectrum, 225, 231, 232
 digestion in gel,
 contamination prevention, 221, 230
 gel washing, 222, 230
 peptide extraction, concentration, desalting, 222, 223
 proteolysis, 222, 230, 231
 spot excision, 221, 230
 isoform differentiation, 354
 MASCOT database search, 225, 226, 229, 232, 233
 mass spectrometry, 224, 231
 materials, 220, 221
 matrix-assisted laser desorption ionization, 219
 phosphoprotein identification, 309
 principles, 219, 220, 223
 sample deposition,
 classical dried droplet method, 224, 231
 dried droplet method on prestructured hydrophobic target, 224, 231
 tandem mass spectrometry strategies, 229, 230
- Peptide *N*-glycosidase A (PNGase A),
 glycoprotein treatment, 331
- Peptide *N*-glycosidase F (PNGase F),
 glycoprotein treatment, 329, 331, 338
- Percoll gradient centrifugation,
 mitochondria fractionation, 54, 59
- Phenol extraction,
 advantages, 9, 10
 extraction conditions, 11–13
 materials, 10–13
 principles, 9, 10
 seed proteins, 18–20, 22, 23
 solubilization and quantification, 12
 xylem sap proteins, 30

- Phloem sap,
collection, 31, 32
protein composition, 28
protein extraction,
depletion of filament protein and
lectin, 32, 33
materials, 28
precipitation with acetone/
methanol/dithiothreitol, 32, 33
sieve element transport, 27, 28
- Phosphoproteins,
electrospray ionization mass
spectrometry, 309
identification approaches, 305, 306
immobilized metal affinity
chromatography enrichment,
306–308, 314
isotope labeling and relative
quantification of peptides, 309,
310, 312
kinase substrate screening with
protein microarrays,
kinase assay, 383, 387, 388
materials, 380, 381
overview, 379, 380
recombinant kinase purification
under native conditions, 381,
382, 387
target selection, 383, 385
target verification, 385, 387
liquid chromatography-mass
spectrometry, 312, 314
materials for identification, 306
trypsinization,
desalting of peptides, 308, 309, 314
membrane fractions, 307, 314
methylation of peptides, 308
soluble fractions, 307
- Plasma membrane,
functions, 93
protein extraction,
hydrophobic protein enrichment,
102, 107
materials, 95–99, 104
plasma membrane isolation,
microsomal fraction isolation
from *Arabidopsis*, 99, 100
two-phase partitioning, 100–
102, 105–107
purification approaches, 94, 95
two-dimensional gel electrophoresis,
running conditions, 104, 107
solubilization of proteins, 102–104,
107
- PLSR, *see* Partial least squares
regression
- PMF, *see* Peptide mass fingerprinting
- PNGase A, *see* Peptide N-glycosidase
A
- PNGase F, *see* Peptide N-glycosidase F
- Principal component analysis (PCA),
two-dimensional gel data,
biological interpretation, 203
loading plot interpretation, 203
principles, 196
score plot interpretation, 203
software, 197, 198
spot list,
analysis, 199–201
generation, 198, 209, 210
importing into software, 198, 199,
210
validation method, 201, 210
- Protein extraction, *see* Cell wall protein
extraction; Chloroplast;
Nuclear protein extraction;
Phenol extraction; Phloem sap;
Plasma membrane; Seed
protein extraction;
Trichloroacetic acid/acetone
extraction; Wood; Xylem sap
- Protein microarray,
antibody screening,
monoclonal antibodies, 371, 372
polyclonal antibodies, 372, 373
applications, 365, 366

- high-throughput protein expression and purification, 367–369, 373, 374
 - image analysis, 373
 - materials, 366, 367, 373
 - microarray preparation, 369–371, 374, 375
 - principles, 365, 366
 - protein kinase substrate screening, kinase assay, 383, 387, 388 materials, 380, 381 overview, 379, 380 recombinant kinase purification under native conditions, 381, 382, 387 target selection, 383, 385 target verification, 385, 387
 - Protein recovery, *see* Electroelution
 - Protein sequencing, *see* Cleveland peptide mapping; Edman sequencing
 - Protein solubilization, two-dimensional gel electrophoresis samples, chaotrope requirements, 112 detergent requirements, 112, 113 materials, 114, 115, 117 nucleic acid interference, 112 pH effects, 111, 112 phenol-extracted proteins, 12 plasma membrane proteins, 102–104, 107 trichloroacetic acid/acetone-extracted proteins, 3, 4, 7 urea solubilization, 116, 117 urea/thiourea solubilization, 116, 117 wood proteins, 40 zone electrophoresis samples, 117
 - PROTICdb database, alimentation of database via web forms, image file submission, 283, 284, 301 new project creation, 282, 300, 301 output file uploading, 285, 286, 301 project selector, 283 availability, 280 data sharing, 293, 302 file-based database feeding, mass spectrometry identification report file uploading, 292, 301, 302 mass spectrometry spot identification order, 287–290, 301, 302 plant2image file uploading, 287, 301 spot detection output file uploading, 290–292, 301
 - gel browser, alimentation of knowledge base, 297, 298, 300, 302, 303 previously identified spot identification, 295–297, 302 quantitative data export, 300, 302 overview, 279–281 server installation, 281 software requirements, client side, 280, 281 server side, 280
- R,S**
- Rice, *see* Mudpit analysis; Nuclear protein extraction
 - Sap, *see* Phloem sap; Xylem sap
 - SDS-polyacrylamide gel electrophoresis, *see* Blue-native polyacrylamide gel electrophoresis; Membrane proteins; Two-dimensional gel electrophoresis
 - Seed protein extraction, albumins, 20, 21, 23 amphiphilic proteins, 21, 23 globulins, 20, 21, 23 granulometry considerations, 16 materials, 17, 18, 22 protein types, 16 seed composition, 15, 16

