

Contents

Preface	<i>XV</i>
List of Contributors	<i>XVII</i>
List of Abbreviations	<i>XXIII</i>

Part I Induced Mutations 1

1	Physically Induced Mutation: Ion Beam Mutagenesis	3
	<i>Shimpei Magori, Atsushi Tanaka, and Masayoshi Kawaguchi</i>	
1.1	Introduction	3
1.1.1	LET	4
1.1.2	Mutational Effects of Ion Beams on Plants	5
1.2	Methods and Protocols	7
1.2.1	Ion Beam Irradiation	8
1.2.2	Dose Determination for Ion Beam Irradiation	9
1.2.3	Plant Radiation Sensitivity	10
1.2.4	Population Size of the M1 Generation	11
1.3	Applications	11
1.3.1	Ion Beams for Forward Genetics	12
1.3.2	Ion Beams for Plant Breeding	13
1.3.3	Limitations of Ion Beams	13
1.4	Perspectives	14
	References	14
2	<i>Ds</i> Transposon Mutant Lines for Saturation Mutagenesis of the <i>Arabidopsis</i> genome	17
	<i>Takashi Kuromori and Takashi Hirayama</i>	
2.1	Introduction	17
2.2	Methods and Protocols	18
2.3	Applications	26
2.4	Perspectives	28
	References	28

3	Use of Mutants from T-DNA Insertion Populations Generated by High-Throughput Screening	31
	<i>Ralf Stracke, Gunnar Huep, and Bernd Weisshaar</i>	
3.1	Introduction	31
3.2	Methods and Protocols	34
3.2.1	Plant Material and Growth Conditions	34
3.2.2	Plasmid Design	34
3.2.3	<i>Agrobacterium</i> Culture	35
3.2.4	Plant Transformation and T1 Seed Harvesting	35
3.2.5	Sulfadiazine Selection of Transgenic T ₁ Plants	36
3.2.6	DNA Preparation from Sulfadiazine-Selected T1 Plants	36
3.2.7	FST Production	37
3.2.8	Sequencing and Computational Sequence Analysis	40
3.2.9	Genetic Analysis of T-DNA Insertions	41
3.2.10	DNA-Preparation for Confirmation of FST Predicted Insertion Sites	41
3.2.11	Confirmation PCR	42
3.2.12	Sequencing and Computational Sequence Analysis	44
3.2.13	Seed Donation	45
3.2.14	Identification of Homozygous Mutants	46
3.3	Applications and Considerations for Work with T-DNA Insertion Mutants	47
3.3.1	Unconfirmed T-DNA Insertion Lines	48
3.3.2	Use of Selectable Marker	48
3.3.3	Aberrant T-DNA Insertions	48
3.3.4	Multiple T-DNA Insertions	49
3.3.5	T-DNA-Induced Dominant Effects	49
3.3.6	Allelic Series of Mutants	49
3.3.7	Lethal Knockout Mutants	50
3.3.8	Search for Knockout Phenotype	50
3.3.9	Handling of Non-Single-Copy Genes	50
3.4	Perspectives	51
	References	52
4	Making Mutations is an Active Process: Methods to Examine DNA Polymerase Errors	55
	<i>Kristin A. Eckert and Erin E. Gestl</i>	
4.1	Introduction	55
4.2	Methods and Protocols	56
4.2.1	Overview of the Genetic Assay	56
4.2.2	Overview of the Biochemical Assay for TLS	67
4.3	Applications	73
4.3.1	General Features of the <i>In Vitro</i> Genetic Assay	73
4.3.2	Polymerase Accuracy in the Absence of DNA Damage	74

4.3.3	Mutational Processing of Alkylation Damage by DNA Polymerases	75
4.3.4	DNA Lesion Discrimination Mechanisms	75
4.4	Perspectives	78
	References	79
5	<i>Tnt1</i> Induced Mutations in <i>Medicago</i>: Characterization and Applications	83
	<i>Pascal Ratet, Jiangqi Wen, Viviane Cosson, Million Tadege, and Kirankumar S. Mysore</i>	
5.1	Introduction	83
5.2	Methods and Protocols	84
5.2.1	Identification of <i>Tnt1</i> Insertion Sites	84
5.2.2	Reverse Genetic Approach	94
5.2.2.1	FST Sequencing	94
5.2.2.2	Screening DNA Pools	94
5.3	Applications	95
5.3.1	Line with a Mutant Phenotype – No FSTs Identified	96
5.3.2	Line with a Mutant Phenotype and FSTs Already Identified	96
5.3.3	FST Sequence in the <i>Tnt1</i> Database Matches a Gene of Interest – No Mutant Phenotype is Described in that Line	97
5.3.4	Have a Gene to Work With – No FST or Mutant Phenotype	97
5.4	Perspectives	98
	References	98
Part II	Mutation Discovery	101
6	Mutation Discovery with the Illumina Genome Analyzer	103
	<i>Abizar Lakdawalla and Gary P. Schroth</i>	
6.1	Introduction	103
6.1.1	Overview of the Illumina Genome Analyzer Sequencing Process	103
6.1.2	Resequencing Strategies	104
6.1.2.1	Resequencing Whole Genomes	105
6.1.2.2	Targeted Genome Selection	105
6.1.2.3	Sequencing Transcriptomes	107
6.2	Methods and Protocols	107
6.3	Applications	116
6.4	Perspectives	118
	References	118
7	Chemical Methods for Mutation Detection: The Chemical Cleavage of Mismatch Method	121
	<i>Tania Tabone, Georgina Sallmann, and Richard G.H. Cotton</i>	
7.1	Introduction	121
7.2	Methods and Protocols	125