- starch granule proteins, 21–23
- trichloroacetic acid/acetone
 - extraction, 18–20, 22, 23
- Sequencing, *see* Cleveland peptide mapping; Edman sequencing
- Silver nitrate, gel staining, 146, 148, 150–154
- Solubilization, *see* Protein solubilization, two-dimensional gel electrophoresis samples
- Staining, polyacrylamide gel electrophoresis,
 - C16 fluorescein, 147, 148, 152–154
 - Coomassie blue, 145, 148–150, 152, 153
 - CyDyes, *see* Differential in-gel electrophoresis
 - Deep Purple, 146, 148, 152–154
 - dye selection in proteomic analysis, 147
 - ElectroBlue, 359–361
 - materials, 147–149, 152, 153
 - overview of dyes, 145–147
 - silver nitrate, 146, 148, 150–154
 - Sypro Ruby, 146, 148, 151–154
 - two-dimensional blue-native polyacrylamide gels, 349, 350
- Starch granule proteins, seed protein extraction, 21–23
- Sypro Ruby, gel staining, 146, 148, 151–154
- T**
- Tandem mass spectrometry, *see* Mudpit analysis; Nano liquid chromatography-tandem mass spectrometry; Peptide mass fingerprinting
- TCA/acetone extraction, *see* Trichloroacetic acid/acetone extraction
- Tomato, *see* Differential in-gel electrophoresis
- Trees, *see* Phloem sap; Xylem sap; Wood
- Trichloroacetic acid (TCA)/acetone extraction,
 - advantages for two-dimensional gel electrophoresis, 1, 2
 - isoelectric focusing sample preparation, 4, 7
 - materials, 2, 3, 7
 - phenol extraction comparison, 9, 10
 - precipitation and denaturation, 3, 7
 - protease inhibition, 1
 - rinsing with, 2-mercaptoethanol/acetone, 3
 - solubilization, 3, 4, 7
 - tomato proteins for differential in-gel electrophoresis, 162, 171
- Two-dimensional gel electrophoresis,
 - applications, 121, 122, 124, 125
 - blue-native gel electrophoresis, *see* Blue-native polyacrylamide gel electrophoresis
 - challenges in proteome analysis, 124, 125, 158
 - databases, *see* PROTiCdb database
 - differential in-gel electrophoresis, *see* Differential in-gel electrophoresis
 - imaging, *see* Image analysis, two-dimensional gel electrophoresis
 - isoelectric focusing on immobilized pH gradient strips,
 - cup loading strip holders, 136, 137
 - equilibration of strips for second dimension electrophoresis, 137, 138
 - in-gel rehydrated samples, 135, 136
 - IPGphor unit, 133–135, 141
 - Multiphor II unit, 129–133

- pH ranges, 127, 128
 - rehydration and sample application, 128, 129, 141
 - materials, 125–127, 140, 141
 - plasma membrane proteins, *see* Plasma membrane
 - principles, 124, 125, 157
 - protein identification, *see*
 - Cleveland peptide mapping;
 - Edman sequencing;
 - Mudpit analysis;
 - Nano liquid chromatography-tandem mass spectrometry;
 - Peptide mass fingerprinting
 - protein recovery, *see* Electroelution
 - protein solubilization, *see* Protein solubilization, two-dimensional gel electrophoresis samples
 - SDS-polyacrylamide gel electrophoresis,
 - casting of gels, 138, 139
 - Ettan Dalt II vertical electrophoresis unit, 140
 - staining, *see* Staining, polyacrylamide gel electrophoresis
 - Two-dimensional nanoflow liquid chromatography-tandem mass spectrometry, *see* Mudpit analysis
- U**
- Urea, protein solubilization for two-dimensional gel electrophoresis, 116, 117
- W**
- Western blot,
 - glycoproteins,
 - antibody detection, 327, 337, 338
 - lectin affinodetection, 325, 326, 337
 - kinase substrate verification, 385, 387
 - Wood,
 - cell types, 37
 - formation, 37, 38
 - protein extraction,
 - materials, 38–40
 - solubilization, 40
 - total protein extraction, 39, 40
- X**
- Xylem sap,
 - collection, 28, 29, 33
 - protein composition, 27
 - protein extraction,
 - concentration with filter units, 30
 - materials, 28
 - trichloroacetic acid/acetone precipitation, 30