7.3	Applications	127
7.4	Perspectives	127
	References	128
8	Mutation Detection in Plants by Enzymatic Mismatch Cleavage	131
	<i>Bradley J. Till</i>	
8.1	Introduction	131
8.2	Methods and Protocols	136
8.3	Applications	143
8.4	Perspectives	144
	References	145
9	Mutation Scanning and Genotyping in Plants by High-Resolution DNA Melting	149
	<i>Jason T. McKinney, Lyle M. Nay, David De Koeyer, Gudrun H. Reed, Mikeal Wall, Robert A. Palais, Robert L. Jarret, and Carl T. Wittwer</i>	
9.1	Introduction	149
9.2	Methods and Protocols	150
9.2.1	LightScanner Instrument	151
9.2.2	LightScanner for Variant Scanning	151
9.2.3	LightScanner for Lunaprobe™ (Unlabeled Probe) Genotyping	156
9.3	Applications	159
9.3.1	Sensitivity and Specificity for SNP Heterozygote Detection	159
9.3.2	Variant Scanning by High-Resolution Melting	160
9.3.3	Bell Pepper Multiplex Genotyping with Two Unlabeled Probes	161
9.3.4	Potato Tetraploid Genotyping including Allele Dosage using an Unlabeled Probe	161
9.4	Perspectives	162
	References	163
10	<i>In Silico</i> Methods: Mutation Detection Software for Sanger Sequencing, Genome and Fragment Analysis	167
	<i>Kevin LeVan, Teresa Snyder-Leiby, C.S. Jonathan Liu, and Ni Shouyong</i>	
10.1	Introduction	167
10.2	Mutation Detection with Sanger Sequencing using Mutation Surveyor	168
10.3	Mutation Detection with NextGENE™ and Next-Generation Sequence Technologies	175
10.4	Mutation Detection with DNA Fragments Using GeneMarker®	180
10.5	Perspectives	182
	References	182

Part III	High-Throughput Screening Methods	185
11	Use of TILLING for Reverse and Forward Genetics of Rice	187
	<i>Sujay Rakshit, Hiroyuki Kanzaki, Hideo Matsumura, Arunita Rakshit, Takahiro Fujibe, Yudai Okuyama, Kentaro Yoshida, Muluneh Oli, Matt Shenton, Hiroe Utsushi, Chikako Mitsuoka, Akira Abe, Yutaka Kiuchi, and Ryohei Terauchi</i>	
11.1	Introduction	187
11.2	Methods and Protocols	188
11.3	Perspectives	196
	References	197
12	Sequencing-Based Screening of Mutations and Natural Variation using the KeyPoint™ Technology	199
	<i>Diana Rigola and Michiel J.T. van Eijk</i>	
12.1	Introduction	199
12.2	Methods and Protocols	202
12.3	Applications	206
12.3.1	EMS Mutation Screening and Validation	206
12.3.2	Natural Polymorphism Screening and Validation	208
12.4	Perspectives	211
	References	211
Part IV	Applications in Plant Breeding	215
13	Natural and Induced Mutants of Barley: Single Nucleotide Polymorphisms in Genes Important for Breeding	217
	<i>William T.B. Thomas, Brian P. Forster, and Robbie Waugh</i>	
13.1	Brief Review of Barley Mutants	217
13.2	Applications in Breeding	221
13.3	Single Nucleotide Polymorphism Genotyping to Identify Candidate Genes for Mutants	223
13.3.1	Resources	223
13.3.2	Case Study: Two/Six-Row Locus in Barley	224
13.3.3	Case Study: Graphical Genotyping of a Disease Resistance Locus	227
13.3.4	General Protocol for using High-Throughput Genotyping to Localize Mutants	227
	References	229
14	Association Mapping for the Exploration of Genetic Diversity and Identification of Useful Loci for Plant Breeding	231
	<i>André Beló and Stanley D. Luck</i>	
14.1	Introduction	231
14.2	Methods and Protocols	233
14.2.1	Population for Association Mapping	233

14.2.2	Genotyping	234
14.2.3	Phenotyping	234
14.2.4	Statistical Procedures	235
14.3	Applications	238
14.3.1	QTL Mapping versus Association Mapping	240
14.3.2	Limitations	241
14.4	Perspectives	242
	References	243
15	Using Mutations in Corn Breeding Programs	247
	<i>Anastasia L. Bodnar and M. Paul Scott</i>	
15.1	Introduction	247
15.1.1	Factors to Consider Before Starting a Breeding Program	248
15.1.2	Alternatives to Breeding	249
15.2	Methods and Protocols	249
15.2.1	Backcross Breeding	249
15.2.2	Forward Breeding	252
15.2.3	Supplementary Protocols	253
15.2.3.1	Determining How Many Seeds to Plant	253
15.2.3.2	Working with Recessive Mutations	255
15.2.3.3	Intermating	256
15.2.4	Complication: Pleiotropic Effects	257
15.3	Applications	258
15.3.1	Breeding with a Natural Mutation: QPM	258
15.3.2	Breeding with a Transgene: GFP	259
15.4	Perspectives	259
15.4.1	Marker-Assisted Selection	259
15.4.1.1	Marker-Assisted Selection in Backcross Breeding	259
15.4.1.2	Marker-Assisted Selection in Forward Breeding	260
15.4.2	Doubled Haploids	260
	References	261
16	Gene Targeting as a Precise Tool for Plant Mutagenesis	263
	<i>Oliver Zobell and Bernd Reiss</i>	
16.1	Introduction	263
16.2	Methods and Protocols	266
16.3	Applications	279
16.4	Perspectives	280
	References	280
Part V	Emerging Technologies	287
17	True Single Molecule Sequencing (tSMS)TM by Synthesis	289
	<i>Scott Jenkins and Avak Kahvejian</i>	
17.1	Introduction	289
17.2	Methods, Protocols, and Technical Principles	291

17.2.1	Single Molecule Sequencing Technical Challenges and Solutions	291
17.2.2	Flow Cell Surface Architecture	294
17.2.3	Cyclic SBS	295
17.2.4	Optical Imaging of Growing Strands	297
17.2.5	Mechanical Operation	300
17.2.6	System Components	300
17.2.7	Data Analysis	301
17.3	Applications	301
17.3.1	Single Molecule DGE and RNA-Seq	301
17.3.1.1	DNA Sequencing Applications	303
17.3.2	Single Molecule Sequencing Techniques under Development	303
17.4	Perspectives	304
	References	306
18	High-Throughput Sequencing by Hybridization	307
	<i>Sten Linnarsson</i>	307
18.1	Introduction	307
18.2	Methods and Protocol	308
18.3	Discussion	316
18.4	Applications	316
	References	317
19	DNA Sequencing-by-Synthesis using Novel Nucleotide Analogs	319
	<i>Lin Yu, Jia Guo, Ning Xu, Zengmin Li, and Jingyue Ju</i>	
19.1	Introduction	319
19.2	General Methodology for DNA SBS	321
19.3	Four-Color DNA SBS using CF-NRTs	323
19.3.1	Overview	323
19.3.2	Design, Synthesis, and Characterization of CF-NRTs	324
19.3.3	DNA Chip Construction	326
19.3.4	Four-Color SBS using CF-NRTs	326
19.4	Hybrid DNA SBS using NRTs and CF-ddNTPs	329
19.4.1	Overview	329
19.4.2	Design and Synthesis of NRTs and CF-ddNTPs	331
19.4.3	Four-Color Hybrid DNA SBS	333
19.5	Perspectives	335
	References	336
20	Emerging Technologies: Nanopore Sequencing for Mutation Detection	339
	<i>Ryan Rollings and Jiali Li</i>	
20.1	Introduction	339
20.1.1	Nanopore Detection Principle	340
20.1.2	Important Parameters and Nanopore Sensing Resolution	341
20.1.3	Biological Nanopore History	341
20.1.4	Solid-State Nanopore History	343

20.1.5	Nanopore Promise	343
20.2	Current Developments in Nanopore Sequencing	344
20.2.1	Improving Biological Nanopores	344
20.2.2	Improving Solid-State Nanopores	345
20.2.3	Slowing Translocation and Trapping	347
20.2.4	Modification of the DNA	347
20.2.5	Resequencing Applications	349
20.3	Work Done in Our Lab	350
20.4	Perspectives	351
	References	352

Glossary 355

Index 427